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REVIEWS

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## ANALYTICAL REVIEW OF BIOTECHNOLOGICAL PROBLEM OF UKRAINIAN HARD CHEESES

Yu. SKRIL<sup>1</sup>, O. SHVED<sup>1</sup>, Z. HUBRII<sup>1</sup>, O. VICHKO<sup>2</sup>, T. KUPKA<sup>3</sup>

<sup>1</sup>Lviv Polytechnic National University, Ukraine <sup>2</sup>Ternopil Ivan Puluj National Technical University, Ukraine <sup>3</sup>Opole University, Institute of Chemistry, Polska

E-mail: olha.v.shved@lpnu.ua

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An analytical review of the biotechnological process of production of various hard and semi-hard cheeses in the EU and Ukraine, as well as domestic recipes of fermented cheeses for production at craft cheese factories and at home was conducted. An analysis of the conditions of the key stages of production, including fermentation, coagulation and ripening was carried out. The composition and type of lactic acid bacteria in sourdough for fermented cheeses, as well as enzymes for fermentolysis and coagulation of milk casein was studied. Because of a complex study, 73 types of hard and semi-hard cheeses were analyzed: 35 recipes of the New England Cheesemaking Supply Company by Jim Wallace; 30 production processes of hard and semi-hard cheeses from the EU; 8 technical conditions of hard cheeses of Ukrainian producers. It was shown that the prospects for optimizing the development of new types of hard and semi-hard cheeses in Ukraine are mainly related to the regulation of the time and temperature of fermentation, cooking and ripening of cheeses, as well as the expansion of the biodiversity of the primary and secondary microbiome of starter cultures to improve the taste and aroma of the ready-to-use product.

*Purpose* to analyze the fermentation process and recipes for the production of hard cheeses in Ukraine with the main world samples, to compare the composition and type of lactic acid bacteria in industrial and craft starters, as well as the types of enzymes for fermentolysis and casein coagulation of milk in order to optimize production to improve the taste and aroma of ready-to-use product.

*Materials and methods.* Methodical analysis and abstract-logical method for summarizing the evaluation criteria of the biotechnological process of various hard and semi-hard domestic cheeses with world samples according to recommendations, requirements and standards with the development of patents, technical conditions of their production in the EU and Ukraine; DSTU 6003 (Solid cheeses); New England Cheesemaking Supply Company cheese recipes by Jim Wallace. The obtained data were processed by the methods of statistical analysis, systematization, comparison and generalization of information.

*Results.* The documentation was examined and the data of standards, regulations, requirements and recommendations regarding the biotechnology of hard and semi-hard cheeses were analyzed. An analysis of the market of hard cheeses and the peculiarities of the fermentation process of their production were carried out. The determination of critical points and key stages of production using industrial deposited fermentation producers and protein enzymes coagulation and biodiversity of lactic acid bacteria in sourdoughs for fermented cheeses at world productions were given. An assessment of the prospects for developing new and improving the biotechnology of Ukrainian cheese of high quality and safe hard cheeses for healthy nutrition were done.

*Key words:* hard cheese; semihard cheese; acidification; mesophilic and thermophilic lactic bacteria; coagulation; rennet; chymosin; renin; pepsin; cheese maturation; microbiome.

Production of fermented cheeses is represented in every country of the world, as consumers choose them for their nutrition, rich taste and aroma. The oldest and largest producers of authentic cheeses are European countries such as the Netherlands, Switzerland, Denmark, France, Italy and Spain.

The production of dairy products is one of the important sectors of global agribusiness and a point of innovative implementation of the food bioindustry [1]. According to the Union of Dairy Enterprises of Ukraine in 2019, milk production amounted to 8 million tons, and 35 Ukrainian producers were added to the list of exporters of dairy products to Saudi Arabia [2]. The results of monitoring by experts of the state and development prospects of the domestic market of milk and dairy products in 2021 in Ukraine produced 8.72 million tons of milk, including farm enterprises produced 2.75 million tons of milk. Today, there are about 200 milk fermentation enterprises in Ukraine, with the concentration of production at large milk processing enterprises. In 2020, compared to 2019, the volume of exports of dairy products to Moldova, Georgia, Azerbaijan and Kazakhstan increased by 9-11% in each country. [3].

However, there are many factors that negatively affect the innovative development of enterprises in the food industry of Ukraine, in particular, in cheese making, which reduce their competitiveness. Thus, as of January 1, 2022, the total volume of the shadow segment on the Ukrainian dairy market was estimated at 20-25%. Among the falsified goods, various types of cheese, butter, and sour cream prevailed, which were dominated by substances of vegetable origin, and the content of milk raw materials did not exceed 26% [4], and, under the influence of war uncertainty, these indicators can be even worse [5].

In the structure of dairy production in Ukraine for 2020, according to the marketing research of the Pro-Consulting Company, influencing factors, problems and development trends of one of the most important food industries of our country — the dairy market in Ukraine, including fermented milk products, were determined and studied. 4%, cheeses — 9.4%. The cheese market was characterized by multidirectional trends: the volume of fresh cheese production continued its growth in 2021 (+7.4%); the production volume of other (fermented) cheese in 2021 continued its decline (-14.2% to the level of 2020, 70 thousand tons); processed cheese production volumes in 2021 decreased slightly from their 2020 levels, returning to 2018 levels. Mainly dessert yogurt products and cheeses are imported into Ukraine. The main suppliers of dairy products to the domestic market are Poland, Germany, the Netherlands, France, Italy, and Belgium. [6].

Fermented cheeses are produced all over the world. Europe has the richest history of producing authentic cheese recipes, namely countries such as the Netherlands, Switzerland, Denmark, France, Italy and Spain. As defined by cheesemakers on the food market today, the most popular and included in the TOP-10 types of cheese in the world are Mozzarella, Emmentaler, Parmigiano reggiano, Brie, Chedar, Camembert, Gouda, Feta, Swiss cheese, and Grujere [7].

World production of cheese according to the International Dairy Federation -(International Dairy Federation (IDF)) in 2017 reached 20.5 million tons, in 2019 it was estimated at 25 million tons. The main producers of cheese in the world are the EU and the USA. Together, they produce about 16 million tons of cheese. The largest importers of cheese are Germany, Great Britain, Italy, France, and the United States, according to the agricultural information agency Agravery [8]. By 2020, over the past 50 years, the global cheese market has grown fourfold. The main consumers, in fact, as well as producers, remain European countries, although cheeses from the USA, Canada and Japan enter the market. There are currently no hard cheeses with bifidobacteria on the market.

The dairy industry of Ukraine is one of the leading ones in the agro-industrial complex, and cheese production is an important component of it. The production of hard cheeses by Ukrainian milk processors and transnational companies (Almira, Terafood, Milk Alliance, Como, Rud, Milkiland Ukraine, Lustdorf) with Ukrainian enterprises in Ukraine in 2015 decreased by a quarter (landlord.ua.). Until 2018, in Ukraine, 12 large enterprises produced 60% of cheese and cheese products (108 thousand tons), 87 dairy enterprises of medium capacity produced 50 thousand tons of cheese and cheese products, which was  $28\,\%$  of the total volume of production, and the rest — 22 thousand tons (12%), products are produced by small enterprises. Among the cheese-making enterprises there are large Ukrainian producers according to the Kompass Ukraine business directory (Kharkiv, info@kompass.ua) — Company ZhSZ —

Zhydachiv cheese factory, TDV (Zhydachiv, 1946, 1994); Company Buchatsky syrzavod, LLC (Buchach, Ternopilska, 2002); Company ZSK — Zvenigorod cheese factory, PrJSC of the SAVENCIA international group. They are such as Zvenigorodka, Cherkassy, 1931, TM Zveny Gora, 2001; Globinsky maslosirzavod, Globino Corporation LLC (Globino, Poltava, 1929, 2006); Cheese factories of the Lactalis company in Ukraine (Mykolaiv, 1996, Pavlograd, 2007, Shostka, 2021); Tulchyn cheese factory, branch of Terra Food (Tulchyn, Vinnytsia, 1999); Chertkiv cheese factory, company Chortkivsyr, PP (Chertkiv, 1967); Pyryatyn cheese factory, Milk Alliance holding LLC (Pyryatyn, Poltava, 1973); Okhtyr cheese factory, a branch of PP Ros of the Milkiland company (Okhtyrka, Sumy, 1988); Myrhorod Cheese Factory, LLC, a member of the State Enterprise "Milkyland-Ukraine" (Myrhorod, Poltava 1982, 2002); Andrushivsky maslosyrzavod, LLC(Andrushivka, Zhytomyrska, 2000); Novograd-Volyn cheese factory, PJSC Zhytomyrmoloko (Zhytomyrska, 1982); Losynivskyi maslosyrzavod, LLC (Nizhynskyi, Chernihivska, 1939, 1999); Euro-milk Joint Ukrainian-Czech Enterprise (Rohatyn, Ivano-Frankivsk, 1997, 2004); private cheese factories — "Forest goat", "Selyska cheese factory", "Farmer's dairy products", "Stanislavskaya cheese factory", "Obereg", "Stary Porytsk", "Cheeses from the farmer", "European cheese factory", "Chesnikov cheese factory", "Lviv Cheese Factory", and others [9].

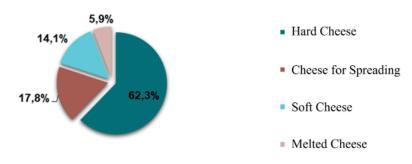
In the structure of the country's dairy industry, cheese production makes up about 10%. More than 150 enterprises are engaged in the production of cheeses in Ukraine, 2/3 of which produce hard rennet cheeses, the rest — soft and processed (melted) [6].

The basis of the assortment of cheeses in Ukraine are hard cheeses that are pressed with a low temperature of the second heating. In August 2018, 17,125 tons were produced in Ukraine [10]. The output of cheese has increased significantly, but the further increase in the volume of its production is already in doubt. According to the data of the State Statistics Service of Ukraine for 2020. only 5-6 thousand tons of goods have been sold to foreign markets. With the total production of cheese in France of 1.8 million tons per year, one French person consumes 26.8 kg per person, and in Ukraine -4.2 kg per person (the total volume of production is 140 thousand tons) according to the agricultural information agency Agravery [8].

In 2022, for the first time in the competition's 34 years of existence, the world cheese community, thanks to Ardis Group and ProCheese, got to know 39 Ukrainian cheeses with the slogan "Freedom tastes great" from 22 cheese factories at the World Cheese Awards 2022 in Great Britain, where 4,434 cheeses from 42 countries of the world. Thirteen Ukrainian cheeses received awards [11].

Classic fermented cheeses are classified by hardness into extra-hard, hard, se mihard and soft from the use of microcultures (0.5-10%) of starter and enzyme for curdling and curd formation, and also differ in the type of secondary microbiome — that is, those ripening with the help of lactic acid bacteria, propionic sourdough, blue and white mold. Hard cheeses are also divided by cooking temperature (second heating): high (thermophilic sourdough), medium (mixed sourdough), and low (mesophilic sourdough)[12].

Modeling of the general biotechnological system of the fermented cheese production



Source: Pro-Consulting

Fig. 1. Segmentation of the cheese market by product types in Ukraine, 2021 according to Pro-Consulting (hard cheese, pasty cheese, soft cheese, processed cheese)

process consists of the main stages: preliminary preparation of milk, fermentation, curdling, coagulation, grinding, cooking, washing, pressing, salting, forming, ripening, with the establishment and definition of technological parameters for each stage of production with specific modes the flow of physico-chemical and biochemical processes with the further search for opportunities for regulation and optimization of parameters, as well as the introduction of innovative methods of the technological process and successful entry into the commercial market of food products.

Global innovations in milk production are based on the tendency to increase the volume of milk production on the world market and promote the development of new functional preparations from live probiotic cultures in the field of biotechnology; products based on baking concentrates, including traditional fermented products, including cheeses and cheese products. Innovative methods of processing (ultrafiltration) of milk and cheese grain, processing time, coagulation and syneresis, etc. are increasingly used to standardize milk during cheese production.

The milk preparation process includes stages: selection, standardization, normalization, thermalization, pasteurization, microfiltration, ultrafiltration, bactofugation, homogenization. The quality of cheeses is determined, first of all, by the type and component composition of milk. The milk of cows, sheep, goats, and buffaloes is used for the production of cheese. Selection, standardization and pasteurization of milk is mandatory, because the sensory characteristics of cheese depend on the quality and freshness (acidity of 19 °T) of milk. Any milk consists of casein, whey proteins, lipids, minerals, lactose and other oligosaccharides.

The nature of cheeses depends on the content of milk fat (hydrolysis produces 41 fatty acids, including butyric, stearic, oleic, and glycerol) and milk proteins, which make up 3.3% of nitrogenous compounds of four fractions (casein -2.8%; lactoalbumin -0.43%, lactoglobulin — 0.06%, proteose peptone -0.01%). The majority of proteins (85%) is case in ( $\alpha - 60\%$ ,  $\beta - 25\%$ ,  $\gamma - 10\%$ fractions), which is in the form of a calcium phosphate complex micelle and is released during milk acidification and coagulation of  $\alpha$ and  $\beta$  fractions with rennet enzyme (pH = 4.6): the rest is albumin, which when heated turns into serum and precipitates (at 70–80 °C) and globulin, which also turns into serum and is dissolved in water and precipitates (at 75 °C) in an acidic environment [13].

The production of hard 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 and biological value of the final product, but also its safety. During ripening, all components of the cheese mass undergo deep changes, because of which the specific taste, aroma of the cheese, its consistency and pattern are formed. An important feature of hard rennet cheeses made by traditional technology is their suitability for long-term storage. The formation of cheese quality is largely determined by the composition and properties of raw milk, microbiological and biochemical features of product maturation, and technological parameters of production [14].

Fermentation of milk is carried out with the help of lactic coagulation microbial producers of fermentation (lactic acid bacteria of the microflora of starter cultures), lactic acid coagulation enzymes (plant, animal, in particular, rennet, and microbial proteolytic enzymes, in particular renin) and for special cheeses — solid-phase ripening (propionic bacteria, yeast and molds). Therefore, milk may also contain other substances that affect the quality of milk and the final product cheese: enzymes, in particular proteases (plasmin and cathepsin D), and lipases; antibiotics and mycotoxins. To prevent the growth of pathogenic microorganisms Campylobacter, Staphylococcus, Salmonella spp., Clostridium tyrobutyricum, thermal (thermalization (50-70  $^\circ \mathrm{C},~5\text{--}30~\mathrm{s})$  and pasteurization (72-75 °C, 20-15 s) and physical (microfiltration, homogenization and ultrafiltration are used to regulate the content of fats and proteins in milk.

During the acidification of milk, several phases of biochemical transformations take place, namely:

- bactericidal phase with the formation of natural milk antibiotics lactenins (Streptococcus lactis — nisin; Streptococcus cremovis — diplococcin; Lactobacillus acidophilus — acidophilin, lactocidin; Lactobacillus plantarum — lactolin; Lactobacillus brevi — brewin, lysozyme);

- the incubation phase with a decrease in acidity by the initial microflora of milk (at  $37 \degree$ C for 6 hours);

- a mixed microbial phase with the decomposition of lactose into galactose and glucose by all present microflora, lactic acid with an increase in the biomass of *Lactobacillus* and a further decrease in pH;

- symbiotic phase with an increase in pH under the influence of yeast and mycelial fungi [15].

Sourdough starters are used for controlled fermentation. European cheese producers in the European Union most often choose mesophilic starters — in 53.3% of cases, thermophilic and mixed less often — in 20% of the analyzed cheeses. All the Ukrainian producers we studied use mesophilic starter cultures.

Selected starter cultures are used for milk fermentation: mesophilic (Lactococcus lactis subsp. Lactis, Lactococcus lactis subsp. Cremoris, Lactococcus lactis subsp. Diacetylactis); thermophilic (Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii subsp. Lactis, Lactobacillus bulgaricus); mixed [16].

The use of thermophilic lactic acid bacteria allows the use of a higher fermentation and coagulation temperature, and also shortens the ripening period of the cheese. It is also possible to directly acidify the addition of lactic acid and use natural starters from authorized farms for some cheeses. In this study, a comparative analysis of the qualitative composition of starters used in the recipes and production of various fermented cheeses, as well as the temperature and duration of action of lactic acid bacteria, was carried out.

Coagulation is carried out with the help of rennet of young herbivores — calves, lambs, goats, consisting of chymosin, pepsin in a small amount, lipase, and pregastric esterase. Beef pepsin, fungal and bacterial proteinase are also used in industry. The coagulation process is affected by acidity, calcium concentration, protein content, and temperature. The product of the coagulation process is the cheese mass, which is further crushed and subjected to further processing for the production of mature cheese. This study examines the use of enzymes and the temperature regime and duration of coagulation in various cheese productions.

By processing the curd, conditions are created for microbiological and enzymatic processes necessary for the production of cheese of the appropriate consistency. Shredding of cheese mass is carried out with special knives in horizontal cheese baths or vertical fermenters. The size of the granules depends on the technology of cheese production and can be from 2 to 30 mm. Grinding increases the surface area of the curd granules, mixes it well and improves further ripening.

Boiling (second heating) is carried out at a temperature of 33-55 °C, depending on the amount of moisture in the final product and the type of starter. Cheddarization is characteristic of some types of cheese, such as Cheddar and Cheshire. During cheddarization, the cheese mass is once again chopped into pieces of 5 kg each, salted and cheddarized to a pH of 5.4. During cheddarization, the cheese mass acquires a fibrous structure, like chicken breast meat, and becomes elastic.

The finished cheese mass is taken from the cheese vat and distributed among perforated molds of different sizes depending on the type of cheese. The size of the cheese head depends not only on the product appearance, but is also necessary for proper ripening. A cheese ripened by a surface microbiome must be small so that the enzymes secreted by the microorganisms growing on the surface can penetrate the entire volume of the cheese. However, cheeses that use propionic acid fermentation must be large enough to hold carbon dioxide in the middle to ripen the cells.

Pressing is used for extra-hard, hard and semi-hard cheeses. Mostly, the pressing takes place under a pressure of 1200 kg per 1 surface of the cheese head for hard cheeses and 2000 kg for extra hard cheeses. To improve the taste, table salt (sodium chloride) is added to some cheeses. The salt concentration depends on the type of cheese and can be from 0.3 to 10%, but most cheeses contain 2.5% salt. Pickling can be done in two ways: wet (17–30% salt solution) and dry (wrapping with dry salt).

Ripening of fermented cheese is necessary for the formation of its characteristic shape, texture, taste and aroma. Maturation involves enzymes (rennet, renin, plasmin, proteases, lipases, peptidases) and microorganisms of the secondary microbiome (bacteria Propionibacterium freudenreichii, Propionibacterium acidipropionici, mold fungi Penicillium camemberti, Penicillium roqueforti, yeast Geotrichum candidum)[17].

The chemical composition and sensory characteristics of cheese depend on: the chemical composition of the cheese mass; temperature; humidity; type of enzymes; origin of milk; species composition of the primary and secondary microbiome at each technological stage [18].

To conduct an analytical review of the technological process of production of various hard and semi-hard cheeses in the EU and Ukraine, as well as recipes for fermented cheeses for production at craft cheese factories and at home from the USA. Conduct an analysis of the conditions and terms of key stages of production, such as fermentation, coagulation, boiling, pressing, salting, ripening. To investigate the composition and type of lactic acid bacteria in starter cultures used in the production of fermented cheeses. as well as enzymes used for fermentolysis and coagulation of milk casein. To propose recommendations for the development of new types of hard and semi-hard cheeses in Ukraine, which would mainly be related to the regulation of the time and temperature of fermentation, cooking and ripening of cheeses, as well as the expansion of the biodiversity of the microbiome of primary lactic acid starters to improve the taste and aroma of ready-to-use product.

#### **Materials and Methods**

The object of the study is the technological process of production of various hard and semi-hard cheeses in the EU and Ukraine, as well as recipes for fermented cheeses for production at craft cheese factories and at home from the USA. Information from patents, technical conditions for the production of hard and semi-hard cheeses in the EU and Ukraine was used to evaluate the production technology of hard and semihard cheeses; DSTU 6003 (Solid cheeses); New England Cheesemaking Supply Company (NECSC) cheese recipes by Jim Wallace. The obtained data were processed by methods of statistical analysis, systematization, comparison and generalization of information.

#### **Results and Discussion**

In this study, hard and semi-hard cheeses were examined. An analysis of the market of hard cheeses and an overview of the biotechnological process of the production of fermented cheese and a comparative analysis of the key stages of production were completed. A study of the use of biodiversity of lactic acid bacteria in starters and enzymes used in the production of fermented cheeses at world productions, and an attempt to show prospects for the development and improvement of Ukrainian hard cheeses were done. At the first stage of research, an analysis of the microbiome of lactic starters used in the production of fermented cheeses was carried out.

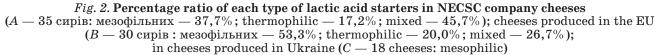
Milk fermentation is carried out using mesophilic and thermophilic yeasts, and it was established that in the recipes of the NECSC company, mesophilic yeasts are used in 37.3% of cases, thermophilic yeasts in 17.1%, and mixed yeasts in 45.7% (Fig. 2, A), (Table 1). Cheese producers in the European Union most often choose mesophilic starters — 53.3% of cases, thermophilic — 20.0% of cases, and mixed — 26.7% of cases (Fig. 2, B). In Ukraine, mesophilic starters are used in 100% of the analyzed cheeses (Fig. 2, C).

A comparative study of the species composition of lactic acid bacteria in starter cultures of lactic acid bacteria in NECSC company recipes by Jim Wallace was carried out, the results are presented in Table 1. According to the results of the study, mesophilic bacteria are most often used Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, in 29/35 spilled cheese recipes, in 22/35 analyzed cheeses, a thermophilic bacterium is used *Streptococcus* thermophilus, mesophilic bacterium Lactococcus lactis subsp. diacetylactis is used in 17/35 analyzed cheeses. Other thermophilic bacteria Lactobacillus helveticus are used less often in 8/35 cheeses, Lactobacillus delbrueckii subsp. lactis in 7/35 cheeses, Lactobacillus bulgaricus in 2/35 cheeses (Table 1, Fig. 3).

According to the conducted research, Propionibacterium freudenreichii subsp. shermanii was used as additional components of starter cultures and components of the secondary microbiome in 4/35 cheeses — a bacterium that carries out propionic acid fermentation, the yeast Geotrichum candidum and the brevibacterium Brevibacterium linens — in 2/35 cheeses and the fungus Trichothecium domesticum — in 2/35 cases (Table 1).

To study the technology of cheese production in the EU, the production technology of 30 cheeses was analyzed, the species composition of bacteria was described in 15 types of cheeses, and natural starters from certified farms were used in five types of analyzed cheeses. In the largest number of examined cheeses, 10/15, mesophilic bacteria Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, in 5/15 of the analyzed cheeses, the thermophilic bacterium Streptococcus thermophilus, the mesophilic bacterium Lactococcus lactis subsp.





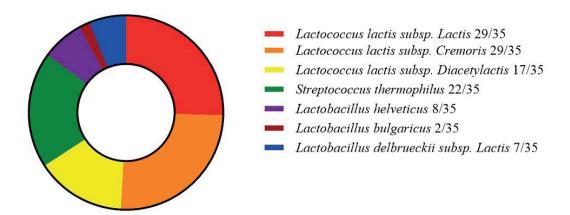


Fig. 3. Ratio of lactic acid bacteria species in starters from NECSC recipes by Jim Wallace

Table 1. Comparative analysis of the species composition of lactic acid bacteria	
in starter cultures of lactic acid bacteria recipes of the NECSC company	

No.	The name of the cheese	Type of starter	Type of sourdough starter	Types of microorganisms	
1	2	3	4	5	
	Alpine Style Cheese Альпійський сир	mixed	MA011	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris	
1			TA61	$Streptococcus\ thermophilus$	
			FLAV54	Lactobacillus helveticus	
2	Alpine Tomme Альпійський порожній	льпійський thermophilic C201 Lactobacillus helveti		Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii subsp. lactis	
	Alpine Washed	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus	
2	Rind Альпійський з промитою		Propionic Shermanii	Propionibacterium freudenreichii subsp. shermanii	
	шкіркою	propionic	GEO17	Geotrichum candidum	
			Bacteria Linens	Brevibacterium linens	
3	Appenzeller Апенцеллер thermophilic		Thermo B	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus	
4	Asiago Asiaro	thermophilic	C201	Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii subsp. lactis	
			LH100	Lactobacillus helveticus, Lactobacillus lactis	
	Beaufort Бофор	5		MA011	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
5		mixed	TA61	Streptococcus thermophilus	
			LH100	Lactobacillus helveticus, Lactobacillus lactis	
6	Brick Брусковий	mesophilic	MM100	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis biovar diacetylactis	
			Bacteria Linens	Brevibacterium linens	
7	Cabra al Vino П'яна коза	mixed MA 4002 Lactococcus lactic Lactococcus lactic		Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus	
8	Caerphilly Карфіллі	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus	
		fungal	Mycodore	Trichothecium domesticum	
	Canestrato Italian Basket Канестрато	mixed	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris	
9			Y1	Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacterium lactis, Lactobacillus rhamnosus	

Table 3 (continued)

1	2	3	4	Table 3 (continued)   5
10	Cantal Канталь	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus
11	Cheddar Чеддер	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
12	Colby Колбі	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
13	Cheshire Чешир	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
14	Dunlop Данлоп	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
15	Edam Едам	mesophilic	MM 100	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. lactis biovar diacetylactis
16	Farmstead Фермерський	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
17	Gouda Гауда	mesophilic	MM 100	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. lactis biovar diacetylactis
18	Sweet Gouda Солодка Гауда	mesophilic	MM 100	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. lactis biovar diacetylactis
	Gruyere Грюєр	thermophilic	TA61	Streptococcus thermophilus
19		propionic	C6	Propionibacterium freudenreichii subsp. shermanii
20	Hispanico Іспанський	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus
21	Ibores Іборес	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus
22	Jack Джек	mesophilic	MA011	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris
23	Jarlsberg Ярлсберг	mesophilic	Flora Danica	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. diacetylactis
24	Lancashire Ланкашир	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris Lactococcus lactis subsp. Diacetylactis. Streptococcus thermophilus
	Maasdam Мааздам	mixed	Su Casu	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. lactis, Lactobacillus helveticus
25			C6	Propionibacterium freudenreichii subsp. shermanii
		propionic	MM 100	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis biovar diacetylactis

Table 3 (end)

1	2	3	4	5			
	Mutschli Herdsman's Маленький пастуший сир	thermophilic	C201	Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii subsp. lactis			
26		propionic	C6	Propionibacterium freudenreichii subsp. Shermanii			
			GEO17	Geotrichum candidum			
			Bacteria Linens	Brevibacterium linens			
27	Red Leicester Червоний Лестер	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris			
28	Romano Романо	thermophilic	C201	Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii subsp. lactis			
29	Sao Jorge Сан-Жоржі	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus			
30	Toma Ossolano Тома			mixed	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus
00		mixeu	GEO17	Geotrichum candidum			
			Bacteria Linens	Brevibacterium linens			
31	Тотте Томе		mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus		
					LH100	Lactobacillus helveticus, Lactobacillus lactis	
			Mycodore	Trichothecium domesticum			
	Toscano Pepato Тосканський		C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris			
32		mixed	C201	Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii subsp. lactis			
33	Yorkshire Йоркшир	mesophilic	C101	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris			
34	Queso de Mah n Mao	mixed	MA 4002	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Streptococcus thermophilus			

diacetylactis is used in 9/15 analyzed cheeses. Other thermophilic bacteria Lactobacillus helveticus are used less often in 6/15 cheeses, Lactobacillus delbrueckii subsp. lactis in 3/15 cheeses, Lactobacillus bulgaricus in 3/15 cheeses, the bacterium Leuconostoc mesenteroides was also used in production in 4/15 analyzed cheeses (Table 2).

According to the study, *Propionibacterium* freudenreichii subsp. shermanii was used as an additional leavening component in 3/30 cheeses — a bacterium that carries out propionic acid fermentation. Because of the research, 8 types of hard cheeses of Ukrainian producers were analyzed. Mesophilic bacteria *Lactococcus lactis subsp. lactis* were used in all types of examined cheeses, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. diacetylactis* (Table 3).

Hard rennet cheeses are evaluated according to organoleptic indicators according to a 100-point system. Depending on the amount of points scored, the grade of cheese is determined: higher or first. Hard rennet cheeses "Russian", "Poshekhonsky",

# *Table 2.* Comparative analysis of the species composition of lactic acid bacteria in starter cultures of lactic acid bacteria in cheeses produced in the EU

No.	The name of the cheese	Producer/ Patent owner/ Region	Type of starter	Types of microorganisms
1	Gouda Гауда	BC2 Friesland B.V.	mixed	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis subsp. diacetylactis, Lactobacillus helveticus, Lactobacillus lactis
2	Gouda Гауда	DSM IP Assets B.V	mesophilic	Lactococcus lactis subsp. lactis & cremoris Lactobacillus helveticus, Lactobacillus lactis
3	Swiss-type Швейцарський	Шройнарський Енгиппени		Streptococcus thermophilus, Lactobacillus delbrueckii subsp. lactis, Lactobacillus helveticus
		B.V.	propionic	$Propionibacterium\ freuden reichii\ {\rm subsp.\ } shermanii$
4	Berner Alpk se Бернський	Berner Hobelk se PDO	thermophilic	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. lactis, Lactobacillus helveticus
5	Graviera Kritis Грав'єра	Crete, Greece	thermophilic	Streptococcus thermophilus, Lactobacillus delbrueckii subsp bulgaricus, Lactobacillus helveticus
6	Kefalotyri Кефалотирі	Greece	mixed	Streptococcus thermophilus, Lactobacillus subsp delbrueckii bulgaricus, Lactobacillus helveticus, Lactococcus lactis subsp. lactis & cremoris
7	Le Gruy re Грюєр	La Neuveville, Switzerland	thermophilic	Streptococcus thermophilus, Lactobacillus delbrueckii subsp lactis Lactobacillus helveticus
8	V sterbottensost Вестерботтен	V sterbotten, Sweden	mesophilic	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis, Leuconostoc mesenteroides
9	Appenzeller Апенцеллер	Appenzell, Switzerland	thermophilic	Streptococcus thermophilus, Lactobacillus. delbrueckii subsp bulgaricus, Lactobacillus casei
10	Havarti Хаварті	Denmark	mesophilic	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis, Leuconostoc mesenteroides.
11	Herrgard Шведський	Sweden	mesophilic	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis, Leuconostoc mesenteroides
12	Pr st Прастост	Sweden	mesophilic	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis, Leuconostoc mesenteroides.
13	San Sim n Сан Симон	Galicia, Spain	mesophilic	Lactococcus lactis subsp. lactis & cremoris
14	Svecia Швеція	Sweden	mesophilic	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis, Leuconostoc mesenteroides
15	Gouda Гауда	Netherlands	mesophilic	Lactococcus lactis subsp. lactis & cremoris, Lactococcus lactis subsp. lactis biovar. diacetylactis

No.	The name of the cheese	Producer/ Patent owner	Type of starter	Types of microorganisms
1	Костромський Kostroma	«Жидачівський сирзавод» «Zhydachiv cheese factory»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
2	Голландський круглий Dutch round	«Жидачівський сирзавод» «Zhydachiv cheese factory»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
3	Голландський брусковий Dutch bar	«Жидачівський сирзавод» «Zhydachiv cheese factory»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
4	Голландський Dutch	Бучацький сирзавод Buchatsky cheese factory	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
5	Ейдам Aidam	«Євро-мілк» «Euro-milk»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
6	Рогатинський Rohatynskyi	«Євро-мілк» «Euro-milk»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
7	Прикарпатський Carpathian	«Євро-мілк» «Euro-milk»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis
8	Явір Sycamore	«Євро-мілк» «Euro-milk»	mesophilic	Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis

Table 3. Comparative analysis of the species composition of lactic acid bacteria in starter cultures
of lactic acid bacteria in cheeses produced in Ukraine

"Picantny", reduced fat and accelerated ripening (1 month), as well as soft and processed cheeses are not divided into varieties. Their quality is determined by the compliance of their indicators with the requirements of standards or technical conditions.

During the certification of cheeses, in addition to organoleptic ones, the main physico-chemical indicators characterizing the nutritional value of cheeses are determined, including the mass fraction of fat, salt, moisture, titrated and active acidity, sodium nitrate content.

At the second stage of the research, an analysis of production conditions and the duration of the key stages of production of fermented cheeses was carried out. The recipes of the NECSC company, authored by Jim Wallace, and the technical conditions of production in Ukraine and the EU were also analyzed. 35 types of fermented cheeses were analyzed according to the temperature of cheese fermentation and coagulation; cooking (second heating); maturation.

Based on the analysis of the obtained data, it was found that the temperature of fermentation and coagulation is in the range from 29 °C to 37 °C. A lower temperature of 29-33  $^\circ \! \mathrm{C}$  is used for cheeses with a mesophilic type of sourdough, the highest temperature of 35-37 °C is used for cheeses with thermophilic sourdoughs, in recipes for cheeses with mixed sourdoughs, an intermediate temperature regime is used (Fig. 4, A). The temperature of the second heating depends on the desired moisture content in the final product and the type of starter used and is 30-55 °C. For semihard cheeses with mesophilic sourdough, a temperature of 30-35 °C is used, for hard cheeses with thermophilic sourdough, a temperature of 45–55 °C is used. The ripening temperature is low and is set at 12-14 °C, only for 3 cheese recipes the recommended temperature is  $16-21 \degree C$  (Fig. 4, *A*).

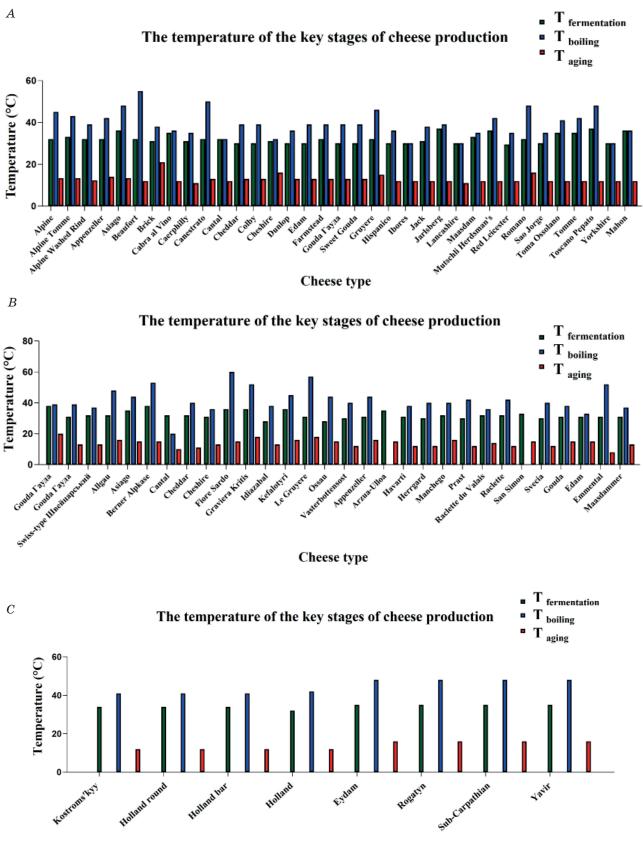


Fig. 4. Quantitative ratio of the temperature of the key stages of cheese production in the recipes of the NCSC company (A), produced in the EU (B), produced in Ukraine (C)

An analysis of 30 types of fermented cheeses produced in the EU was carried out according to the temperature of cheese fermentation and coagulation; cooking (second heating); maturation. Based on the analysis of the obtained data, it was found that the temperature of fermentation and coagulation is in the range from 30 °C to 38 °C. A lower temperature of 30–33 °C is used for cheeses with a mesophilic type of sourdough, the highest temperature of 35–38 °C is used for cheeses with thermophilic sourdoughs, in recipes for cheeses with mixed sourdoughs, an intermediate temperature regime is used (Fig. 4, B). The temperature of the second heating depends on the desired moisture content of the final product and the type of starter used, and is higher in largescale production than in recipes for craft cheese factories and is 33–60 °C. For semi-hard cheeses with mesophilic sourdough, a temperature of 33-40 °C is used, for hard cheeses with thermophilic sourdough, a temperature of 45–60 °C is used. The ripening temperature in production varies more among different cheeses than in recipes, because in large enterprises it is possible to build several types of thermostatic shops with a temperature characteristic for a certain type of cheese, ranging from 8–20 °C and humidity. A higher ripening temperature reduces its duration and improves the taste characteristics of cheese due to the higher activity of enzymes and microorganisms of the secondary microbiome (Fig. 4, *B*).

8 types of fermented cheeses produced in Ukraine were studied. For Ukrainian cheeses, the fermentation temperature is approximately the same, since only mesophilic sourdoughs are used and is 34 °C for cheeses produced by the Zhydachiv cheese plant and 35 °C for cheeses from the Evro-milk enterprise. The cooking temperature is 41 °C for cheeses produced by "Zhydachiv cheese plant" and 48 °C for cheeses from the "Euro-Milk" enterprise. The ripening temperature is 12 °C for cheeses produced by the "Zhydachiv cheese factory" and 16 °C for cheeses from the "Euro-milk" enterprise (Fig. 3, C). At the third stage of research, an analysis of the duration of the stages of fermentation and coagulation, as well as ripening, was carried out. All NECSC recipes by Jim Wallace use fermentation before coagulation. That is, lactic acid starter is first added to milk heated to the appropriate temperature, and only after 30–60 minutes rennet is added, and in large-scale production, lactic acid starter and enzyme are added at the same time, pre-fermentation is characteristic only for 5 types of European and none of the analyzed Ukrainian cheeses.

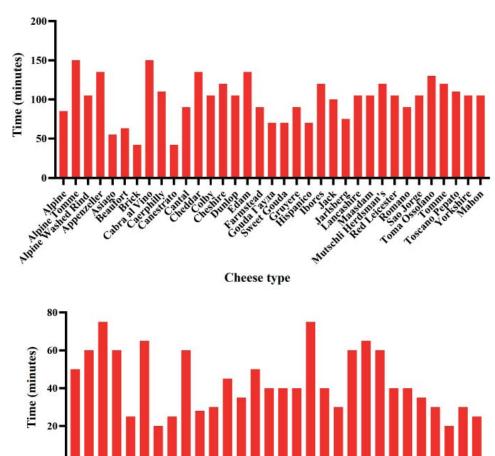
Therefore, as a result of the study, two comparative analyzes were made: the first includes the time of preliminary fermentation and coagulation, which is presented in the graph as the time of fermentation with the help of lactic acid bacteria (Fig. 5), and the second — the time of coagulation with the help of rennet (Fig. 6).

In the NECSC recipes by Jim Wallace, the fermentation time with lactic acid bacteria ranges from 40 to 140 min (Fig. 5, A), in the technical conditions of EU productions from 25 to 75 min (Fig. 5, B), in Ukrainian technical conditions, lactic acid fermentation lasts 25–35 min (Fig. 5, C).

In the recipes of the NECSC company by the authorship of Jim Wallace, the time of hydrolysis with the help of rennet was from 30 to  $60 \min$  (Fig. 6, A), in the technical conditions of EU productions from 25 to  $60 \min$  (Fig. 6, *B*), in the Ukrainian technical conditions, lactic acid fermentation lasts  $25 - 35 \min (Fig. 6, C)$ . Because of the comparative analysis, the type of rennet used for coagulation was also investigated. In all NECSC recipes, calf rennet is recommended, but microbial rennin can be used, which does not affect the structure and taste characteristics of the cheese, as noted by the author. In the technical conditions of EU productions, calf rennet is used in most cases, if sheep milk is used, the technology requires the use of lamb rennet, and beef rennet occurs with a small frequency, for most cheeses produced in large volumes, there are analogues with microbial renin. In the studied technical conditions, beef rennet is used for most cheeses of Ukrainian producers, but microbial rennin occurs with a small frequency.

According to the analysis of the duration of ripening, it was established that the duration of ripening of hard and semi-hard cheeses in the recipes of the NECSC Company ranges from 70 to 730 days. The exception is only some cheeses with propionic leavens (Edam, Ibores, Farmersky), the duration of which is less than 45 days of ripening (Fig. 7, A). In cheeses produced in the EU, the duration of ripening ranges from 14 to 730 days. The average duration of maturation of European cheeses is 223 days. Cheeses with the addition of microorganisms of the secondary microbiome described in the introduction: propioic acid bacteria, yeast, and mold fungi have a short ripening period of up to 40 days (Fig. 7, B). In the studied cheeses of Ukrainian producers, the duration of ripening ranges from 35 to 60 days (Fig. 7, C).

As the study of technological schemes for the production of hard cheeses shows, the





A



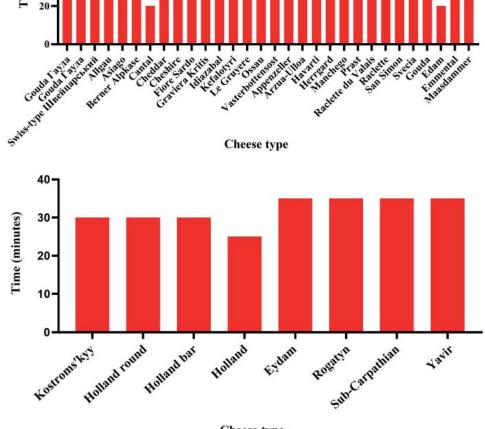


Fig. 5. Quantitative ratio of the duration of lactic acid fermentation in the technical conditions of cheeses, in the recipes of the NCSC company (A), produced in the EU (B), produced in Ukraine (C)

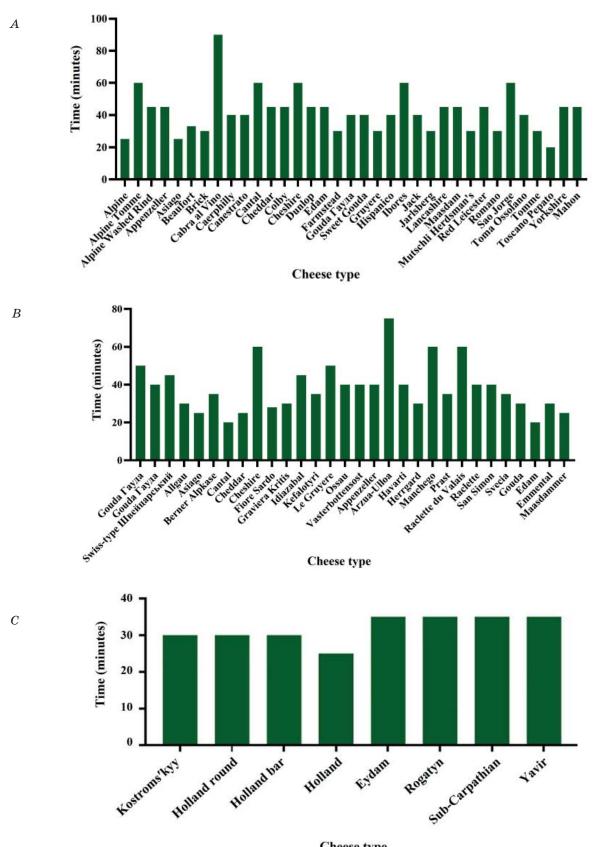


Fig. 6. Quantitative ratio of duration of coagulation in technical conditions of cheeses, NCSC (A), produced in the EU (B), produced in Ukraine (C)

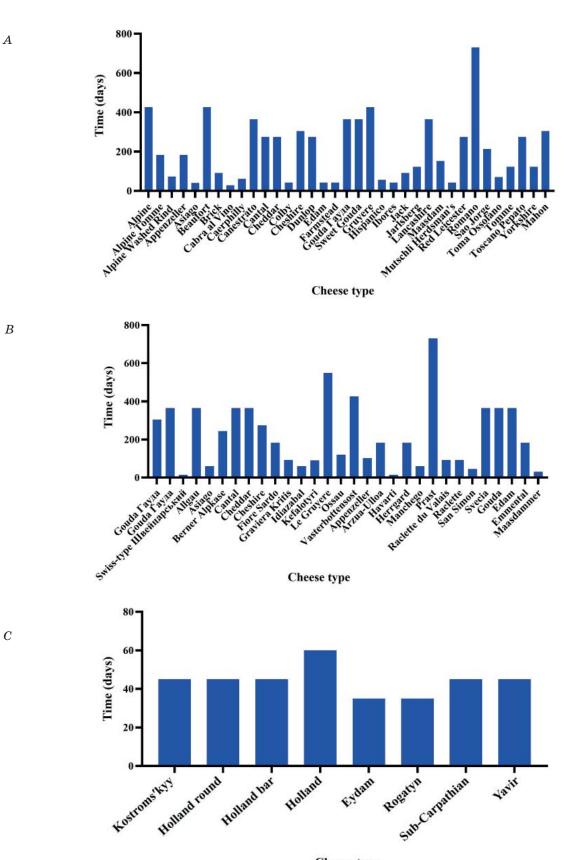


Fig. 7. Quantitative ratio of ripening duration in the technical conditions of cheeses of the NCSC company (A), produced in the EU (B), produced in Ukraine (C)

general principle stages are mostly similar and involve the implementation of optimization changes and innovative methods of improving production in terms of product quality and obtaining commercial benefits.

#### Conclusions

As a result of a comprehensive study of the technological process of the production of various hard and semi-hard cheeses in the EU and Ukraine, as well as recipes of fermented cheeses for production at craft cheese factories and at home from the USA, a total of 73 types of hard and semi-hard cheeses were analyzed: 35 recipes of the New England Cheesemaking Supply Company by Jim Wallace; 30 production processes of hard and semi-hard cheeses from the EU; 8 technical conditions of hard cheeses of Ukrainian producers.

It is shown that the prospects for the development of new types of hard and semihard cheeses in Ukraine should include the following recommendations: if possible, apply short-term fermentation with the help of lactic acid starters before adding rennet, which will increase the activity of rennet, since it will be immediately added to milk with a pH of 4.6; the use of thermophilic bacteria in the composition of primary starters shortens the ripening period of cheese and improves its taste characteristics; when using thermophilic or mixed leavens, it is necessary to increase the temperature of fermentation and cooking; the use of rennet from young animals is only

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advisable, because beef enzyme contains little chymosin and a lot of pepsin and has a lower optimal pH of 2, it is only advisable to replace calf rennet with microbial renin; for better fermentation with the help of microorganisms of the secondary microbiome and enzymes, it is necessary to increase the ripening period to at least 180 days for classic hard cheese, the ripening period can be shortened by adding propionic acid bacteria, yeast and mold fungi as additional components of starter cultures, as well as by increasing the ripening temperature.

By applying the recommendations for optimization of fermentation processes, introduction of innovative biotechnologies, with an assessment of the prospects for the development of new biotechnological methods in Ukraine, it is possible to produce the final product of Ukrainian good-quality and safe hard cheeses for healthy eating, which, in terms of sensory characteristics and texture, will obviously not differ from cheeses produced in EU countries.

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### АНАЛІЗ ТЕХНОЛОГІЧНОГО ПРОЦЕСУ ВИРОБНИЦТВА ТВЕРДИХ ТА НАПІВТВЕРДИХ СИРІВ В ЄС ТА УКРАЇНІ

Ю. А. Скріль<sup>1</sup>, О. В. Швед<sup>1</sup>, З. В. Губрій<sup>1</sup>, О. І. Вічко<sup>2</sup>, Т. Купка<sup>3</sup>

<sup>1</sup>Національний університет «Львівська політехніка», Україна <sup>2</sup>Тернопільський національний технічний університет імені Івана Пулюя, Україна <sup>3</sup>Університет Ополє, Інститут Хімії, Польща

#### E-mail: olha.v.shved@lpnu.ua

Проведено аналітичний огляд технологічного процесу виробництва різноманітних твердих і напівтвердих сирів в ЄС та Україні, а також вітчизняних рецептур ферментних сирів для виробництва на крафтових сироварнях та у домашніх умовах. Здійснено аналіз умов ключових стадій виробництва, зокрема сквашування, коагуляція та дозрівання. Досліджено склад та тип молочнокислих бактерій у заквасках для ферментних сирів, а також ферментів для ферментолізу та коагуляції казеїну молока. Показано, що перспективи оптимізації розроблення нових видів твердих та напівтвердих сирів в Україні переважно пов'язані з регулюванням часу та температури сквашування, варіння та дозрівання сирів, а також розширенням біорізноманіття первинного та вторинного мікробіому заквасок для покращення смаку та аромату готового до вживання продукту.

*Mema* — проаналізувати ферментаційний процес та рецептури виробництва твердих сирів в Україні з основними світовими зразками, порівняти склад та тип молочнокислих бактерій у промислових та крафтових заквасках, також типи ферментів для ферментолізу та коагуляції казеїну молока, щодо оптимізації виробництв для покращення смаку та аромату готового до вживання продукту.

Mamepianu ma memodu. Методичний аналіз й абстрактно-логічний метод для узагальнення критеріїв оцінки біотехнологічного процесу різноманітних твердих і напівтвердих вітчизняних сирів зі світовими зразками за рекомедаціями, вимогами та стандартами з опрацюванням патентів, технічних умов їх виробництва в ЄС та Україні; ДСТУ 6003 (Сири Тверді); рецепти сирів компанії New England Cheesemaking Supply Company за авторством Джима Валласа. Отримані дані опрацьовані методами статистичного аналізу, систематизації, порівняння та узагальнення інформації.

*Результати.* У даному досліджені розглянуто докумендацію та проаналізовано дані стандартів, положень, вимог та рекомедації щодо біотехнології твердих та напівтвердих сирів, проведено аналіз ринку твердих сирів та особливостей ферментаційного процесу їх виробництва з визначенням критичних точок та ключових стадій виробництва з використанням промислових депонованих продуцентів сквашування та ферментів білкової коагуляції та біорізноманіття молочнокислих бактерій у заквасках для ферментованих сирів на світових виробництвах, з оціненням можливостей перспективи розроблення нових та удосконалення біотехнології українських доброякісних та безпечних твердих сирів для здорового харчування.

*Ключові слова:* твердий сир; напівтвердий сир; сквашування; мезофільні та термофільні молочнокислі бактерії; коагуляція; сичужний фермент; хімозин; ренін; пепсин; дозрівання ферментного сиру; мікробіом.