potravinárstvo®
OPEN ACCESS
Received: 21.8.2024
Revised: 20.10.2024
Accepted: 22.10.2024
Published: 22.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 899-918
https://doi.org/10.5219/2011
ISSN: 1337-0960 online
www.potravinarstvo.com

Analysis of the hard rennet cheese microbiota at different stages of the technological process

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ABSTRACT

The purpose of the research was microbiological screening using MALDI-TOF technology starting from bulk raw milk to the finished dairy product and analyzing microorganisms that were being detected during the technological process of production of Ukrainskyi hard rennet cheese and which were clinically significant for human and animal health. Methods. Microbial detection was performed by accumulation and inoculation using the sector inoculation method on differential media for aerobic and anaerobic microorganisms with further MALDI-TOF identification. Sampling was carried out at 7 stages of cheese production: starting from bulk raw milk to bactofugation, after bactofugation to a mixture normalized in fat content, a pasteurized mixture, a mixture prepared for coagulation, cheese after pressing, and cheese after maturation. Microflora studies were repeated three times, with 405 samples examined. Microbiological studies of Ukrainskyi hard rennet cheese using Maldi TOF technology starting from raw materials to finished dairy products showed the presence of microorganisms at all stages of production – from bulk milk to the finished product. During the entire period of experiments, 43 species of various microorganisms have been isolated and identified. However, the number and individual types of microorganisms differed at different stages of production. Some microorganisms that have been isolated in raw milk are also found in the final product, such as *Acinetobacter baumannii* and Escherichia coli. In total, 18 types of microorganisms have been isolated and identified in the final product – hard rennet cheese, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*, which are of particular concern in the context of safe consumption of this cheese.

Keywords: MALDI-TOF, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, hard rennet cheese.

INTRODUCTION

Milk and dairy products are important components of human nutrition because they are an important source of protein, lactose, milk fat, and biologically active substances **[1]**. Due to its rich chemical composition and optimal physical properties favourable for most microorganisms, milk is an ideal environment for their growth and development.

The main part of the milk microbiota consists of mesophilic aerobic and facultatively anaerobic microorganisms that grow in the presence of oxygen. This group of bacteria is a microbiological indicator of food quality, showing the effectiveness of heat treatment and compliance with sanitary and hygienic requirements during production, primary raw milk processing, transportation, and storage. The presence of mesophilic aerobic microorganisms may also suggest sources of contamination during milk processing **[2]**.

In the dairy industry, the microbiological safety of raw milk is the basis for the technology of producing a proper-quality product. So, the permanent microflora of raw milk is represented by Corynebacterium spp. **[3]**,

certain species that can be pathogenic to animals and humans. These bacteria can also be found in finished dairy products, particularly cheeses, and this can also pose a danger to human health **[4]**.

Raw milk supplied to milk processing enterprises may contain various microorganisms **[5]**. Still, special attention should be paid to opportunistic and pathogenic ones since they can threaten the life and health of people and animals. One study shows the diversity of the microflora found in raw milk throughout the year and the dependence of milk quality indicators on the microflora composition. Bacterial contamination of milk was higher in May and June, with its lowest indicator in October and December. The following types of bacteria have been isolated: *Firmicutes*, and *Bacteroidote*. The most common genera were represented by *Pseudomonas*, *Acinetobacter*, *Streptococcus*, and *Lactobacillus* **[6]**.

Opportunistic pathogenic microorganisms, such as *Staphylococcus aureus*, can multiply in dairy products, affect their organoleptic parameters, and accumulate toxins **[7]**. Most often, the presence of this pathogen is registered in raw milk due to poor hygiene and sanitation on the farm, but hygiene during milking and hygiene of service personnel, as well as mastitis in cows, have an impact, too **[8]**.

Pathogenic microorganisms – pathogens of infectious diseases – in milk and dairy products can maintain their viability long and pose a danger to consumers. In particular, bacteria of the Enterococcus genus, isolated from raw milk and finished dairy products, can form a biofilm, which confirms the need for continuous monitoring of microbial adhesion in dairy production facilities **[9]**.

The bacteria that can be present in milk may cause a variety of bacterial infections. Pathogenic or opportunistic pathogenic microorganisms, such as *Acinetobacter* **[6]**, *Escherichia coli* **[7]**, *Klebsiella spp* **[8]**, and so on are especially dangerous for humans.

Klebsiella pneumoniae is one of the most common species of the *Klebsiella* genus. It is the causative agent of infectious diseases of animals, in particular, mastitis in cows **[9]**, **[10]**. Increasing antibiotic resistance of K. pneumoniae, especially strains producing extended-spectrum β-lactamases (ESBL) and/or carbapenems, is of worldwide concern today **[11]**, **[12]**.

In cattle, *Klebsiella spp* are transmitted through contact with udder teats with manure, bedding, and other agricultural accessories. The infection affects the epithelial cells of the teat and can persist in the udder for a long time. Besides, *Klebsiella spp* strains affect the safety and quality of milk, as well as the productivity of adult cows, and pose a threat to the survival of newborn calves **[13]**, **[14]**.

Over the past decade, the number of cases of *Klebsiella spp* detection in milk samples obtained from cows with mastitis has increased dramatically worldwide **[15]**, **[16]**.

Klebsiella pneumoniae is a zoonotic pathogen that often becomes a source of nosocomial infections **[17]**. *Klebsiella pneumoniae* mainly causes pneumonia, liver abscess, meningitis **[16]**,**[17]** urinary tract diseases **[18]**, toxemia, septicemia, and other symptoms of infection **[19]**, **[20]**. Researchers point out that in the United States of America, *Klebsiella pneumoniae* accounts for 3% to 8% of all nosocomial infections (nosocomium) - an infection that the patient did not have at the time of admission to a hospital or other healthcare facility **[19]**.

The World Health Organization (WHO) controls Klebsiella spp., carriers of many drug-resistance genes **[21]**, **[22]**.

Bacteria of the *Acinetobacter* genus are identified in milk as a result of contamination through milking equipment, which may contain water residues, or due to improper cleaning of milk pipelines or coolers, contaminated udders, and teats, non-compliance with hygienic requirements during the transportation and storage of milk, and improper cleaning of dairy equipment **[23]**, **[24]**. Most representatives of the Acinetobacter genus are opportunistic pathogenic clinically insignificant commensals with limited virulence. However, the severity of infections caused by Acinetobacter has recently increased due to the frequent use of mechanical breathing devices, venous catheters, and antibiotics, which pose a significant public health concern. *Acinetobacter baumannii (A. baumannii)* is an opportunistic pathogenic microorganism that causes various nosocomial infections **[25]**. Studies conducted in animal models and clinical data have shown that *A. baumannii* is a virulent species. It is a dangerous pathogen, especially due to the emergence of multi-resistant (MLR) strains and their association with many nosocomial and community-acquired infections **[26]**. Researchers claim that dairy products contaminated with *A. baumannii* can be community-acquired reservoirs as underestimated pathogens that pose health risks for immunocompromised adults and children **[27]**, **[28]**.

Escherichia coli, which causes subclinical or clinical mastitis in cattle, accounts for transmitting antimicrobial resistance through human consumption of raw milk or raw dairy products **[25]**.

The spread of E. coli's antimicrobial resistance has recently become increasingly recognised, with concern about human and animal health growing even more. In particular, the constant use of antimicrobial agents for the treatment and prevention of bovine mastitis has contributed to the emergence of antimicrobial resistance of E. coli due to genetic mutation or horizontal gene transfer that can potentially pose a health threat **[26]**.

One of the health protection problems worldwide is the presence of pathogenic bacteria *Escherichia coli* in milk and dairy products, which produces Shiga toxin (STEC) **[27]**, causing intestinal diseases. Therefore, monitoring milk and dairy products at all stages of production for contamination with *Escherichia coli* is important for ensuring the safety of dairy products **[28]**. Besides, *Escherichia coli* can form biofilms, which pose a significant risk during dairy production. Also, researchers leave open the possibility that biofilms can withstand milk pasteurisation regimes **[29]**.

Raw milk may also contain other microorganisms that risk human and animal health **[30]**. To neutralise unwanted microorganisms during raw milk processing, it is exposed to a number of technological factors, such as bactofugation, pasteurisation, and the like **[31]**.

Scientific Hypothesis

Dairy products and their production chains can become a depot for the transmission of bacteria that contaminate raw milk, circulate in processing plants, live in finished dairy products, and pose a threat to consumer health. That is why it was our objective to conduct microbiological screening using Maldi TOF technology, starting from raw milk to the finished dairy product, and to conduct an analysis of microorganisms that are being detected during the technological process of Ukrains hard rennet cheese's production and are clinically significant for human and animal health.

MATERIAL AND METHODOLOGY

Samples

Samples of bulk raw milk were taken at Haisyn Dairy Plant LLC, located in Haisyn Town, Vinnytsia Oblast. Raw milk was supplied from five dairy farms. The plant's technological capacity allows it to produce 300 tons of hard and 180 tons of soft cheeses, 540 tons of butter and spreads, and 360 tons of dry dairy products monthly. The company has implemented a food safety management system to the international standard ISO 22000 requirements.

Chemicals

Nutrient media, reagents, and materials were used in the work.

Blood Agar (BA). Produced by BioMérieux, France.
Buffered Peptone Water (BPW). Produced by HiMedia, India.
Baird Parker Agar (BPA). Produced by HiMedia, India.
Endo Agar (Endo). Produced by Farmactiv, Ukraine.
Pseudomonas Agar (Pseudo). Produced by HiMedia, India.
Enterococcus Agar. Produced by Farmactiv, Ukraine.
Bacillus Cereus Agar. Produced by HiMedia, India.
Bismuth Sulfite Agar (BCA). Produced by HiMedia, India.
Xylose-lysine deoxycholate Agar (XLD Agar). Produced by HiMedia, India.
Packages for creating anaerobic conditions. Produced by BioMérieux, France.
HCCA mass spectrometer matrix (art. 255344). Produced by Bruker, Germany.
Bacterial calibrator (art. 255343). Produced by Bruker, Germany.
Peptone salt solution (PSS). Produced in Ukraine.

Materials

Loops made of platinum/iridium or nickel/chromium, with a diameter of 3 mm. Produced in Ukraine.
Graduated glasses. Produced by Simax, Czech Republic.
Volumetric flasks. Produced by Duran, Germany.
Test tubes P2 16x150 mm. Produced by Sklopryład, Ukraine.
Petri dishes, with a diameter of 90 mm. Produced by Vorwarts Diagnostic, Ukraine.
Plastic sterile Pasteur pipettes. Produced by Labexpert, China.
Sterile glass vials, 500 ml. Produced by Simax, Czech Republic.
Sterile tips with filter for Eppendorf dispensers for 0,1-10 µL. Produced by Eppendorf, Germany.
Metal chips for MALDI-TOF. Produced by Bruker Daltonics, Germany.

Instruments

During the study, we used the equipment as follows:

MALDI-TOF MS mass spectrometer. Produced by Bruker Daltonics, Germany.

Wet sterilisation device (autoclave), capable of maintaining temperatures from 120□C to 134□C (vertical autoclave 5050 ELV D-line). Produced by Tuttnauer, Israel.

The drying chamber maintains a temperature of 160ºC (dry heat steriliser ED115). Produced by Binder, Germany.

Incubator (thermostat) capable of maintaining temperatures of 30 and 37ºC - 1ºC (incubator with natural convection, Binder BD 115). Produced by Binder, Germany.

pH meter with calibration accuracy of 0.1 units of hydrogen index for 25 ºC (laboratory pH meter/ionomer, Mettler Toledo). Produced by Mettler Toledo, Switzerland.

Variable volume piston dispenser for 0.1-2.5 µL. Produced by Eppendorf, Germany.

Loop steriliser. Produced by SteriMaks, Germany.

Vortex (vibration mixer), Biosan. Produced by Biosan, Latvia.

Leica DM500 LED binocular microscope. Produced by Leica, Germany.

Refrigerator and freezer compartments. Produced by Liebherr, Switzerland.

Laboratory Methods

Microbiological milk screening was performed using the MALDI-TOF PV.BLS 7.2-08.15 method **[32]**. The method's principle lies in detecting existing microorganisms in any group of foods, feed, and water by accumulation and inoculation on differential media for aerobic and anaerobic microorganisms, with identification conducted on the MALDI-TOF device. The sector inoculation method was applied following PV to count microorganisms.BLS 7.2-09/08 Investigation of biological fluids by microbiological method (semi-quantitative method) **[33]**.

Identification of microorganisms was carried out according to RI.BLS 7.2-09.13 Standard Operating Procedure "Working with MALDI-TOF Bruker Biotyper" **[34]**. Pathogenic/opportunistic pathogenic bacteria were isolated with their identification on MALDI-TOF **[35]**, which corresponds to the DSTU ISO 16140:2006 Standard **[36]**.

The preparation of media was carried out according to the manufacturer's instructions: preparation of samples for microorganism identification and preparation of standard solutions of reagents.

To prepare 1 cm^3 of basic solvent (OS), we added to the Eppendorf microtube 1.5 cm^3 475 µL of ultrapure deionised water; 500 µL of acetonitrile (ACN); 25 µL of 100% trifluoroacetic acid (TFA). The solvent was thoroughly mixed;

Preparation of the matrix (Bruker IVD HCCA Matrix): we added 250 (±5) µL of OS to a test tube containing 2.5 mg of IVD HCCA matrix (or its analogue) (in a 1:100 ratio, with a final concentration of 10 mg of matrix/cm3), and closed the test tube tightly. The solvent was thoroughly mixed on the vortex (vibration mixer) until the crystals were completely dissolved. The finished solution was stored at room temperature (20-25°C) in a place protected from light for up to one week (test tubes with the precipitate in the form of a crystal are no longer suitable for use);

Preparation of a working solution of the Bruker Bacterial Test Standard (BTS, cat. No. 255343): we added 50 µL of basic solvent (OS) to a BTS test tube. We dissolved it by pipetting about 20 times at room temperature. We piped slowly (about once every 2 seconds) with the tip in the solution during the procedure. We kept it for 5 minutes at room temperature and repeated the procedure as indicated above. We centrifuged it at 13,000 rpm for 2 minutes at room temperature if necessary. Then, we counted the number of colonies growing in different sectors (Table 1).

Table 1 Determining the degree of bacterial contamination by the number of isolated colonies.

A	Number of CFU per sector			Number of CFU in 1 cm^3 of the product
	I	II	III	
1 - 6	-	-	-	up to 1,000
8 - 20	-	-	-	3,000
20 - 30	-	-	-	5,000
30 - 60	-	-	-	10,000
70 - 80	-	-	-	50,000
100 - 150	5 - 10	-	-	100,000
-	20 - 30	-	-	500,000
-	40 - 60	-	-	1 million (10^9)
-	100 - 140	10-20	-	5 million ($5*10^9$)
-	-	30-40	-	10 million (10^{10})
-	-	60-80	single colonies	100 million (10^{11})

Each time, before starting work with a new chip, the device was calibrated with a 100% concentration bacterial test standard (BTS) per the operating instructions to control the compliance and intensity of peaks.

Sector inoculation method: The sample was thoroughly mixed before inoculation. The cup containing agar was conventionally divided into 4 sectors. We used a platinum loop with a diameter of 2 mm and a capacity of

0.005 ml to inoculate the sample (30-40 streaks) on Sector A of a petri dish containing blood agar. After that, the loop was sterilised 4 streak inoculations were made from Sector A to Sector I and similarly – from Sector I to Sector II, and from Sector II to Sector III. The cups were incubated at 37°C for 18-24 hours.

Description of the Experiment

Samples were taken at various technological stages of production: raw milk before bactofugation, raw milk after bactofugation, a normalised mixture from the tank, a pasteurised mixture, a mixture from the cheese maker prepared for coagulation, Ukrainskyi cheese after pressing, Ukrainskyi hard cheese after ripening. At each stage of production, 15 samples were taken for microbiological studies. The studies were repeated three times. A total of 405 samples were taken at seven stages of hard rennet cheese production to study the microflora. Water (10 samples) and brine were examined before and after pasteurisation (10 samples each).

Sample preparation: For sampling milk at Haisyn Dairy Plant LLC, we used sterile disposable plastic dishes with a volume of 100 cm^3. The sampling site was flamed, and the first portion of milk was drained into a separate container; then, we took the samples for examination in a disposable sterile container tightly closed. Immediately after sampling, all samples were placed into a container with a temperature of 2°C and delivered within 2.5 hours to the Biolights Expert Centre for Diagnostics and Laboratory Support LLC, Ternopil City (accreditation according to ISO/IEC 17025).

Appropriate reagents and solutions were prepared before starting work.

The samples to be examined were in liquid form (milk before and after bactofugation, normalised and pasteurised mixture) and semi-solid form (cheese mixture from the cheese maker and cheese after pressing).

Dilutions in a solvent (10 g of product per 90 cm^3) were prepared in peptone salt solution (PSS). This dilution was the source for direct inoculation of samples on cups with blood agar performed by sector inoculation method and further incubated under aerobic and anaerobic conditions at a temperature of 37±1 °C within 24±1 hours.

The liquid product was directly inoculated on the surface of blood agar (by the sector inoculation method) and incubated under aerobic and anaerobic conditions at 37±1°C within 24±1 h. To accumulate a small amount of microflora in the 10 g product sample (liquid or solid), we added up to 90 cm^3 of buffered peptone water (BPW). We incubated it at a temperature of 37±1°C within 24±1 h.

After accumulation, the sample was once again inoculated into differential diagnostic media by sector inoculation method (Baird-Parker Agar, XLD Endo Agar, Pseudomonas Agar, Enterococcus Agar, Bacillus Cereus Agar) and grown within 24±1 or 48±1 h at a temperature of 37±1 or 30±1°C (depending on the requirements of incubation of the nutrient medium).

After cultivation, we examined the inoculations, with the resulting colonies identified on MALDI TOF according to RI.BLS 7.2-09.13 "Working with MALDI-TOF Bruker Biotyper" **[37]**.

An isolated colony (1-2 μL in volume) was taken from a petri dish using a loop or toothpick. In a circular motion, the bacterial mass was evenly applied in a thin layer directly to the surface of the chip hole. After drying up, an automatic dispenser applied a matrix solution in a volume of 1 μL to the sample.

The chip was transferred to MALDI-TOF, and isolated cultures were identified using MBT Compass MALDI Biotyper 3.1 and Compass 1.4 for FLEX—Volume 1 and 2 Software and Manuals (Bruker Daltonik, Bremen, Germany). The studies' outcomes included bacteria identified with a scope value of 2.00.

Number of samples analyzed: We analysed 12 samples.

Number of repeated analyses: All measurements of instrument readings were performed two times.

Number of experiment replications: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: At the initial stage, samples of milk and dairy products were taken during the technological process for microbiological screening to study microorganisms identified in raw milk and detected in subsequent technological processes. After the first experiment, it became clear that individual microorganisms found in raw milk were also found in the final product. It should be noted that spore-forming microorganisms appear after milk pasteurisation. Therefore, at the next stages, we investigated the probable sources of contamination of the intermediate and final product at the processing stages. For this purpose, we conducted microbiological studies of water and brine. These studies aimed to exclude or confirm the bacteria circulation at the milk processing stages in the milk processing plant. The research findings were subjected to statistical processing and analysis.

Statistical Analysis

Statistical processing of the obtained results was performed using the ANOVA program, with the data in the tables presented as x ± SD (mean ± standard deviation). The difference between the groups was probable at $P < 0.05$ (considering the Bonferroni correction).

RESULTS AND DISCUSSION

At the first stage of the technological process of making Ukrainskyi hard rennet cheese, 18 types of microorganisms were isolated during the study of samples of bulk raw milk before bactofugation (Table 2).

Table 2 Types of bacteria isolated from raw milk at the first stage of the technological process – before bactofugation.

Types of microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/cm^3
Escherichia coli	100	$2.5\pm0.2\times10^4$
Kurthia gibsonii	33	$1\pm0.1\times10^1$
Acinetobacter baumannii	17	$1\pm0.01\times10^1$
Lactococcus lactis	17	$1\pm0.04\times10^1$
Enterobacter bugandensis	33	$1\pm0.02\times10^1$
Hafnia alvei	17	$1\pm0.001\times10^1$
Acinetobacter nosocomialis	17	$1\pm0.03\times10^1$
Lactococcus garvieae	66	1±0.04×101
Citrobacter freundii	17	$1\pm0.02\times10^2$
Staphylococcus aureus	66	$1\pm0.01\times10^1$
Streptococcus uberis	66	$1\pm0.02\times10^1$
Streptococcus uberis	17	$1\pm0.01\times10^1$
Enterococcus faecalis	50	$1\pm0.001\times10^1$
Citrobacter break	17	$1\pm0.05\times10^1$
Macrococcus caseolyticus	17	$1\pm0.001\times10^1$
Enterobacter cloacae	33	$1\pm0.03\times10^1$
Enterococcus faecium	17	$1\pm0.002\times10^1$
Enterobacter ludwigii	33	$1\pm0.004\times10^1$

Note: M±m, n=45.

A gram-negative bacteria, *Escherichia coli*, was isolated in all raw milk samples, which indicates its significantly dominant amount over the rest of the microflora. However, apart from *Escherichia coli*, *Streptococcus uberis*, and *Staphylococcus aureus* were isolated in raw milk in 66% of cases, and mastitis pathogens in cows. *Streptococcus uberis* is considered non-pathogenic to humans, possibly due to difficulties in identifying this type of infection. Besides, *Streptococcus uberis* is identified by classical microbiology as *Staphylococcus aureus* **[38]**.

A significant amount of the microflora identified in milk was of fecal origin. *Enterococcus faecalis* was identified in 50% of the samples, with *Enterobacter cloacae* and *Enterobacter bugandensis* identified in 33%. This speaks for non-compliance with hygienic requirements when obtaining raw milk on farms supplying it to the milk processing plant **[39]**, **[40]**. Other bacteria have also been identified: *Kurthia gibsonii*, *Lactococcus lactis*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Citrobacter freundii*, *Enterobacter kobei*, *Citrobacter braaki*, *Macrococcus caseolyticus*, *Enterococcus faecium*, *Enterobacter ludwigii*. Among them, clinically significant human diseases *Citrobacter braaki*i, since it is classified as wound microflora (especially the content of wounds) **[41]**, and *Acinetobacter baumannii*, which is the causative agent of infections of the respiratory tract, blood, abdominal cavity, urinary tract, traumatic infections, central nervous system infections, skin infections, accompanied by the risk of severe complications. This bacterium is resistant to antibiotics and disinfectants, soitoftenbecomes a nosocomial flora **[42]**, **[43]**.

In the second stage of the investigation, raw milk samples were taken after their bactofugation. At this technological stage, 13 microorganisms were identified (Table 3).

Table 3 Bacteria isolated in raw milk at the second stage of the technological process – after bactofugation.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	100	$1.6 \pm 0.1 \times 10^3$
Kurthia gibsonii	33	$1 \pm 0.01 \times 10^1$
Lactococcus lactis	33	$1 \pm 0.01 \times 10^1$
Enterobacter bugandensis	17	$1 \pm 0.03 \times 10^1$
Citrobacter freundii	17	$1 \pm 0.001 \times 10^1$
Staphylococcus aureus	33	$1 \pm 0.01 \times 10^1$
Enterococcus faecalis	66	$1 \pm 0.01 \times 10^1$
Enterobacter cloacae	50	$1 \pm 0.02 \times 10^1$
Enterococcus faecium	50	$1 \pm 0{,}01 \times 10^2$
Enterobacter ludwigii	50	$1 \pm 0.02 \times 10^1$
Staphylococcus chromogens	17	$1 \pm 0.01 \times 10^1$
Streptococcus gallolyticus	17	$1 \pm 0.01 \times 10^1$
Chryseobacterium bovis	17	$1 \pm 0.001 \times 10^1$

Note: M±m, n=45.

Several bacteria were detected at the first stage of research, in particular: *Acinetobacter baumannii*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Streptococcus uberis*, *Enterobacter kobei*, *Citrobacter braaki*, Macrococcus caseolyticus. However, bacteria that had not been detected at the first research stage and isolated, such as *Staphylococcus chromogenes*, and *Streptococcus gallolyticus*.

In 100% of the samples taken after bactofugation, the gram-negative bacterium *Escherichia coli* was isolated, but in concentrations slightly lower than in raw milk, by 1.6 times. Also, the causative agent of cow mastitis, *Staphylococcus aureus*, and faecal contamination flora, *Enterobacter cloacae*, was isolated in 33% of milk samples, with *Enterococcus faecalis* isolated in 66%. We should note that in the second stage, *Staphylococcus aureus* was isolated 2 times from several samples. *Enterobacter cloacae* was isolated 1.5 times, with *Enterococcus faecalis* isolated 1.3 times from larger samples. The increase in the number of samples from which *Enterobacter cloacae* and *Enterococcus faecalis* were isolated may be due to the formation of biofilms of these microorganisms on the equipment or due to its unsatisfactory hygiene **[44]**.

We identified *Streptococcus gallolyticus* (old name Streptococcus bovis) in 15% of the samples. This opportunistic pathogenic microorganism can occasionally enter the human bloodstream and cause various diseases. As a rule, this microorganism only colonises pregnant women in the intestines and genitourinary tract. However, there is evidence that this bacterium is associated with infectious endocarditis and human colon cancer. It has the property of adhering to the extracellular matrix, such as collagen, fibronectin, and fibrin, which is a pathogenesis mechanism **[45]**.

Streptococcus gallolyticus is part of the bovine rumen biota and causes diseases in ruminants, particularly mastitis in cows. That is why there is a reason to believe this bacterium got into raw milk **[46]**.

Other bacteria were detected in a smaller percentage of samples. Still, in general, the isolation of 13 types of microorganisms after bactofugation without a significant decrease in their number may indicate unsatisfactory hygienic treatment of technological equipment or the presence of biofilms **[47]**.

Observe in Table 4 the absence of bacteria that were detected at the first stage, before bactofugation, and were not identified after bactofugation: *Acinetobacter baumannii*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Streptococcus uberis*, *Enterobacter kobei*, *Citrobacter braaki*, *Macrococcus caseolyticus*. However, we observed the appearance of bacteria that were not detected at the first stage but were fixed after bactofugation: *Staphylococcus chromogenes*, *Streptococcus gallolyticus*.

Table 4 Comparative table: Bacteria isolated before and after bactofugation.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
	before bactofugation		after bactofugation	
Escherichia coli	100	$2.5\pm0.2\times10^4$	100	$1.6\pm0.1\times10^3$
Kurthia gibsonii	33	$1\pm0.1\times10^1$	33	$1\pm0.01\times10^1$
Lactococcus lactis	17	$1\pm0.04\times10^1$	33	$1\pm0.01\times10^1$
Enterobacter bugandensis	33	$1\pm0.02\times10^1$	17	$1\pm0.03\times10^1$
Citrobacter freundii	17	$1\pm0.001\times10^1$	17	$1\pm0.001\times10^1$
Staphylococcus aureus	33	$1\pm0.01\times10^1$	33	$1\pm0.01\times10^1$
Enterococcus faecalis	50	$1\pm0.001\times10^1$	66	$1\pm0.01\times10^1$
Enterobacter cloacae	33	$1\pm0.03\times10^1$	50	$1\pm0.02\times10^1$
Enterococcus faecium	17	$1\pm0.002\times10^1$	50	$1\pm0,01\times10^2$
Enterobacter ludwigii	33	$1\pm0.004\times10^1$	50	$1\pm0.02\times10^1$
Staphylococcus chromogens	-	-	17	$1\pm0.01\times10^1$
Streptococcus gallolyticus	-	-	17	$1\pm0.01\times10^1$
Chryseobacterium bovis	-	-	17	$1\pm0.001\times10^1$
Acinetobacter nosocomialis	17	$1\pm0.03\times10^1$	-	-
Acinetobacter baumannii	17	$1\pm0.01\times10^1$	-	-
Hafnia alvei	17	$1\pm0.001\times10^1$	-	-
Lactococcus garvieae	66	$1\pm0.04\times101$	-	-
Streptococcus uberis	66	$1\pm0.02\times10^1$	-	-
Citrobacter braaki	17	$1\pm0.05\times10^1$	-	-
Macrococcus caseolyticus	17	$1\pm0.001\times10^1$	-	-

Note: M±m, n=45.

The next step was to study samples of milk mixture normalised in fat content. The study results showed that 18 types of microorganisms had been re-isolated at this stage of production (Table 5).

At this research stage, a gram-negative bacteria *Escherichia coli* was isolated from 66% of samples in a milk mixture normalised in fat content, which, in contrast to the previous stage, is 34% lower. *Streptococcus uberis*, isolated in raw milk after bactofugation and in the normalized mixture, was not detected. *Staphylococcus aureus*, found in raw milk and after bactofugation, was also not detected in the the normalized mixture. In 33% of the normal samples, we found *Enterococcus faecalis*, which was 50% lower than the indicator of the previous stage. The number of *Enterobacter cloacae* and *Enterobacter ludwigii* had also reduced, which might be explained by the suppression of some bacterial species by others.

We also noted the appearance of microorganisms that had yet to be isolated at the previous two stages, namely: *Lelliottia amnigena*, *Streptococcus parauberis*, and *Citrobacter gillenii*. This might be due to both the presence of biofilms and the increase in the quantity of some bacterial species and the decrease in the number of others, and the suppression of some bacteria, depending on their number or the entry of these bacteria from washing water or other potential sources in the milk processing plant.

Special attention should be paid to *Lelliottia amnigena* – a gram-negative facultative anaerobic bacterium. It is usually detected in water sources and then in food (onions, cream, unpasteurised milk, and Spanish pork sausages), which, under favourable conditions, may cause infectious diseases in humans, especially in immunocompromised patients. Several cases of human infection have been described in published research papers, such as endophthalmitis, urinary tract infections, pyonephrosis, and sepsis **[48]**.

In the next, third stage, we studied pasteurised mixtures. Types of microorganisms were detected in pasteurised milk (Table 6).

Table 5 Bacteria isolated from samples of milk mixture normalized in fat content.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	66	$1\pm0.1\times10^4$
Kurthia gibsonii	50	$1\pm0.1\times10^1$
Enterobacter bugandensis	33	$1\pm0.01\times10^1$
Hafnia alvei	33	$1\pm0.04\times10^1$
Lactococcus garvieae	33	$1\pm0.02\times10^1$
Citrobacter freundii	50	$1,4\pm0.7\times10^4$
Enterococcus faecalis	66	$3.4\pm0.4\times10^2$
Citrobacter braaki	66	$1\pm0.3\times10^3$
Macrococcus caseolyticus	50	$1\pm0.01\times10^1$
Enterobacter cloacae	33	$1\pm0.01\times10^1$
Enterobacter ludwigii	17	$1\pm0.02\times10^1$
Moraxella osloensis	17	$1\pm0.01\times10^1$
Buttiauxella gaviniae	17	$1\pm0.001\times10^1$
Aeromonas media	17	$1\pm0.03\times10^1$
Citrobacter koseri	17	$1\pm0.01\times10^1$
Lelliottia amnigena	33	$1\pm0.02\times10^1$
Streptococcus parauberis	17	$1\pm0.002\times10^1$
Citrobacter gillenii	17	$1\pm0.02\times10^4$

Note: M±m, n=45.

Table 6 Bacteria isolated from pasteurized milk samples.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	17	$1\pm0.01\times10^3$
Kurthia gibsonii	17	$1\pm0.02\times10^1$
Hafnia alvei	17	$1\pm0.01\times10^1$
Enterococcus faecalis	17	$1\pm0.01\times10^1$
Streptococcus gallolyticus	17	$1\pm0.02\times10^1$
Acinetobacter Pitti	17	$1\pm0.01\times10^1$
Bacillus cereus	33	$1\pm0.02\times10^1$
Bacillus subtilis	33	$1\pm0.001\times10^1$
Bacillus licheniformis	66	$1\pm0.01\times10^1$
Bacillus megaterium	17	$1\pm0.002\times10^1$

Note: M±m, n=45.

We found *Escherichia coli* again in 17% of the studied samples but in a much smaller amount ($1\pm0.01\times10^3$).

Streptococcus gallolyticus was isolated and identified in 83% of the samples, which is a gram-positive, opportunistic pathogen that can cause bacteremia and endocarditis in humans.

Also, after the pasteurisation process, the appearance of various types of bacteria of the Bacillus family was detected (*Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*), which had not been identified in samples taken during previous technological processes. *Bacillus cereus* is a spore-forming microorganism, a common contaminator of dairy products (Table 7). Because the microorganism is widespread in the environment, it can contaminate milk during the milking process and enter dairy products at every stage of processing, storage, and hard rennet cheese ripening. Pasteurisationofmilkisineffectiveifitiscontaminatedwith *Bacillus cereus*. Moreover, it may act as a spore germination activator **[49]**.

In one example, *Acinetobacter pitty*, which belongs to the *Moraxellaceae* family, was isolated. *Acinetobacter pitty*, in association with other species of *Acinetobacter*, may cause various infectious diseases in humans, such as pneumonia, bacteremia, wound infections, meningitis, and urinary tract infections. *Acinetobacter* types of bacteria have a natural resistance to antibiotics and easily acquire this resistance, with their clinical isolates being able to spread rapidly among patients and survive in a hospital environment **[50]**.

Table 7 Comparative table: Bacteria isolated before and after pasteurization.

Microorganisms that have been identified	**% of samples with isolated bacteria**	**Number of microorganisms, CFU/ cm^3**	**% of samples with isolated bacteria**	**Number of microorganisms, CFU/ cm^3**
	before pasteurization		after pasteurization	
Escherichia coli	66	$1\pm0.1\times10^4$	17	$1\pm0.01\times10^3$
Kurthia gibsonii	50	$1\pm0.1\times10^1$	17	$1\pm0.02\times10^1$
Enterobacter bugandensis	33	$1\pm0.01\times10^1$	-	-
Hafnia alvei	33	$1\pm0.04\times10^1$	17	$1\pm0.01\times10^1$
Lactococcus garvieae	33	$1\pm0.02\times10^1$	-	-
Citrobacter freundii	50	$1,4\pm0.7\times10^4$	-	-
Enterococcus faecalis	66	$3.4\pm0.4\times10^2$	17	$1\pm0.01\times10^1$
Citrobacter braaki	66	$1\pm0.3\times10^3$	-	-
Macrococcus caseolyticus	50	$1\pm0.01\times10^1$	-	-
Enterobacter cloacae	33	$1\pm0.01\times10^1$	-	-
Enterobacter ludwigii	17	$1\pm0.02\times10^1$	-	-
Moraxella osloensis	17	$1\pm0.01\times10^1$	-	-
Buttiauxella gaviniae	17	$1\pm0.001\times10^1$	-	-
Aeromonas media	17	$1\pm0.03\times10^1$	-	-
Citrobacter koseri	17	$1\pm0.01\times10^1$	-	-
Lelliottia amnigena	33	$1\pm0.02\times10^1$	-	-
Streptococcus parauberis	17	$1\pm0.002\times10^1$	-	-
Citrobacter gillenii	17	$1\pm0.02\times10^4$	-	-
Streptococcus gallolyticus	-	-	17	$1\pm0.02\times10^1$
Acinetobacter Pitti	-	-	17	$1\pm0.01\times10^1$
Bacillus cereus	-	-	33	$1\pm0.02\times10^1$
Bacillus subtilis	-	-	33	$1\pm0.001\times10^1$
Bacillus licheniformis	-	-	66	$1\pm0.01\times10^1$
Bacillus megaterium	-	-	17	$1\pm0.002\times10^1$

Note: M±m, n=45.

Data from the literature also indicate the fixation of bacteria of the Bacillus family in pasteurized milk **[51]**. Pasteurisation of milk is ineffective in reducing contamination and may instead act as an activator of spore germination. **[52]**, **[53]**. In the next, fourth stage, we studied the mixture from the cheese maker prepared for curdling. At this stage, we isolated three types of microorganisms. The main bacterial component in all samples was the bacterium *Lactococcus lactis*, which was isolated at $1.7\pm0.02\times10^4$. *Escherichia coli* was isolated in 17% of samples, and Enterobacter cloacae was isolated in 33% of samples, $1\pm0.01\times10^1$.

The next, fifth stage, was dedicated to studying cheese after pressing. At this stage, 15 types of bacteria were isolated (Table 8).

Table 8 Bacteria isolated from samples of pressed cheese mixture.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	83	$7.4 \pm 0.01 \times 10^3$
Kurthia gibsonii	17	$1 \pm 0.1 \times 10^1$
Acinetobacter baumannii	50	$1 \pm 0.01 \times 10^1$
Lactococcus lactis	66	$6 \pm 0.02 \times 10^1$
Enterococcus faecalis	50	$1 \pm 0.01 \times 10^1$
Citrobacter break	17	$1 \pm 0.01 \times 10^1$
Macrococcus caseolyticus	33	$1 \pm 0.01 \times 10^1$
Enterobacter cloacae	33	$5.05 \pm 0.01 \times 10^2$
Enterococcus faecium	50	$1.3 \pm 0.01 \times 10^1$
Bacillus cereus	33	$1 \pm 0.01 \times 10^1$
Klebsiella pneumoniae	100	$1 \pm 0.01 \times 10^3$
Enterobacter xiangfangensis	17	$1 \pm 0.01 \times 10^1$
Pseudomonas putida	17	$1 \pm 0.02 \times 10^1$
Enterobacter hormaechei	17	$1 \pm 0.1 \times 10^1$
Klebsiella variicol	17	$1 \pm 0.01 \times 10^1$

Note: M±m, n=45.

After pressing, *Klebsiella pneumoniae was isolated in* 100% of the mixture samples. *Escherichia coli* and various forms of enterococci (*Enterococcus faecalis, Enterococcus faecium*) were isolated in most samples (83%). Half of the studied samples had *Acinetobacter baumannii*, and various forms of Enterobacteria (*Enterobacter cloacae, Enterobacter hormaechei, Enterobacter xiangfangensis*).

Unlike previous samples, *Klebsiella pneumoniae*, *Klebsiella varietal*, *Enterobacter xiangfangensis*, *Pseudomonas putida*, and *Enterobacter hormaechei* were found in the mixture for making cheese after pressing, which might indicate internal contamination during cheese production.

Special attention should be paid to *Klebsiella pneumonia*, as it is a zoonotic pathogen that generally causes various infectious conditions in both humans and animals **[54]**. We are concerned that this pathogen is detected in all samples at the penultimate stage of cheese production, which suggests that *Klebsiella pneumoniae* has entered the final product.

Considering that microorganisms can also enter milk at all technological stages with water, we examined 15 samples of water used in production. No microorganisms were detected in all the studied samples.

Studies were also conducted on the presence of microorganisms in the brine for salting cheese before and after pasteurisation. We identified 7 types of microorganisms (Table 9).

Table 9 Bacteria isolated from cheese brine samples.

Microorganisms that have been identified	Number of microorganisms in brine before pasteurization, CFU/cm^3	Number of microorganisms in brine after pasteurization, CFU/cm^3
Escherichia coli	$2 \pm 0.01 \times 10^5$	$2 \pm 0.02 \times 10^5$
Lactococcus garvieae	$1 \pm 0.01 \times 10^1$	
Enterococcus faecalis	$1 \pm 0.01 \times 10^1$	$1 \pm 0.001 \times 10^1$
Enterobacter cloacae	$1 \pm 0.02 \times 10^3$	$2 \pm 0.03 \times 10^3$
Enterococcus faecium	$1 \pm 0.001 \times 10^1$	
Klebsiella pneumoniae	$1 \pm 0.02 \times 10^3$	$1 \pm 0.01 \times 10^3$
Enterobacter xiangfangensis		$1 \pm 0.001 \times 10^1$

Note: M±m, n=15.

Study Shav found is contaminated with various bacteria, particularly *Klebsiella pneumoniae*, which we have previously found in cheese samples after pressing. Also, brine, both before and after pasteurization, contains *Escherichia coli* in a fairly large number of 2×10^5 CFU/cm^3.

The final stage was studying the cheese after it had matured for 30 days. Eighteen types of microorganisms were identified in the finished hard rennet cheese (Table 10).

Table 10 Bacteria isolated from samples of Ukrainskyi hard rennet cheese.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	83	$5.6\pm0.01\times10^2$
Kurthia gibsonii	33	$1\pm0.02\times10^1$
Acinetobacter baumannii	50	$1\pm0.002\times10^1$
Lactococcus lactis	66	$1.28\pm0.02\times10^2$
Enterococcus faecalis	66	$1\pm0.002\times10^1$
Macrococcus caseolyticus	33	$1\pm0.002\times10^1$
Enterobacter cloacae	33	$5\pm0.06\times10^2$
Enterococcus faecium	50	$1.33\pm0.001\times10^1$
Bacillus cereus	50	$1\pm0.002\times10^1$
Klebsiella pneumoniae	100	$1\pm0.05\times10^3$
Enterobacter xiangfangensis	17	$1\pm0.02\times10^1$
Pseudomonas putida	17	$1\pm0.01\times10^1$
Micrococcus luteus	17	$1\pm0.03\times10^1$
Corynebacterium flavescens	17	$1\pm0.02\times10^1$
Staphylococcus hominins	17	$1\pm0.01\times10^1$
Lysinibacillus sphaericus	17	$1\pm0.01\times10^1$
Enterobacter asburiae	17	$1\pm0.01\times10^1$

Note: M±m, n=15.

Considering the above, the finished product also contains many microorganisms. According to the search findings, a high content of *Escherichia coli* was detected—10^2. *Klebsiella pneumoniae* was identified in cheese after pressing and in finished cheese in 100% of samples, although this bacterium had not been detected at previous stages. Most likely, the brine was contaminated with bacteria at the final stages of production.

In total, 18 types of microorganisms were isolated and identified in the final product – Ukrainskyi hard rennet cheese, including *Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli*, which are of particular concern in terms of the safety of cheese consumption.

It should be noted that at the final stage, bacteria that had not been identified at the previous stages of milk processing wereisolated, such as *Micrococcus luteus*, *Corynebacterium flavescens*, *Staphylococcus hominis*, *Lysinibacillus sphaericus, Enterobacter asburiae, Corynebacterium flavescens*. Most likely, they were part of the starter cultures of microorganisms added during cheese production. However, *Lysinibacillus sphaericus* may be found in the human gastrointestinal tract, but most commonly it is found in soil. Also, this bacterium is classified as a causative agent of insect diseases, which is present everywhere in the environment. They contaminate bacteria, which relatively rarely cause infectious diseases in humans **[55]**.

So, bulk raw milk that had been delivered for processing contained 18 types of microorganisms, including fecal contamination bacteria – *Enterobacter cloacae, Enterobacter ludwigii, Citrobacter braaki, Enterobacter kobei* and pathogens of cow mastitis: *Escherichia coli, Staphylococcus aureus, Streptococcus uberis*. This speaks for the inadequate sanitary quality of raw milk. Of particular concern are bacteria that are of clinical significance to humans, which may cause various infectious diseases, such as *Lactococcus garvieae* **[54]**, *Acinetobacter baumannii* **[56]**, *Enterobacter bugandensis* **[57]**, *Enterobacter ludwigii* **[58]**, *Acinetobacter baumannii* and *Enterococcus faecalis* **[59]**. All of them relate to bacteria that can become resistant to antibiotics pose a serious threat to people in various infectious conditions and circulate as nosocomial infections. It is dangerous that Acinetobacter identified the very first stage – in raw milk before bactofugation, and then it appears at the final stages of the technological process more often than at the beginning. This fact indicates the possible circulation

of these bacteria in the equipment or auxiliary accessories of the milk processing plant. The bacterium's ability to resist disinfectants allows it to survive even when milk pipelines and other equipment are thoroughly washed and disinfected. Controlling only coliform bacteria (CB) as sanitary indicative microorganisms may not show the real situation with bacterial contamination of equipment, pipelines, water, etc. We should also note that the bacterium retains its viability in cheese after 30 days of storage, even with salt. *Acinetobacter* spp. Bacteria can transmit genes resistant to other bacteria, thus posing a significant risk to humans. However, due to the difficulty of isolation and the absence of official standard methods, there is a lack of work on epidemiological data on foodborne diseases caused by this microorganism.

The Infectious Diseases Society of America (IDSA) classified *A. baumannii* as ESKAPE type (acronym of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter)* which cause most nosocomial infections in the United States and around the world, being usually antibiotic-resistant **[60]**.

Enterococcus faecalis was isolated at all stages of the technological process except for the mixture prepared for curdling. *Enterococcus faecium* was isolated at the beginning of the technological process (before bactofugation and after bactofugation) and at the end of the technological process (cheese after pressing, cheese after maturation). Apart from the fact that enterococci are one of the external pathogens of mastitis, the opportune pathogenic bacteria are part of humans' and animals' normal physiological intestinal flora. However, in recent years, they have become one of the main pathogens that cause numerous infections in humans, mainly nosocomial ones, such as bacteremia and infections of the urinary tract, skin, soft tissues, abdominal cavity, pelvis, and central nervous system. These infections are mostly caused by *E. faecalis* (about 80.0%) and *E. faecium* (10.0%–15.0%) **[61]**. The high resistance of enterococci to adverse conditions allows them to survive in the environment, particularly, in slaughterhouses. Potential mammary gland infections caused by the bacteria are normally mild. It is typical for Enterococci to have high resistance to many anti-bacterial substances, both by internal and acquired mechanisms. Due to their ability to acquire and transmit genes that determine resistance to other bacteria, they are perceived as a good indicator of antimicrobial resistance in the environment. The possibility of enterococci transmission through milk to humans raises concern **[62]**. *Streptococcus gallolyticus* was isolated after bactofugation.

This opportunistic pathogenic microorganism can occasionally enter the human bloodstream and cause various diseases. As a rule, this microorganism only colonises pregnant women's intestines and genitourinary tract. However, there is evidence that this bacterium is associated with infectious endocarditis and human colon cancer. It can adhere to the extracellular matrix, such as collagen, fibronectin, and fibrin, a pathogenesis mechanism. *Streptococcus gallolyticus* is part of the rumen biota, but it also causes various diseases of ruminants. In particular, it can cause mastitis in cows. That is why there is a reason to suggest that this bacterium was present in raw milk **[63]**.

Special attention should be paid to Lelliottiaamnigena, isolated in a mixture of normal fat content. It is a gram-negative facultative anaerobic bacterium, usually found in water sources and then in food (onions, cream, unpasteurisedmilkandSpanish pork sausages), which, under favourable conditions, can cause infectious diseases in humans, especially in immunocompromised patients. Several cases of human infection with endophthalmitis, urinary tract infections, pionephrosis, and sepsis have been highlighted in the published research papers **[61]**. Since this microorganism was not detected in previous studies, we may assume it got into samples from potential sources at the milk processing plant.

After pasteurisation, we detected the appearance of various types of bacteria belonging to the Bacillus family (*Bacillus cereus, Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus megaterium*). These bacteria were not isolated in samples taken during previous processes. *Bacillus cereus* is a spore-germinating microorganism that is ten isolated in dairy products. Since the microorganism is widespread in the environment, it can contaminate milk during the milking process. It can enter the dairy product at every cheese processing, storage, and maturation stage. Pasteurisation of milk is not effective for *Bacillus cereus.* Instead, pasteurisation may act as a spore germination activator **[62]**.

Acinetobacter pitty, belonging to the *Moraxellaceae f*amily, has also been identified. In association with other Acinetobacter species, Acinetobacter pitty causes various infectious conditions in humans, such as pneumonia, bacteremia, wound infections, meningitis, and urinary tract infections. Acinetobacter types of bacteria have a natural resistance to antibiotics and easily acquire this resistance, with their clinical isolates being able to spread rapidly among patients and survive in a hospital environment **[42]**.

Also of great concern is the appearance of *Klebsiella pneumonia*, which is detected at the last two stages of the technological process and found in the final product after maturation.

Table 11. Bacteria isolated from raw milk to final product (Ukrainskyi hard rennet cheese).

Microorganisms that have been identified	Raw milk	After bactofugation	Milk mixture normalized	After pasteurization	Pressed cheese mixture	Final product
	% of samples with isolated bacteria					
Escherichia coli	100	100	66	17	17	83
Kurthia gibsonii	33	33	50	17	17	17
Acinetobacter baumannii	17				50	33
Lactococcus lactis	17	50			66	66
Enterobacter bugandensis	33	17	33			
Hafnia alvei	17	33	17			
Acinetobacter nosocomialis	17					
Lactococcus garvieae	66		33			
Citrobacter freundii	17	17	50			
Staphylococcus aureus	66	50				
Streptococcus uberis	33					
Enterobacter kobei	17					
Enterococcus faecalis	50	66	66	17	50	50
Citrobacter braaki	17		66			
Macrococcus caseolyticus	17		50		33	33
Enterobacter cloacae	33	50	33		33	33
Enterococcus faecium	17	50			50	17
Enterobacter ludwigii	33	50	17			
Staphylococcus chromogens		17				
Streptococcus gallolyticus		17		100		
Chryseobacterium bovis		17				
Moraxella osmosis			17			
Buttiauxella gaviniae			17			
Aeromonas media			17			
Citrobacter koseri			17			
Lelliottia amnigena			33			
Streptococcus parauberis			17			
Citrobacter genii			17			
Acinetobacter Pitti				17		
Bacillus cereus				33	33	50
Bacillus subtilis				33		
Bacillus licheniformis				66		
Bacillus megaterium				17		
Klebsiella pneumoniae					100	100
Enterobacter xiangfangensis					17	17
Pseudomonas putida					17	17
Enterobacter hormaechei					17	
Klebsiella varietal					17	
Micrococcus luteus						17
Corynebacterium flavescens						17
Staphylococcus hominins						17
Lysinibacillus sphaericus						17
Enterobacter asburiae						17

Note: n=45.

A total of 43 species of various microorganisms have been isolated and identified during the entire experimental period (Table 11). We have found the following bacteria: *Escherichia coli*, *Kurthia gibsonii*, *Acinetobacter baumannii*, *Lactococcus lactis*, *Enterobacter bugandensis*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Citrobacter freundii*, *Staphylococcus aureus*, *Streptococcus uberis*, *Enterobacter kobei*, *Enterococcus faecalis*, *Citrobacter braaki*, *Macrococcus caseolyticus*, *Enterobacter cloacae*, *Enterococcus faecium*, *Enterobacter ludwigii*, Staphylococcus chromogenes, *Streptococcus gallolyticus*, *Chryseobacterium bovis*, *Moraxella osloensis*, *Buttiauxella gaviniae*, *Aeromonas media*, *Citrobacter koseri*,

Lelliottia amnigena, *Streptococcus parauberis*, *Citrobacter gillenii*, *Acinetobacter pittii*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Klebsiella pneumoniae*, *Enterobacter xiangfangensis*, *Pseudomonas putida*, *Enterobacter hormaechei*, *Klebsiella variicol*, *Micrococcus luteus*, *Corynebacterium flavescens*, *Staphylococcus hominis*, *Lysinibacillus sphaericus*, *Enterobacter asburiae*.

For a more in-depth understanding of the sources of microbial entry into samples at the stages of the technological process of cheese production, the study of the microbiota of equipment, starter cultures, and brine for salting cheese, as well as the sensitivity of the most significant microorganisms to antibacterial agents is looking rather promising.

CONCLUSION

Microbiological screening of Ukrainskyi hard rennet cheese using MALDI-TOF technology, starting from bulk raw milk to the finished dairy product, showed the presence of microorganisms at all stages of production.

During the experiments, 43 species of various microorganisms were isolated and identified. However, the number and individual types of microorganisms differed at different stages of production. Of the 43 types of microorganisms identified during the entire experiment, only 18 were fixed at the first production stage, that is, in raw milk. The other 25 types of bacteria were detected at the next stages of production. Some microorganisms isolated in raw milk are also identified in the final product: *Acinetobacter baumannii* and *Escherichia coli*. In this regard, the production conditions must monitor these microorganisms to control them and prevent their appearance in the finished product, hard cheese.

A clinically significant microorganism such as *Klebsiella pneumonia* most likely got into the final product from brine. This is caused by improper control during production and insufficient sanitary actions to avoid contamination with foreign microflora.

This experiment requires further research, particularly the study of the sensitivity of isolated bacteria to antibiotics and how technological processes affect their significance. The fact that bacteria that can cause various diseases get into the final product will pose an even greater threat if these bacteria remain resistant to antibiotics.

In total, 18 types of microorganisms were isolated and identified in the final product—Ukrainskyi hard rennet cheese—including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*, which are of particular concern regarding the safe consumption of cheese.

The presence of opportunistic microorganisms in finished dairy products can lead to: the risk of potentially dangerous bacteria entering the human body, spoilage of products before the expiration date, deterioration of the taste qualities of products, and, accordingly, economic losses for the manufacturer. Based on the experimental data, it is possible to recommend food industry manufacturers conduct additional monitoring (microbiological screening) during the technological process to identify potential sources of contamination by various types of microorganisms. According to the obtained data, it can be concluded that more is needed to control microbiological indicators regulated by legislation. In the production process, a wide list of microorganisms can circulate, which are not included in the basic indicators of the control of dairy products.

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Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. Larysa Bal-Prylypko

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

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