The object of research is patterns in a technological process related to the fermentation of milk clots from dairy raw materials obtained from cows with different genotypes by the kappa-casein gene. The task still unresolved is the need to adjust the technological process in the manufacture of fermented milk products from dairy raw materials obtained from cows with different genotypes by this gene. This is associated with the interest of animal owners to create herds of cows with the BB genotype in order to improve milk suitability for cheese making.

Changes in physical-chemical, microbiological, and organoleptic parameters in the process of targeted use of mesophilic lactic acid bacteria of biotechnological processing and during storage have been studied.

Based on the results of the study of fermented clots, their physical-chemical and microbiological parameters, the absence of dependence of the fermentation process of milk clots by mesophilic lactic acid streptococci on the cow genotype according to the kappa-casein gene has been found.

The study has resulted in confirming the hypothesis that genetic variants of the kappa-casein (CSN3) gene in cows (AA, AB, BB) affect the physical-chemical characteristics of milk, the dynamics of fermentation, and the properties of milk clots formed under the action of mesophilic lactic acid streptococci, and therefore the quality of finished fermented milk products. The use of milk from the cow genotype based on the kappa-casein gene is possible using classical technology and does not require adjustment of technological conditions.

The results of the work could be used in the milk processing industry in the development of technology for fermented milk products from raw milk of cows with different genotypes based on the kappa-casein gene

Keywords: kappa-casein, milk clot, lactic acid bacteria, consistency, viscosity, biotechnological processing, lactococci, technological properties, storage of fermented milk products

UDC 637.1:637.3
DOI: 10.15587/1729-4061.2025.333997

ESTABLISHING THE DEPENDENCE OF FERMENTATION PROCESS OF MILK CLOTS WITH MESOPHILIC LACTIC ACID STREPTOCOCCI ON THE GENOTYPE OF COWS IN TERMS OF THE KAPPA-CASEIN GENE

Volodymyr Ladyka
Doctor of Agricultural Sciences, Professor, Academician of NAAS
Department of Technology of Production and Processing of Animal Products and Cinology**
Yuriy Skliarenko
Correspoding author
Doctor of Agricultural Sciences
Laboratory of Animal Husbandry and Fodder Production
Institute of Agriculture of the Northeast of the National Academy of Sciences
Zelena str., 1, Sad vill., Sumy reg., Ukraine, 42343
E-mail: Sklyrenko9753@ukr.net
Natalia Bolgova
PhD, Associate Professor
Department of Technology and Food Safety**
Viktoriia Vechorka
Doctor of Agricultural Sciences, Professor
Department of Industrial Engineering and Agroengineering
Khmelnytskyi National University
Instytuts'ka str., 11, Khmelnytskyi, Ukraine, 29016
Serhiy Tereshchenko
PhD Student*
Vitalii Pysariev
PhD Student*
*Department of Animal Genetics, Breeding and Biotechnology**
**Sumy National Agrarian University
Herasyma Kondratieva str., 160, Sumy, Ukraine, 40000

Received 17.05.2025
Received in revised form 17.07.2025
Accepted date 25.07.2025
Published date 26.08.2025

How to Cite: *Ladyka, V., Skliarenko, Y., Bolgova, N., Vechorka, V., Terechenko, S., Pysariev, V. (2025). Establishing the dependence of fermentation process of milk clots with mesophilic lactic acid streptococci on the genotype of cows in terms of the kappa-casein gene. Eastern-European Journal of Enterprise Technologies, 4 (11 (136)), 49–56. https://doi.org/10.15587/1729-4061.2025.333997*

1. Introduction

Economic and political processes that occur all over the world threaten the food security of humankind. Scientists in various sectors of the national economy are constantly working on solving this problem. One of the directions for addressing the food problem is the development of such sciences as biotechnology and genetics. Scientists have long been study-

ing the issue of increasing the quantity of livestock products and their quality. In order to improve the technological properties of milk, scientists pay attention to the genotype of cows according to the kappa-casein gene. Depending on it, the yield of cheeses can be increased and their quality improved. However, the issue of the influence of the genotype according to this gene on the technological properties of milk in the production of other dairy products remains poorly studied.

One of such products is fermented milk products, which remain the most widely used segment among dairy products [1, 2]. However, there are no studies on the influence of the genotype according to the kappa-casein gene on their production.

It should be remembered that one of the main conditions for dairy raw materials in the dairy production is its compliance with certain technological properties. They determine the direction of processing and the specificity of the technological process for a particular product. Technological properties such as rennet coagulation, the ability to ferment, and heat resistance determine the direction of the technological process [3]. The biochemical composition of milk has a significant impact on its technological qualities [4]. Therefore, scientists believe that different variants of milk proteins can affect the technological properties of the raw material [5]. The creation of dairy herds with the BB genotype according to the kappa-casein gene requires research to determine its impact on the production of lactic acid products. The relevance of the research is associated with resolving the issue of practical use of milk raw materials from cows with different genotypes according to the kappa-casein gene in the production of lactic acid products.

2. Literature review and problem statement

In [6] it is stated that the genetic markers associated with the technological properties of milk include the genes of alpha-lactalbumin, beta-lactoglobulin, and others. However, in dairy cattle, research is mainly aimed at identifying the influence of genetic markers on milk productivity indicators and technological properties of milk in cheese production. Their influence on the technological properties of raw milk in the production of fermented milk products is not defined.

In works [7, 8] it was established that the kappa-casein protein is one of the main proteins of milk. However, they do not indicate the influence of these proteins on the qualitative and technological characteristics of milk. This approach was used in [9], which investigated the dependence of milk composition on the genotype of the kappa-casein gene. However, the authors do not provide information on the technological characteristics of milk from cows of different origins. In [10], the dependence of the coagulation properties of milk depending on the polymorphism of the kappa-casein gene was studied. In this case, the authors did not examine the influence of genotype on the technological characteristics of milk in the production of fermented milk products. In [11], a reliable influence of the genotype for kappa-casein on the heat resistance of milk in the alcoholic test was established. This also leaves the issue of the production of fermented milk products from dairy raw materials of different origins unresolved.

The results of studies prove that the "desired" alleles and genotypes of whey proteins (alpha-lactalbumin) in the genome of cows have a significant influence on the technological properties of milk [12]. But the authors only consider the influence on the technological properties in the production of cheese. The influence of the genotypes of alpha-casein, beta-casein, kappa-casein on the properties of cow's milk during its processing has been confirmed in [13]. However, their influence has been studied only in the production of hard cheeses. Other researchers associate the genotype for alpha-casein and beta-casein only with the biochemical composition of milk [14]. The researchers do not report results of studies to determine the influence of genotype on associated genes on the fermentation process of milk clots.

Each breed has a certain genetic structure that affects the technological properties of milk. Therefore, the identification of milk proteins as markers in the animal genome contributes to the creation of a herd of cows with a high protein content in milk, which is necessary for the production of high-quality dairy products [15]. At the same time, the cited work did not study the influence of genotype on the fermentation process, which is the basis of most technologies for the production of fermented milk products [16]. Fermentation of milk mixtures is a process of biochemical changes in the components of milk mixtures under the influence of enzymes [17, 18]. Raw milk contains about 100 enzymes of different classes, and if the primary processing processes of milk are disturbed, they can lead to its spoilage. The main source of enzymes in pasteurized milk is starter microorganisms [19]. The cited works [17–19] did not study the influence of raw milk proteins on the process of its fermentation.

To activate the directed fermentation process, specially selected strains of lactic acid, propionic acid bacteria, yeast, as well as prebiotics and their combinations are added to the pasteurized milk mixture. As a result of the vital activity of the starter microorganisms, significant biochemical changes occur in all components of milk with the formation of a number of chemical substances [20]. However, the cited work does not mention the dependence of the fermentation process on the characteristics of the milk protein raw material.

Lactose undergoes the greatest changes in the process of directed fermentation. Under the influence of β-galactosidase, it is broken down to galactose and glucose, which, in the case of anaerobic oxidation of carbohydrates, along the Embden-Meyerhof-Parnas pathway, forms lactic acid as the final fermentation product [21]. However, the cited work does not resolve the issue of the influence of enzymatic hydrolysis of lactose on the process of milk clot formation.

Milk proteins also undergo enzymatic changes due to the influence of proteolytic enzymes of microbial origin. The most significant enzymatic transformation is undergone by protein due to the action of proteases [22, 23]. Proteases catalyze the hydrolysis of peptide bonds in protein molecules. In the production of fermented milk products, strains of starter cultures that carry out proteolysis moderately are used. Proteases formed by the starter microflora attack αs-casein more easily than β- and κ-casein, but individual strains of microorganisms cleave α- and β-fractions equally effectively [23]. The question of the influence of the proteolytic activity of lactobacteria on the formation of milk clots depending on the raw milk remains unresolved.

Studies have shown that in the production of fermented milk products, starter cultures of mixed mesophilic cultures of *Lactococcus lactis ssp.* of different strains are usually used. It was established that lactococci do not have high proteolytic activity, but shallow casein breakdown provides optimal consistency and structure, but at the same time increases the digestibility of casein in the gastrointestinal tract and ensures stability during storage. No significant difference in the

use of *L. Lactis subsp. Cremoris* Y15 or *L. Lactis subsp. Lactis* KLDS4.0325 for the production of fermented milk products was determined in study [24]. The question of the influence of mesophilic lactococcal cultures on raw milk from cows with different genotypes according to the kappa-casein gene remains unresolved.

As a result of biotechnological processing of milk mixtures, the fat component also undergoes changes, but less significantly. The degree of lipolysis depends on the composition of the microflora, both initial and introduced, and on the number and condition of fat globules. Uncontrolled lipolysis leads to the emergence of defects and spoilage of products [25]. The question of the effect of starter cultures on milk fat globules, which contain complex lipids and proteins, remains unresolved.

Therefore, it is advisable to conduct a study aimed at determining the effect of the protein genotype of raw milk on the processes of its biotechnological processing in order to understand the significance of the effect and adjust the technological process if necessary.

3. The aim and objectives of the study

The purpose of our work is to establish the dependence of the fermentation process of milk clots, carried out by mesophilic lactic acid streptococci, on the genotype of cows according to the kappa-casein genome, in order to identify the influence of genetic variants on the properties of clots and the potential quality of final dairy products. This will make it possible to determine the features of technology in the production of fermented milk products from dairy raw materials of different origins according to the CSN3 kappa-casein genotype.

To achieve this aim, the following objectives were accomplished:

– to investigate the physicochemical characteristics of raw milk from cows with different genotypes according to the kappa-casein genome;

– to investigate the process of fermentation of milk mixtures with a starter culture based on mixed mesophilic cultures of *Lactococcus lactis ssp* and changes in the indicators of fermented milk clots during storage from raw milk from cows with different genotypes according to the kappa-casein genome.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of our study is patterns in the technological process of fermentation of milk clots from raw milk from cows with different genotypes according to the kappa-casein gene.

The hypothesis of the study assumes the influence of genetic variants of the kappa-casein gene in cows (AA, AB, BB) on the features of the technological process of fermentation.

It was assumed that the studied raw milk has differences according to the kappa-casein gene and this affects the fermentation and formation of milk clot. Other factors that can affect fermentation (temperature, pH, composition of microflora, storage mode) are constant, focusing the analysis on the influence of the genotype.

The use of a small sample of data simplifies the research. However, the sample size is sufficient for statistical analysis and does not claim to be fully representative of the entire livestock.

4. 2. Research base

The research was conducted at the breeding plant for breeding the Sumy intrabreed type of the Ukrainian black-and-white dairy breed, the State Enterprise "Research Farm of the Institute of Agriculture of the North-East of NAAS", which is located in the Sumy oblast (the village of Sad, Ukraine).

4. 3. Determination of genotypes by the kappa-casein genome

Genetic studies were conducted at the laboratory of the Bogomolets Institute of Physiology of NAS using generally accepted methodologies. Blood samples were taken from 2.7 ml monovets ("Sarstedt", Germany) with subsequent freezing of the samples and their storage at –20 °C. To obtain DNA from biological samples for the purpose of genotyping, the appropriate kit for purification of genomic DNA Monarch® New England BioLab (USA) was used. For allelic discrimination, the TagMan@Genotyping system and a set of primers and probes were used [26].

4. 4. Principles to form research groups

Laboratory studies were conducted in the fourth quarter of 2024 at the interdepartmental laboratories, Faculty of Food Technologies, Sumy National Agrarian University. In order to establish the influence of the genotype of cattle according to the kappa-casein gene on the fermentation process of milk clots, cow's milk obtained from cows with different genotypes was used (Table 1).

For this purpose, 9 milk samples were taken from the above-mentioned farm during morning milking from cows with the following genotypes for the kappa-casein gene: AA, AB, BB. The milk samples were grouped according to the genotype for the studied gene. Milk samples numbered 1–3 were obtained from animals with the AA genotype, 4–6 with the AB genotype, and 7–9 with the BB genotype.

Table 1

Examined groups

Sample No.	Kappa-casein genotype	Cow ID number	The name of the cow	Fat content, %	Content of dry skimmed residue	Protein content, %
No. 1	AA	UA 8013741149	Maklushka	4.54	8.06	2.88
No. 2	AA	UA 8014100955	Vorotka	4.24	7.91	2.82
No. 3	AA	UA 8014072559	Smolka	3.83	8.09	2.88
No. 4	AB	UA 8013741119	Melashka	2.67	8.10	2.87
No. 5	AB	UA 8013741133	Estafeta	4.54	8.86	3.17
No. 6	AB	UA 8015717535	Volnitsa	5.25	8.64	3.10
No. 7	BB	UA 8014072555	Havana	4.69	8.48	3.03
No. 8	BB	UA 8015405598	Kubanya	4.69	9.10	3.26
No. 9	BB	UA 8015717506	Kukla	4.32	8.75	3.13

4. 5. Methodology for determining sample quality indicators

Experimental studies were conducted in accordance with current standards, modern, generally accepted methodologies, taking into account requirements [27] and in accordance with the Procedure for conducting experiments on animals by scientific institutions [28].

Generally accepted methods for determining physicochemical and microbiological indicators were applied. Mass fraction of fat (%), mass fraction of dry skimmed milk residue (%), mass fraction of protein (%), density (°A) were determined using an EKOMILK ultrasonic analyzer.

Titrated acidity of samples (°T) was determined by titration of selected milk samples diluted 1:2 with distilled water with a 0.1 normal sodium hydroxide solution with the addition, as an indicator, of a 1% alcoholic solution of triphenylmethane dye.

Active acidity (pH) was determined by the Apera Instruments PH8500-DP device according to the standard.

The relative viscosity was determined using a VZ-246 viscometer, by the time of outflow from the nozzle of the viscometer with a diameter of 5 mm of a thoroughly mixed product in an amount of 100 cm^3, in seconds (s).

The total bacterial inoculation was determined from the combined sample by the plate method when sowing 1 cm^3 of the studied sample on a dense nutrient medium and cultivating the crops at 30 ± 1°C for 72 hours.

The most probable number of viable lactococcal cells (CFU/cm^3) in fermented clots was determined by the method of sowing in liquid nutrient media (sterile skim milk). The sample of the studied clot was diluted by tenfold dilutions, and the last three dilutions were sown in 2 test tubes containing 10 cm^3 of sterilized skim milk, so that the last dilution did not contain microorganisms. The sown test tubes were thermostated for 7 days at a temperature of 30 ± 1°C. After the thermostating period, the test tubes were inspected and marked those in which the milk had coagulated, a numerical characteristic was made, using reference data [29] the most probable number of viable cells was calculated.

When preparing milk samples for biotechnological processing, cleaning, separation, and pasteurization were carried out at a temperature of 90 ± 2°C with a holding time of 5 min. To minimize the influence of the fat fraction of milk on the objectivity of the results, it was decided to carry out fermentation of previously skimmed samples. Skimmed milk was separated by separating whole milk heated to 41 ± 1°C on a Motor Sich-100 separator, twice. Skimmed milk was pasteurized and cooled to a fermentation temperature of 29 ± 2°C, bypassing the cooling process. Skimmed milk was fermented at a temperature of 29 ± 2°C with a starter culture of mixed cultures of lactic acid mesophilic lactococci of the trademark "VIVO-Syr kislomochny", consisting of cultures of *L. Lactis ssp.* The starter culture was introduced in an amount that would ensure an initial concentration of lactobacilli cells of at least 1×10^6 CFU/cm^3. The study of the influence of genotype on the growth of lactic acid mesophilic lactococci was carried out by inoculating milk samples into tubes with sterilized skim milk immediately after the end of the fermentation process and on the 3rd, 7th, 10th, and 14th day of storage. The methodology for conducting the inoculations was followed. Organoleptic analysis during the storage of fermented clots consisted of monitoring changes in the taste, smell, and consistency of the studied samples. The duration and frequency of control were carried out similarly to the above-mentioned studies. No scoring was performed.

5. Results of investigating the physicochemical parameters of raw milk and the process of fermentation of milk clots

5. 1. Results of investigating the physicochemical parameters of raw milk

Studies of milk samples from cows with different genotypes of CSN3 kappa-casein prove that samples from cows with the BB genotype have an advantage in terms of average fat content and non-fat dry matter of milk. However, this difference is statistically insignificant. In terms of average protein content in milk, milk samples from cows with the BB genotype prevailed over samples from cows with the AA genotype by 0.28% ($p < 0.05$) (Table 2).

Table 2

Chemical characteristics of raw milk from cows with different genotypes of the CSN3 kappa-casein gene

Groups by genotype	Chemical indicators ($M \pm m$)		
	Fat content, %	Dry fat-free residue content	Protein content, %
AA	4.20 ± 0.206	8.02 ± 0.056*	2.86 ± 0.020*
AB	4.15 ± 0.769	8.53 ± 0.226	3.05 ± 0.091
BB	4.57 ± 0.123	8.78 ± 0.155	3.14 ± 0.058

*Note: *p – significance level according to Student's t-test: $p < 0.05$.*

Samples from cattle with the BB genotype have higher average titrated acidity and average milk density (Table 3).

Table 3

Indicators of acidity and density of raw milk from cows with different genotypes of the kappa-casein gene

Groups by genotype	Indicator ($M \pm m$)		
	Acidity, °T	Density, °A	pH
AA	18.3 ± 1.333	25.8 ± 0.328*	6.63 ± 0.020
AB	18.3 ± 2.028	27.9 ± 0.503	6.59 ± 0.084
BB	21.0 ± 1.000	28.5 ± 0.626	6.49 ± 0.062

*Note: *p – significance level according to Student's t-test: $p < 0.05$.*

Samples from cows with the AA genotype were characterized by a higher average active acidity. However, the difference between the studied samples was not statistically significant.

5. 2. Results of research on the fermentation process of milk clots by mesophilic lactic acid streptococci

The results show that all experimental samples that were fermented have an acidity value that corresponds to a dairy product fermented by mesophilic lactic acid lactococci [24]. The results of the research showed that there were no deviations from the technological process parameters when using raw milk from cows with different CSN3 genotypes. At the end of fermentation (10 hours), regardless of the genotype of the cattle, the raw milk obtained from them had a homogeneous curd mass with moderate acidity and viscosity (Table 4).

After 10 hours of fermentation, the average titrated acidity in all experimental groups was approximately 74°T. The average pH value was close to 4.6 in all experimental

groups. Samples from cows with the BB genotype had a higher average relative viscosity (by 2.6 s), but this difference was statistically insignificant. According to the results of microbiological studies, no statistically significant effect of the CSN3 genotype on the development of lactic acid microorganisms in raw milk was found. However, samples from cattle with the AB genotype had a lower number of lactococci.

Table 4

Characteristics of fermented clots

Groups by genotype	Acidity, °T	pH	Relative viscosity, s	Number of lactococci, CFU/cm^3
AA	74.0 ± 1.53	4.59 ±0.006	25.7 ± 1.45	$5.33 \pm 0.5 \times 10^9$
AB	74.3 ± 1.86	4.59 ±0.030	25.7 ± 28.5	$3.00 \pm 0.5 \times 10^9$
BB	74.0 ± 2.08	4.65 ± 0.035	28.3 ± 0.88	$5.50 \pm 0.5 \times 10^9$

Our results indicate that the viscosity of fermented clots directly depends on the protein content in milk. This is evidenced by the positive value of the correlation coefficient between both indicators in all samples regardless of genotype ($r = 0.79 \pm 0.19$) ($P < 0.001$) (Fig. 1).

Therefore, it can be assumed that the kappa-casein genotype does not affect the quality of clots fermented with lactic acid mesophilic lactococci.

According to the results, it can be concluded that during storage of fermented milk products, a change in titrated and active acidity occurs. It should be noted that such a trend is permissible when using a culture of mesophilic lactococci as a starter.

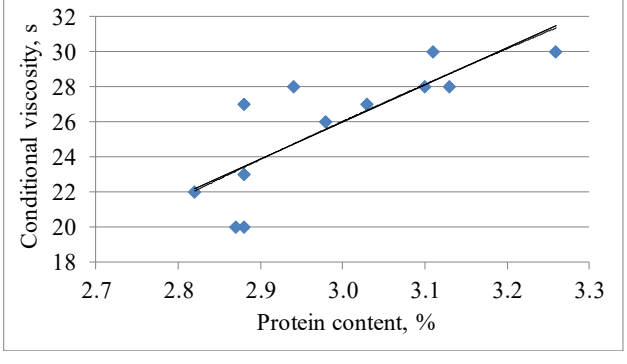

Fig. 1. Correlation between protein content in raw milk and viscosity of fermented clots (regardless of genotype)

During the 14 days of research, the titrated acidity of the studied samples increased unevenly (Table 5).

Table 5

Change in titrated acidity in the studied fermented samples during storage, °T

Groups by genotype	Storage duration, days				
	0	3	7	10	14
AA	75.0 ± 2.08	74.0 ± 1.53	79.3 ± 0.67	89.0 ± 1.53	91.0 ± 1.00
AB	74.0 ± 1.00	74.3 ± 1.86	78.7 ± 2.33	86.7 ± 2.03	92.0 ± 1.53
BB	73.3 ± 2.03	74.0 ± 2.08	80.7 ± 0.88	87.0 ± 2.00	90.7 ± 2.85

In samples from cows with genotype AA, titrated acidity increased by 16 °T, in samples from cows with genotype AB – by 18 °T, in samples from cattle with genotype BB – by 17.4 °T. At the same time, the average value of this indicator at the beginning of the studies and after three days was higher in samples of cattle with genotype AA. On the seventh day, samples of cattle with genotype BB had higher values, on the tenth day – samples of cattle with genotype AA and on the fourteenth – cattle with genotype AB. No statistically significant difference was found.

The level of active acidity also had certain differences by groups of studied samples. Active acidity decreased with increasing storage time. Thus, in samples of cattle with genotype AA it decreased by 0.62, in samples of cattle with genotype AB and BB – by 0.29 (Table 6).

According to the results, it can be concluded that the relative viscosity of fermented clots decreases with storage time.

Table 6

Change in active acidity in the studied fermented samples during storage, pH

Groups by genotype	Storage duration, days				
	0	3	7	10	14
AA	4.59 ± 0.006	4.59 ± 0.006	4.46 ± 0.009	4.42 ± 0.003	3.97 ± 0.327
AB	4.59 ± 0.030	4.59 ± 0.030	4.46 ± 0.027	4.36 ± 0.012	4.30 ± 0.041
BB	4.65 ± 0.035	4.65 ± 0.035	4.49 ± 0.023	4.44 ± 0.009	4.36 ± 0.049

The greatest intensity of the decrease is observed in samples from cows with the AB genotype – by 12%. Over the entire observation period, the greatest value of the studied characteristic is characteristic of samples of cattle with the BB genotype. During storage, the taste of the studied samples changes (becomes sourer), which occurs due to a change in acidity. There are no changes in smell. When characterizing the consistency of the studied sample, it is necessary to note its density with a slight release of whey. The consistency after mixing was homogeneous (Table 7).

Table 7

Change in relative viscosity of fermented samples during storage, s

Groups by genotype	Storage duration, days				
	0	3	7	10	14
AA	27.0 ± 0.57	25.7 ± 1.45	26.3 ± 0.88	27.7 ± 1.45	25.0 ± 1.73
AB	28.3 ± 0.33	25.7 ± 2.85	27.3 ± 2.91	27.0 ± 4.36	25.0 ± 3.60
BB	28.3 ± 0.88	28.3 ± 0.88	28.7 ± 1.33	29.0 ± 2.08	26.0 ± 1.00

A low degree of syneresis was found in the studied samples. Samples of clots obtained from milk of cattle with the BB genotype were distinguished by a higher average number of viable lactococcal cells over the entire period of the studies. With increasing storage time, their number increased (Table 8).

The most intensive growth of lactococcal cells is characteristic of samples from cows with the AB genotype – by 7%.

Table 8

Change in the number of viable lactococcal cells during storage, CFU/cm^3

Groups by genotype	Storage duration, days				
	0	3	7	10	14
AA	$8.87 \pm 0.376 \times 10^9$	$9.00 \pm 0.321 \times 10^9$	$9.53 \pm 0.203 \times 10^9$	$9.87 \pm 0.120 \times 10^9$	$9.13 \pm 0.120 \times 10^7$
AB	$8.97 \pm 0.233 \times 10^9$	$9.40 \pm 0.416 \times 10^9$	$9.83 \pm 0.437 \times 10^9$	$10.4 \pm 0.384 \times 10^9$	$9.57 \pm 0.371 \times 10^7$
BB	$9.47 \pm 0.441 \times 10^9$	$9.80 \pm 0.361 \times 10^9$	$10.4 \pm 0.393 \times 10^9$	$10.6 \pm 0.400 \times 10^9$	$9.97 \pm 0.536 \times 10^7$

6. Discussion of results based on determining the dependence of the technological process on the protein composition of raw milk

The increased interest of breeders in using the achievements of molecular genetics makes it possible to create dairy herds of cattle with an increased protein content in milk and obtain milk with an increased cheese yield during its processing. For this purpose, breeding programs for obtaining cattle with the BB genotype for the CSN3 gene have begun to be implemented at Ukrainian farms. According to the results of our studies, it was found that no significant difference in the fat content in milk was detected in cows with different genotypes for the studied gene. The absence of such a difference does not agree with the results reported by other scientists. However, in terms of the average protein content in milk, milk samples from cows with the BB genotype prevailed over samples from cows with the AA genotype by 0.28% ($P < 0.05$) (Table 2), which to some extent corresponds to the results by other researchers [30].

The unexplored question of the influence of the CSN3 gene genotype on the characteristics of milk fermentation, which is the basis for the production of most dairy products, was studied. Milk from cows with the BB genotype for the kappa-casein gene (Table 3) is characterized by higher acidity and density, lower pH. This indicates its better technological suitability for the production of fermented milk products. This is probably due to the increased content of casein, which is responsible for the formation of a clot during fermentation. Similar results are reported in [5]. This confirms the hypothesis of the influence of the CSN3 genotype on the technological properties of milk. In contrast, paper [19] indicates the influence of different milk protein genotypes on the titrated and active acidity of the dairy product, no significant difference was established. The results given in Table 4 confirm the high technological quality of BB-genotype milk for the production of fermented products, which is consistent with the hypothesis of our study. It was found (Fig. 1) that the viscosity of fermented clots is directly proportional to the protein content in milk, which is consistent with previous studies [3].

The results of our research showed that milk obtained from cows, regardless of the genotype of the CSN3 gene, is suitable for the production of dairy products when using mesophilic lactic acid lactococcal starter. The absence of a statistically significant difference in the studied indicators between dairy raw materials from cows with different genotypes for the kappa-casein gene allows us to state that it does not affect the characteristics of milk fermentation.

The results of the study are limited to one type of starter, although other starters may react to the protein composition of milk differently, as well as to one breed, which requires continued research with the inclusion of other dairy breeds. Further development of this area of research may include other genes associated with the quantitative and qualitative characteristics of milk.

7. Conclusions

1. Based on our theoretical and experimental studies, it was found that all groups of samples of the studied milk are suitable for biotechnological treatment with mixed cultures of mesophilic lactic acid bacteria. According to the results of the study on the physicochemical properties of milk, cows with the BB genotype had higher average values of fat content – 4.57 ± 0.123%, non-fat dry residue – 8.78 ± 0.155%, and protein – 3.14 ± 0.058%, compared with cattle with the AA genotype (4.20 ± 0.206%, 8.02 ± 0.056%, 2.86 ± 0.020%, respectively). The difference in protein content was statistically significant: milk of cattle with the BB genotype exceeded milk of cows with the AA genotype by 0.28% ($p < 0.05$). Milk from cows with the BB genotype also had higher titrated acidity (21.0 ± 1.00 °T) and density (28.5 ± 0.626 °A), which exceeded the corresponding indicators in the AA group (18.3 ± 1.33 °T and 25.8 ± 0.328 °A). At the same time, active acidity (pH) was slightly higher in cows with the AA genotype – 6.63 ± 0.020, but this difference was not statistically significant.

2. Production of fermented milk products from the studied milk using *L. Lactis ssp* cultures can be implemented according to the general technological scheme of production and does not require adjustment of the values of technological parameters. The results of our studies showed that fermented milk raw materials from cows with different CSN3 genotypes had similar quality characteristics, corresponding to the parameters of products fermented with mesophilic lactic acid lactococci. After 10 hours of fermentation, the average titrated acidity of all samples was approximately 74 °T, pH – about 4.6. The highest relative viscosity was observed in the curd from the BB genotype milk (28.3 ± 0.88 s), which correlates with a higher protein content ($r = 0.79$; $p < 0.001$). During 14 days of storage, a gradual increase in titrated acidity (up to 91–92 °T) and a decrease in pH (up to 3.97–4.36) were noted, which caused an increase in the acidity of the product. The smallest decrease in viscosity was observed in samples of the BB genotype, where it remained the highest – 26.0 ± 1.00 s on the 14th day. The consistency of all samples was dense, homogeneous after mixing, with insignificant whey release. In samples of the BB genotype, the largest number of viable lactococcal cells was also recorded throughout the entire observation period – up to 10.6×10^9 CFU/cm^3. At the same time, the CSN3 genotype had no statistically significant effect on the growth of lactococci or acidity stability.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

Funding

The study was conducted without financial support.

Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

References

1. Sakihara, T., Otsuji, K., Arakaki, Y., Hamada, K., Sugiura, S., Ito, K. (2022). Early Discontinuation of Cow's Milk Protein Ingestion Is Associated with the Development of Cow's Milk Allergy. The Journal of Allergy and Clinical Immunology: In Practice, 10 (1), 172–179. https://doi.org/10.1016/j.jaip.2021.07.053
2. Giannetti, A., Toschi Vespasiani, G., Ricci, G., Miniaci, A., di Palmo, E., Pession, A. (2021). Cow's Milk Protein Allergy as a Model of Food Allergies. Nutrients, 13 (5), 1525. https://doi.org/10.3390/nu13051525
3. Čítek, J., Brzáková, M., Hanusová, L., Hanuš, O., Večerek, L., Samková, E. et al. (2020). Technological properties of cow's milk: correlations with milk composition, effect of interactions of genes and other factors. Czech Journal of Animal Science, 65 (1), 13–22. https://doi.org/10.17221/150/2019-cjas
4. Sokoliuk, V. M., Dukhnytsky, V. B., Krupelnytsky, T. V., Ligomina, I. P., Revunets, A. S., Prus, V. M. (2022). Influence of technological factors on milk quality indicators. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 24 (105), 37–43. https://doi.org/10.32718/nvlvet10506
5. Giribaldi, M., Lamberti, C., Cirrincione, S., Giuffrida, M. G., Cavallarin, L. (2022). A2 Milk and BCM-7 Peptide as Emerging Parameters of Milk Quality. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.842375
6. Plivachuk, O. P., Dyman, T. M. (2018). Interdependence of complex genotypes of alfa-lactalbumin and beta-lactoglobulin with composition and technological properties of milk of ukrainian black-and-white dairy cattle. Animal Breeding and Genetics, 51, 124–131. https://doi.org/10.31073/abg.51.17
7. Manguy, J., Shields, D. C. (2019). Implications of kappa-casein evolutionary diversity for the self-assembly and aggregation of casein micelles. Royal Society Open Science, 6 (10), 190939. https://doi.org/10.1098/rsos.190939
8. Khastayeva, A. Zh., Mamayeva, L. A., Abylgazinova, A. T., Zhamurova, V. S., Karimov, N. Zh., Muratbekova, K. M. (2021). RETRACTED ARTICLE: Influence of the kappa casein genotype on the technological properties of cow milk of Simmental and Alatau breeds. Functional & Integrative Genomics, 21 (2), 231–238. https://doi.org/10.1007/s10142-021-00772-1
9. Bonfatti, V., Di Martino, G., Cecchinato, A., Vicario, D., Carnier, P. (2010). Effects of β-κ-casein (CSN2-CSN3) haplotypes and β-lactoglobulin (BLG) genotypes on milk production traits and detailed protein composition of individual milk of Simmental cows. Journal of Dairy Science, 93 (8), 3797–3808. https://doi.org/10.3168/jds.2009-2778
10. Bonfatti, V., Chiarot, G., Carnier, P. (2014). Glycosylation of κ-casein: Genetic and nongenetic variation and effects on rennet coagulation properties of milk. Journal of Dairy Science, 97 (4), 1961–1969. https://doi.org/10.3168/jds.2013-7418
11. Kyselová, J., Ječmínková, K., Matějíčková, J., Hanuš, O., Kott, T., Štípková, M., Krejčová, M. (2019). Physiochemical characteristics and fermentation ability of milk from Czech Fleckvieh cows are related to genetic polymorphisms of β-casein, κ-casein, and β-lactoglobulin. Asian-Australasian Journal of Animal Sciences, 32 (1), 14–22. https://doi.org/10.5713/ajas.17.0924
12. Abeykoon, C. D., Rathnayake, R. M. C., Johansson, M., Silva, G. L. L. P., Ranadheera, C. S., Lundh, Å., Vidanarachchi, J. K. (2016). Milk Coagulation Properties and Milk Protein Genetic Variants of Three Cattle Breeds/Types in Sri Lanka. Procedia Food Science, 6, 348–351. https://doi.org/10.1016/j.profoo.2016.02.070
13. Gustavsson, F., Buitenhuis, A. J., Johansson, M., Bertelsen, H. P., Glantz, M., Poulsen, N. A. et al. (2014). Effects of breed and casein genetic variants on protein profile in milk from Swedish Red, Danish Holstein, and Danish Jersey cows. Journal of Dairy Science, 97 (6), 3866–3877. https://doi.org/10.3168/jds.2013-7312
14. Bijl, E., van Valenberg, H., Sikkes, S., Jumelet, S., Sala, G., Olieman, K. et al. (2014). Chymosin-induced hydrolysis of caseins: Influence of degree of phosphorylation of alpha-s1-casein and genetic variants of beta-casein. International Dairy Journal, 39 (2), 215–221. https://doi.org/10.1016/j.idairyj.2014.07.005
15. Sun, Y., Ding, Y., Liu, B., Guo, J., Su, Y., Yang, X. et al. (2024). Recent advances in the bovine β-casein gene mutants on functional characteristics and nutritional health of dairy products: Status, challenges, and prospects. Food Chemistry, 443, 138510. https://doi.org/10.1016/j.foodchem.2024.138510
16. Bolgova, N. V., Huba, S. O., Sokolenko, V. V., Mazhara, A. K. (2023). Study of the influence of vitamins on the fermentation process in the production of yogurt. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 25 (100), 43–46. https://doi.org/10.32718/nvlvet-f10007

17. Bintsis, T., Papademas, P. (2022). The Evolution of Fermented Milks, from Artisanal to Industrial Products: A Critical Review. Fermentation, 8 (12), 679. https://doi.org/10.3390/fermentation8120679
18. Samilyk, M., Bolgova, N., Samokhina, E., Cherniavska, T., Kharchenko, S. (2024). Use of hop extract in the biotechnology of kefir beverage. Scientific Horizons, 27 (3), 97–106. https://doi.org/10.48077/scihor3.2024.97
19. Wang, X., Yu, Z., Zhao, X., Han, R., Huang, D., Yang, Y., Cheng, G. (2020). Comparative proteomic characterization of bovine milk containing β-casein variants A1A1 and A2A2, and their heterozygote A1A2. Journal of the Science of Food and Agriculture, 101 (2), 718–725. https://doi.org/10.1002/jsfa.10684
20. Liu, T., Li, Y., Yang, Y., Yi, H., Zhang, L., He, G. (2020). The influence of different lactic acid bacteria on sourdough flavor and a deep insight into sourdough fermentation through RNA sequencing. Food Chemistry, 307, 125529. https://doi.org/10.1016/j.foodchem.2019.125529
21. Romanchuk, I., Minorova, A., Rudakova, T., Moiseeva, L. (2020). Regularities of lactose hydrolysis in dairy raw materials. Food Resources, 8 (14), 165–174. Available at: https://iprjournal.kyiv.ua/index.php/pr/article/view/55
22. Gao, D., Helikh, A., Duan, Z. (2021). Functional properties of four kinds of oilseed protein isolates. Journal of Chemistry and Technologies, 29 (1), 155–163. https://doi.org/10.15421/082116
23. Kieliszek, M., Pobiega, K., Piwowarek, K., Kot, A. M. (2021). Characteristics of the Proteolytic Enzymes Produced by Lactic Acid Bacteria. Molecules, 26 (7), 1858. https://doi.org/10.3390/molecules26071858
24. Cheng, T., Wang, L., Guo, Z., Li, B. (2022). Technological characterization and antibacterial activity of Lactococcus lactis subsp. cremoris strains for potential use as starter culture for cheddar cheese manufacture. Food Science and Technology, 42. https://doi.org/10.1590/fst.13022
25. Thum, C., Roy, N. C., Everett, D. W., McNabb, W. C. (2021). Variation in milk fat globule size and composition: A source of bioactives for human health. Critical Reviews in Food Science and Nutrition, 63 (1), 87–113. https://doi.org/10.1080/10408398.2021.1944049
26. Ladyka, V., Drevytska, T., Pavlenko, J., Skliarenko, Y., Lahuta, T., Drevytskyi, O., Dosenko, V. (2022). Evaluation of cow genotypes by kappa-casein of dairy breeds. Acta Fytotechnica et Zootechnica, 25 (1). https://doi.org/10.15414/afz.2022.25.01.1-6
27. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. Available at: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng
28. Nakaz No. 249 vid 01.03.2012 Pro zatverdzhennia Poriadku provedennia naukovymy ustanovamy doslidiv, eksperymentiv na tvarynakh. Available at: https://zakononline.com.ua/documents/show/329995___330060
29. Teliuk, P. A. (2021). Vplyv natriu, kaliu ta kaltsiu na rozvytok Lactococcus lactis. Kyiv. Available at: https://er.nau.edu.ua/items/4e03963d-1bb0-44f0-a01c-d9fa0b65c93f
30. Miluchová, M., Gábor, M., Candrák, J., Trakovická, A., Candráková, K. (2018). Association of HindIII-polymorphism in kappa-casein gene with milk, fat and protein yield in holstein cattle*. Acta Biochimica Polonica, 65 (3). https://doi.org/10.18388/abp.2017_2313