



Volodymyr Ladyka,  
Tetiana Synenko,  
Natalia Bolgova,  
Yuriy Skliarenko,  
Viktoria Vechorka

## DETERMINATION OF THE INFLUENCE OF RAW MILK $\beta$ -CASEIN POLYMORPHISM ON THE EFFICIENCY OF MAKING COTTAGE CHEESE

The positive functional features of A2 milk and the increase in the percentage of animals with the A2A2 genotype will contribute to expanding the choice of dairy products, in particular, cottage cheese. It is expected that determining the influence of the protein composition of raw milk on the quality and yield of cheese will allow for effective selection of dairy breeds of cows. The object of the study is the technological process of producing cottage cheese, produced by the classical acid method of coagulation of milk proteins from cows with different  $\beta$ -casein genotypes (A1A1, A1A2, A2A2). Subject of the study: physical and chemical characteristics of raw milk (A1A1, A1A2, A2A2); yield and quality of cottage cheese. It was experimentally established that the milk samples have a typical composition and comply with DSTU 3662:2018. The average dry matter content in milk from cows with the A1A1 genotype was 12.73%, with the protein-to-fat ratio varying within 0.76–0.83. In raw material samples from animals with the A1A2 genotype, the average dry matter content was 12.72%, and the protein-to-fat ratio was 0.66–0.68. For milk from cows with the A2A2 genotype, the average dry matter content was 13.14%, and the protein-to-fat ratio was in the range of 0.62–0.82. A study of the quality indicators of cottage cheese samples showed that the genetic variation of  $\beta$ -casein does not affect the sensory properties of the final product. The moisture, protein, and fat contents in cheese from milk from cows with the A1A1 genotype were on average 72.27%, 9.77%, and 15.47%, respectively. In samples of cheeses from cows' milk with A1A2 genotype, the average moisture content was 67.17%, protein – 18.30%, fat – 14.37%. For cheeses from cows' milk with genotype A2A2, the average moisture content was 67.47%, protein – 15.30%, fat – 15.40%. It was found that the efficiency of cheese production from cows' milk with A2A2 genotype is the highest and on average is 141.26%, which exceeds similar indicators for A1A1 milk by 13.18% and A1A2 by 2.21%.

**Keywords:** cottage cheese, cheese yield, quality,  $\beta$ -casein, A2 milk, raw milk.

Received: 07.02.2025

Received in revised form: 31.03.2025

Accepted: 22.04.2025

Published: 09.05.2025

© The Author(s) 2025

This is an open access article

under the Creative Commons CC BY license

<https://creativecommons.org/licenses/by/4.0/>

### How to cite

Ladyka, V., Synenko, T., Bolgova, N., Skliarenko, Y., Vechorka, V. (2025). Determination of the influence of raw milk  $\beta$ -casein polymorphism on the efficiency of making cottage cheese. *Technology Audit and Production Reserves*, 3 (3 (83)), 56–62. <https://doi.org/10.15587/2706-5448.2025.328936>

### 1. Introduction

Cottage cheese is an integral part of human nutrition, characterized by high nutritional and biological value. First of all, the value of cottage cheese is due to its high protein content (up to 16%) compared to milk (on average 3.5%) [1].

The chemical composition of raw milk is one of the main factors affecting the production of dairy products, including cottage cheese. Since milk fat and protein are the main ingredients of cheese, the quality of the product depends primarily on their concentration in milk.

The main factor determining the quality of milk and its curdability is the protein content. Raw milk with a high protein content, especially casein, provides a high yield and good quality of cheese products. Fat content is also important, but its comparison with the protein or casein content in milk is especially important. The generally accepted curdability of milk is a protein-to-fat ratio of 0.64 to 0.72. Increasing the protein to fat ratio in milk significantly increases the protein, calcium, and phosphorus content of cheese, and also has a significant impact on reducing the moisture and fat content in dry matter. Thus, the qualitative composition of protein and fat in milk significantly affects the chemical composition of cheese and its yield [2].

Variations in the qualitative and quantitative composition of raw milk depend on many factors, including the type and breed of animal, the stage of lactation. An important breeding trait that affects the technical properties of milk is the  $\beta$ -casein protein composition [3].

The dairy market is actively developing. One of the trends in the dairy industry, which is clearly visible in both the production and scientific spheres, is the differentiation of milk types and attention to the features of using A2 milk [4].

The consumption of A2 milk is also gaining popularity among the population, which, in their opinion, has a preventive effect on a number of diseases, including cardiovascular pathologies, diabetes, and other disorders [5].

Recent studies show that milk with the A2  $\beta$ -casein genotype has different technological properties, in particular, in the production of hard cheese, longer coagulation, in some cases greater curd hardness and cheese yield than A1 milk [6]. Similar results were obtained in the production of soft cheeses, in particular during the study of different methods of protein coagulation in milk characterized by different  $\beta$ -casein genotypes [7].

However, the scientific community has not fully substantiated the feasibility of using A2 milk in the production of fermented dairy products, in particular cottage cheese.

Therefore, in order to promote a rational technological approach to A2 milk and its processing, it is important to expand the study of the influence of the polymorphism of raw milk  $\beta$ -casein on cheese characteristics, in particular, on the production of cottage cheeses.

In cheesemaking,  $\beta$ -casein plays an important role due to its positive correlation with the actual cheese yield [8], and its share in the total protein composition of cows' milk is over 35% [9]. Genetic analysis of dairy cows revealed the presence of 13 genetic variants of  $\beta$ -casein: A1, A2, A3, B, C, D, E, F, G, H1, H2, I and J [10].

The A1 and A2  $\beta$ -casein genotypes are the most common among dairy cattle and differ in amino acid at position 67 (His in A1 or Pro in A2) [11]. The concentrations of A1 and A2  $\beta$ -casein vary depending on the breed of cattle, which can produce A1A1, A2A2 or A1A2 milk [12].

Genetic polymorphisms of  $\beta$ -casein have attracted considerable attention due to their potential impact on the composition and technological properties of milk, and its impact on human health [13].

In [14], it was reported that the difference between A1 and A2  $\beta$ -casein lies in the difference in the amino acid residue of the CSN2 gene (His67 in  $\beta$ -casein A1, Pro67 in  $\beta$ -casein A2). This difference affects the human body differently. In particular, the authors claim that they form different structural units of enzymatic cleavage during digestion [15]. The scientific community also claims that A2 milk is more easily digested, does not cause inflammation and discomfort in the gastrointestinal tract, unlike A1 milk [16]. However, the impact of  $\beta$ -casein genotype variation on the technical properties of raw milk is not considered.

The interest of the dairy industry and the need to develop sound technological solutions for processing A2 milk is confirmed by the increasing proportion of cows with the A2 genotype and the narrow range of available A2 milk products (mainly drinking milk) [17].

The analysis of information sources showed that the issue of using milk with the A2  $\beta$ -casein genotype in the production of fermented milk products, in particular cottage cheeses, is insufficiently considered. Therefore, this study on determining the technological properties of A2 milk is promising for understanding the potential consequences of using this raw material in the technology of fermented milk products.

In [18], it was investigated that the type of  $\beta$ -casein genotype affects the gel strength, water retention capacity in the production of fermented milk products such as yogurt. Studies have shown that genetic variants of  $\beta$ -casein play a crucial role in determining these functional properties, thereby affecting the texture and sensory characteristics of fermented milk products. However, the disadvantage of this work is the narrow range of products studied, which does not allow to fully state the technological properties of A2 milk.

In [19], it is shown that the cheese yield indicator is important for studies aimed at verifying the genetic affiliation of dairy cows to the cheese industry with ensuring a high rate of profitability of production. Since the daily cheese yield, expressed in kilograms of cheese produced, is the ultimate production goal of most dairy enterprises. The authors aimed to develop a model of the cheese production process with cheese yield measurement taking into account the chemical composition of the raw milk, the nutrient recovery index, and the influence of genetic variations in dairy cattle. It was shown that milk with A1A2 and A2A2  $\beta$ -casein genotypes coagulated worse with the formation of a weak clot compared to A1A1 milk. The consequence may be a lower cheese yield during production. Accordingly, genetic variations in protein composition can be used in breeding programs aimed at improving the rennet properties of milk and cheese yield or obtaining milk with an increased content of specific protein fractions. However, a disadvantage of this work is that the study considers the technology of hard cheeses, which is significantly different from the technology of cottage cheeses.

All this indicates the need for research to study the combined effect of A1/A2  $\beta$ -casein polymorphism and the chemical composition of raw milk in the production of cottage cheese made by traditional methods.

The aim of this research is to assess the effect of  $\beta$ -casein polymorphism of raw milk on the efficiency of cottage cheese production. This will allow for the selection of dairy cow breeds based on the protein composition suitable for cheese production.

## 2. Materials and Methods

### 2.1. The object and hypothesis of research

The object of research is the technological process of producing cottage cheese, produced by the classical acid method of coagulation of milk proteins from cows with different  $\beta$ -casein genotypes (A1A1, A1A2, A2A2).

Subject of the study: physical, chemical characteristics of raw milk from cows with different  $\beta$ -casein genotypes (A1A1, A1A2, A2A2); yield of cottage cheese made by the traditional acid method of protein coagulation, as well as its qualitative characteristics.

Hypothesis of research: the technological properties of raw milk are influenced by numerous factors, in particular genetic modifications of proteins. The positive functional properties of A2 milk, an increase in the proportion of cows with the A2A2 genotype contribute to the expansion of the range of dairy products, in particular cottage cheese. It is expected that determining the influence of the protein composition of raw milk on the quality and yield of cottage cheese will allow for effective selection of dairy cow breeds.

### 2.2. Raw materials and finished products used in the experiment

A herd of Ukrainian black-and-white dairy cattle kept on a farm in Sumy district (Ukraine) was selected for the study. Raw milk samples were taken in March-April 2024. As part of the experiment, 10 kg of morning milk was taken from nine cows with different  $\beta$ -casein genotypes (A1A1, A1A2 and A2A2). Immediately after milking, the samples were brought to the university laboratory, immediately cooled in cold water to a temperature of  $(4 \pm 2)^\circ\text{C}$  and kept in a refrigerator ( $(4 \pm 2)^\circ\text{C}$ ) for no longer than 6 hours.

Samples of cottage cheese for the study were obtained from whole milk by the method of acid coagulation of proteins according to classical technology and DSTU 4554:2006 "Cottage cheese. Technical conditions." Nine variants of cheese were simultaneously produced from milk of different genotypes. The test samples of cheeses were labeled as K1-K9 according to the sample number of the raw milk.

10 kg of raw milk was used to produce each sample of cheese. The processes of pasteurization, fermentation and subsequent formation of cheese grains were performed in laboratory conditions.

The technological cycle of cottage cheese production consisted of the following stages: removal of mechanical impurities from milk, temperature treatment  $(78 \pm 2)^\circ\text{C}$  with a holding time of 20–30 seconds. The milk mass cooled to  $(35 \pm 2)^\circ\text{C}$  was inoculated with direct-introduction bacterial starter cultures in the amount recommended by the manufacturer (2.8–4.0 g per 100 kg of milk mixture). The starter preparation included a collection of bacteria of various species: *Lactobacillus lactis*; *Lactococcus lactis* subsp. *cremoris*; *Lactobacillus bulgaricus*; *Streptococcus thermophilus* (mesophilic-thermophilic starter culture XMT-3, manufacturer Chr. Hansen, Denmark). After the introduction of the bacterial complex, the milk mass was thoroughly mixed for 10–12 minutes, after which it was left to form a clot. Protein coagulation took place at a temperature of  $(35 \pm 2)^\circ\text{C}$  until a pH of 4.6–4.7 was reached, which lasted 4.0–4.5 hours. The next stage was cutting the formed clot into fragments measuring  $2 \times 2 \times 2$  cm, which took 5–7 minutes. Next, the mixture was kept for 30–40 minutes, contributing to an increase in acidity and activation of the whey separation process. After that, 40–60% of the whey was removed, and the curd was subjected to gradual heating to  $(38 \pm 1)^\circ\text{C}$  with a holding time of 15–20 minutes for further acidification and acceleration of whey separation. The final stage was the self-pressing of the curd, which contributed to the final

removal of whey and the formation of optimal moisture content of the cottage cheese. The cheese mass was distributed into lamsan bags, filled to three quarters of the volume, and left for self-pressing for 2–3 hours. After that, the finished cheese was cooled to  $(8 \pm 2)^\circ\text{C}$ , packed in plastic containers and stored until analysis for no longer than 5 days at a temperature of  $(6 \pm 2)^\circ\text{C}$ .

### 2.3. Methodology for determining sample quality indicators

The quality assessment of selected samples of milk and cottage cheese was carried out in accordance with generally accepted analysis methods.

The determination of quality parameters of dairy raw materials was carried out in accordance with DSTU 3662:2018, and the quality control of cottage cheese was carried out in accordance with DSTU 4554:2006.

The density of milk was determined by the aerometric method, guided by the standards of DSTU 6082:2009. The acidity (pH) indicators in dairy raw materials and cottage cheese were determined by the potentiometric method in accordance with the regulations of DSTU 8550:2015.

The mass fraction of dry matter in the studied samples was estimated by drying to constant mass in accordance with DSTU 8552:2015. To determine the mass fraction of protein, the Kjeldahl method was used, guided by the standards DSTU ISO 8968-1:2005 and DSTU 5038:2008. The mass fraction of fat was determined by the acid method (Gerber method) in accordance with DSTU ISO 2446:2019.

The evaluation of the organoleptic quality indicators of cottage cheese was carried out in accordance with the provisions of DSTU 4554:2006, taking into account the recommendations of the international standard ISO 22935-2:2023.

To conduct an organoleptic analysis of cottage cheese by such indicators as appearance, consistency, color, taste and smell, an expert commission was formed, which included 15 experts from the Faculty of Food Technologies of Sumy National Agrarian University.

Experts were previously trained to familiarize themselves with the method of conducting organoleptic analysis of cottage cheese. Specific organoleptic quality indicators of cottage cheese were first determined for experts using a reference sensory profile that meets the requirements of DSTU 4554:2006. According to the standard, the appearance and consistency of cottage cheese are defined as soft, smearable or crumbly, slight graininess is allowed and partial separation of whey is possible. The color should be white or with a light cream tint, evenly distributed throughout the mass of the product. The taste and aroma should correspond to the typical characteristics of cottage cheese of this type, without foreign smells or aftertastes. At the next stage, experts assessed the organoleptic quality indicators of cottage cheese using a 5-point scale. In this scoring system, the value 1 corresponds to the level of "very bad", and 5 – "excellent". The average values of the scoring results were interpreted in the form of a profilogram.

Also, the expert commission evaluated the organoleptic quality indicators of cottage cheese using the descriptive method. The results obtained were entered into the tasting sheet, noting the recorded specific descriptors of appearance, consistency, color, taste and smell.

All analytical studies were carried out with three repetitions of measurements. The results obtained are given in SI units.

The efficiency of the cottage cheese production process was assessed according to the recommendations given in the source [20], by analyzing the recovery coefficient of milk components in the cheese mass and comparing the actual product yield with theoretical calculations.

The recovery of milk components (protein and fat) in the final product was calculated by the formula

$$R_{protein/fat} = \frac{(weight\ of\ milk_{protein/fat} - weight\ of\ whey_{protein/fat})}{weight\ of\ milk_{protein/fat}} \cdot 100\%, \quad (1)$$

where  $R_{protein/fat}$  – recovery of milk components, namely protein and fat, in cheese %;  $weight\ of\ milk_{protein/fat}$  – weight of protein and fat in milk, g;  $weight\ of\ whey_{protein/fat}$  – weight of protein and fat in whey, g.

The efficiency of cheese production was calculated by the formula

$$E = \frac{C}{Th_c} \cdot 100\%, \quad (2)$$

where  $E$  – cheese production efficiency, %;  $C$  – experimental cheese yield, %;  $Th_c$  – theoretical cheese yield, %.

The experimental cheese yield from the studied milk samples was calculated by the formula

$$C = \frac{m_{cheese}}{m_{milk}} \cdot 100\%, \quad (3)$$

where  $C$  – cheese yield, %;  $m_{cheese}$  – mass of fresh cheese, kg;  $m_{milk}$  – mass of milk, kg.

The theoretical cheese yield was calculated using the formula

$$Th_c = \frac{(0.93 \cdot \% fat + \% protein - 0.1) \cdot 1.09}{(100 - \% W)} \cdot \frac{100}{100}, \quad (4)$$

where  $Th_c$  – theoretical cheese yield; % fat, % protein – mass fraction of fat, protein in milk, %; %  $W$  – mass fraction of moisture in cheese, %.

The obtained experimental data were processed using computer technology using Excel software from the Microsoft Office 2016 package (Microsoft Corporation, Washington, USA). The probability of statistical error ( $p$ ) did not exceed 0.05, which confirms the high level of accuracy of the obtained results ( $p > 0.95$ ).

## 3. Results and Discussion

### 3.1. Results of studies of physicochemical parameters of raw milk samples

The results of physicochemical analysis of cows' milk samples taken from animals with different  $\beta$ -casein variants (A1A1, A1A2, A2A2) are given in Table 1.

Physicochemical parameters of raw milk samples with different genotypes ( $n=3, p \leq 0.05$ )						
Sample No.	$\beta$ -casein genotype	Acidity, pH units	Density, $\text{kg}/\text{m}^3$	Mass fraction of dry matter, %	Mass fraction of protein, %	Mass fraction of fat, %
1	A1A1	6.55–6.80	$\geq 1027.0$	$\geq 11.5$	3.0	3.4
		$6.61 \pm 0.01$	$1025.0 \pm 1.0$	$12.64 \pm 0.01$	$2.91 \pm 0.01$	$4.86 \pm 0.01$
		$6.63 \pm 0.01$	$1027.0 \pm 1.0$	$12.92 \pm 0.01$	$3.00 \pm 0.01$	$3.62 \pm 0.01$
2	A1A2	$6.63 \pm 0.01$	$1026.0 \pm 1.0$	$12.65 \pm 0.01$	$2.90 \pm 0.01$	$3.55 \pm 0.01$
		$6.79 \pm 0.01$	$1027.0 \pm 1.0$	$12.99 \pm 0.01$	$3.03 \pm 0.01$	$4.58 \pm 0.01$
		$6.66 \pm 0.01$	$1026.0 \pm 1.0$	$12.64 \pm 0.01$	$2.90 \pm 0.01$	$4.27 \pm 0.01$
3	A2A2	$6.76 \pm 0.01$	$1025.0 \pm 1.0$	$12.55 \pm 0.01$	$2.87 \pm 0.01$	$4.36 \pm 0.01$
		$6.67 \pm 0.01$	$1028.0 \pm 1.0$	$13.19 \pm 0.01$	$3.11 \pm 0.01$	$4.26 \pm 0.01$
		$6.67 \pm 0.01$	$1028.0 \pm 1.0$	$12.99 \pm 0.01$	$3.03 \pm 0.01$	$3.71 \pm 0.01$
4						
5						
6						
7						
8						
9						

Note: \* – data according to the standard DSTU 3662:2018 "Raw cows' milk. Technical conditions"

It was established that the studied samples have a typical composition for fresh cows' milk and are consistent with DSTU 3662:2018.

According to the data obtained, the average dry matter content in the cows' milk with the A1A1 genotype was 12.73%, while the protein-to-fat ratio varied within 0.76–0.83.

In raw material samples from animals with the A1A2 genotype, the average dry matter content was 12.72%, and the protein-to-fat ratio was 0.66–0.68.

For milk from cows with the A2A2 genotype, the average dry matter content was 13.14%, and the protein-to-fat ratio was in the range of 0.62–0.82.

### 3.2. Results of studies of the quality indicators of cottage cheese from milk with different genotypes by $\beta$ -casein

Fig. 1 presents a set of external characteristics of cottage cheese made by acid coagulation of proteins from cows' milk with different  $\beta$ -casein genotypes (A1A1, A1A2, A2A2).

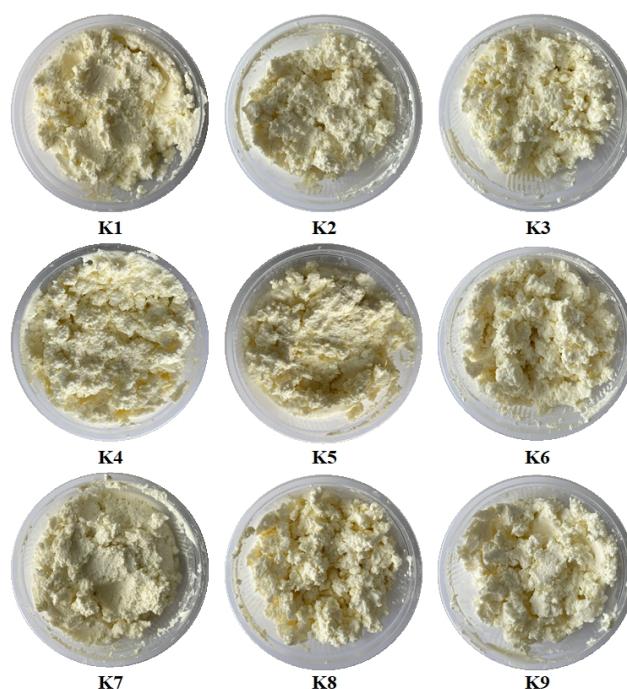


Fig. 1. Appearance of cottage cheese samples

The evaluation of organoleptic quality indicators of cottage cheese (appearance, taste, smell, consistency, color) was carried out by an expert commission, and the results are reflected in a profilogram (Fig. 2).

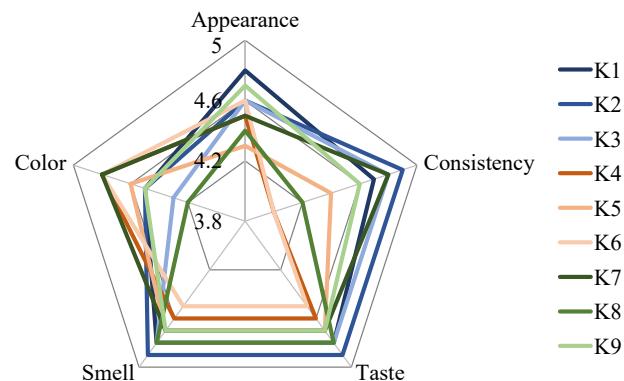


Fig. 2. Sensory profile of samples of cottage cheese from cows' milk with different genotypes by  $\beta$ -casein

According to the obtained data, samples of cottage cheese made from cows' milk with A1A1 genotype received an average score of 4.7 points for appearance, 4.8 points for taste and smell, 4.8 points for consistency, and 4.4 points for color. These samples had a moderately smeary and crumbly consistency with slight graininess, a pronounced fermented milk taste and aroma, and a uniform milky-creamy color. Samples of cheese made from cows' milk with genotype A1A2 received an average score of 4.5 points for appearance, 4.6 points for taste and smell, 4.1 points for consistency, and 4.7 points for color. They were characterized by a delicate consistency, uniform creamy color and pronounced sour milk taste. Cottage cheese from cows' milk with the A2A2 genotype had an average appearance score of 4.5 points, consistency and color – 4.5 points, taste and smell – 4.7 points. It was characterized by a moderate smear, crumbly consistency, pronounced sour milk aroma and uniform creamy color.

The results of the physical and chemical analysis of cottage cheese made by acid coagulation of proteins are given in Table 2.

It was found that the moisture, protein and fat content in cheese from cows with the A1A1 genotype was on average 72.27%, 9.77% and 15.47% respectively. In samples of cheese from cows with the A1A2 genotype, the average moisture content was 67.17%, protein – 18.30%, fat – 14.37%. For cheeses from cows with the A2A2 genotype, the average moisture content was 67.47%, protein – 15.30%, fat – 15.40%.

Table 2

Physical and chemical indicators of samples of cottage cheese from cows' milk with different genotypes by  $\beta$ -casein ( $n=3, p \leq 0.05$ )

$\beta$ -casein genotype	Cheese sample	Moisture content, %	Mass fraction of protein, %	Mass fraction of fat, %	Acidity, pH units
Norm*		65–80	$\geq 14$	2–18	4.62–4.08
A1A1	K1	70.30 $\pm$ 0.01	12.50 $\pm$ 0.01	14.70 $\pm$ 0.01	4.35 $\pm$ 0.01
	K2	74.20 $\pm$ 0.01	9.00 $\pm$ 0.01	14.30 $\pm$ 0.01	4.32 $\pm$ 0.01
	K3	72.30 $\pm$ 0.01	7.80 $\pm$ 0.01	17.40 $\pm$ 0.01	4.29 $\pm$ 0.01
A1A2	K4	66.60 $\pm$ 0.01	20.30 $\pm$ 0.01	12.60 $\pm$ 0.01	4.34 $\pm$ 0.01
	K5	68.20 $\pm$ 0.01	16.80 $\pm$ 0.01	12.50 $\pm$ 0.01	4.30 $\pm$ 0.01
	K6	66.70 $\pm$ 0.01	17.80 $\pm$ 0.01	18.00 $\pm$ 0.01	4.34 $\pm$ 0.01
A2A2	K7	69.20 $\pm$ 0.01	13.70 $\pm$ 0.01	14.60 $\pm$ 0.01	4.39 $\pm$ 0.01
	K8	66.30 $\pm$ 0.01	20.10 $\pm$ 0.01	13.10 $\pm$ 0.01	4.33 $\pm$ 0.01
	K9	66.90 $\pm$ 0.01	12.10 $\pm$ 0.01	18.50 $\pm$ 0.01	4.34 $\pm$ 0.01

Note: \* – data according to the standard DSTU 4554:2006 "Cottage cheese. Technical conditions"

### 3.3. Determination of the efficiency of cottage cheese production from cows' milk with different $\beta$ -casein genotypes

Calculations of the yield of cottage cheese (%) from cows' milk with different  $\beta$ -casein variations (A1A1, A1A2, A2A2) according to formulas (3) and (4) are presented in the histogram (Fig. 3).

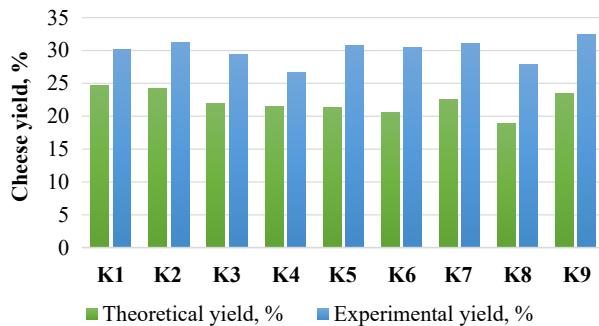


Fig. 3. Cottage cheese yields from cows' milk with different genotypes by  $\beta$ -casein

The average experimental yield of cheese was 30.23% for A1A1 milk, 29.30% for A1A2 and 30.50% for A2A2.

The results of evaluating the efficiency of cottage cheese production from the point of view of the recovery of milk components and the ratio of actual and theoretical yield are presented in Table 3.

Table 3  
Efficiency of cottage cheese production from cows' milk with different genotypes by  $\beta$ -casein

$\beta$ -casein genotype	Cheese sample	Recovery of milk components in cheese, R, %:		Cheese production efficiency, %
		protein	fat	
A1A1	K1	75.90	96.97	122.12
	K2	76.67	99.45	134.32
	K3	75.86	99.72	133.57
A1A2	K4	76.90	99.56	124.04
	K5	75.86	99.77	144.28
	K6	75.61	99.77	148.82
A2A2	K7	77.46	99.76	137.58
	K8	76.86	99.73	147.59
	K9	77.78	99.01	138.62

Analysis of the average content of milk components in the structure of cottage cheese shows that in the process of cheese production from samples of milk of the A1A1 genotype, protein losses are observed at the level of 23.86% and fat losses – 0.62%. For samples of cheeses made from milk of the A1A2 genotype, the average level of protein losses is 23.88% and fat losses – 0.30%. At the same time, in samples obtained from milk of the A2A2 genotype, protein losses are recorded at the level of 22.64% and fat losses – 0.50%.

It was established that the efficiency of cheese production from cows' milk with the A2A2 genotype is the highest and on average is 141.26%, which exceeds the similar indicators for A1A1 milk by 13.18% and A1A2 by 2.21%.

### 3.4. Discussion of the results of determining the influence of $\beta$ -casein polymorphism in raw milk on the soft cheese production

The obtained results of the physicochemical parameters of raw milk samples (Table 1) show that variations in  $\beta$ -casein do not significantly affect the acidity or density of milk. The average dry matter content in milk samples fluctuates at the level of  $(12.87 \pm 0.01)\%$  for all modifications of  $\beta$ -casein genotypes. However, it was found that the

protein content in A2A2 milk is slightly higher (on average by 0.1%) than in A1A1 and A1A2 milk. On the contrary, the fat content in A2A2 milk is lower than in A1A2, but the fat content in A1A1 milk is higher.

The obtained results are consistent with the conclusions of scientists [21–24]. It is shown that the genetic variation in  $\beta$ -casein does not affect the quality parameters of raw milk. In contrast, in [25] cows with the A2A2 genotype had a higher percentage of fat in milk than their A1A1 and A1A2 counterparts.

Calculations of the protein:fat ratio showed that milk samples with the A1A1 genotype had higher values (on average 0.73) compared to milk samples with the A1A2 genotype (0.67) and A2A2 (0.71).

The results of the sensory analysis (Fig. 1) showed that genetic variations of  $\beta$ -casein in raw milk did not significantly affect the organoleptic characteristics of cottage cheese. In all cases, the cheese produced had a moderately soft smear and a crumbly consistency, typical of this group of cheeses. The results obtained are consistent with the results described in [26]. The authors indicated that no significant sensory difference was found between the fermented samples.

Genetic variation of  $\beta$ -casein in milk significantly affected the chemical composition of cottage cheese (Table 2). In particular, cheese samples from A2A2 milk had an increased protein content (on average by 15.30%) and a significantly reduced moisture content (on average by 4.8%) compared to A1A1 milk. However, the results obtained demonstrate that all samples of cottage cheese in terms of quality indicators are consistent with DSTU 4554:2006. The protein:fat ratio in milk raw materials has a significant impact on the efficiency of cheese production. In [23] it is substantiated that the protein:fat ratio as 0.7:0.8 provides an increase in cheese yield and the value of milk component recovery in cheese. It is shown that a high fat content in milk negatively affects cheese quality (increases moisture content), but at the same time increases cheese yield. Conversely, an increase in protein content improves cheese quality but reduces its yield.

The experimental yield of cottage cheeses (Fig. 3) from A2A2 milk was higher (average value 30.50%), which is interconnected with the chemical composition (higher fat content) of the initial raw milk samples. Thus, the obtained results are consistent with the data [23].

The cheese yield per unit of processed milk is a key factor in the profitability of dairy production. And together with the recovery rate of individual milk components in the cheese mass, they determine the efficiency of the cheesemaking process [19].

In [9], it is emphasized that  $\beta$ -casein is a protein fraction of milk with a high impact on the actual yield of cheese and is associated with the recovery of protein and dry matter in the finished product.

The results of calculations of the recovery of milk components (protein and fat) in the cheese mass (Table 3) showed that protein losses are lower in the production of cottage cheese from A2A2 milk compared to A1A1 and A1A2 milk (by 1.22% and 1.24%, respectively). Fat losses are not significant in all cases (within 0.3–0.6%). The obtained data, together with data on the chemical composition of raw milk, affect the efficiency of product production. It was established that the efficiency of the production of cottage cheese from cows' milk with the A2A2 genotype is the highest and on average is 141.26%, which exceeds similar indicators for A1A1 milk by 13.18% and A1A2 by 2.21%.

Thus, the results obtained show that the polymorphism of  $\beta$ -casein of raw milk has a significant impact on the efficiency of cottage cheese production. In addition, it was noted that the cheese-making properties of A2 milk were at a high level, the actual cheese yield was higher compared to A1A1 and A1A2 milk samples.

The main limitations of research are the analysis of raw milk from commercial cattle of the Ukrainian Black and White dairy breed, kept on a farm in the Sumy district (Ukraine). Breeding, keeping and feeding practices of cattle in Ukraine (as a result, the composition of milk) may differ from practices in other countries with different climates and cultures. However, the characteristics of raw milk from the point of view

of genetic variation of milk proteins and cottage cheese made from such milk using classical technology may be applicable to other countries.

The disadvantage of this research is that the influence of  $\beta$ -casein polymorphism of raw milk on the efficiency of production of cottage cheese made from whole milk without standardization of fat content by the method of acid coagulation of proteins according to classical technology was investigated. Further research should be aimed at studying the cheese-making properties of the protein fraction of raw milk without taking into account the effect of fat content.

The conclusions obtained are of practical importance, since it can be taken into account that genetic modifications of  $\beta$ -casein in raw milk can affect the efficiency of cheese production, and therefore, the profitability of production as a whole. Particular attention should be paid to the choice of bacterial starter cultures and the method of production of cottage cheese in further research. Attention should also be paid to the choice of temperature regimes when processing raw milk, since inappropriate heat treatment can affect the protein structure and reduce the practical value of the results obtained.

The study selected cattle of the Ukrainian Black and White dairy breed, which is kept in one of the farms of the Sumy district in Ukraine. It is worth emphasizing that this region has repeatedly been subjected to enemy shelling, which is confirmed by the inclusion of the community in the List of territories where hostilities are (were) conducted or temporarily occupied by the Russian Federation (order of the Ministry of Development of Communities and Territories of Ukraine No. 376 dated 28.02.2025). Changes in animal housing conditions, limited access to high-quality feed and clean water, as well as increased stress due to military operations are key factors that negatively affect the physiological state of livestock. In particular, the inability to provide balanced nutrition, together with the deterioration of care standards, can lead to a decrease in the herd's milk productivity and a deterioration in milk quality. Additionally, stressors such as loud noises, moving animals to safe areas, or other external stimuli can affect both milk yield and milk composition.

#### 4. Conclusions

The conducted studies have established that the studied samples have a typical composition for fresh cow's milk and are consistent with DSTU 3662:2018. According to the results obtained, the average dry matter content in milk from cows with the A1A1 genotype was 12.73%, while the protein-to-fat ratio varied within 0.76–0.83. In raw material samples from animals with the A1A2 genotype, the average dry matter content was 12.72%, and the protein-to-fat ratio was 0.66–0.68. For milk from cows with the A2A2 genotype, the average dry matter content was 13.14%, and the protein-to-fat ratio was in the range of 0.62–0.82. A comprehensive study of the quality indicators of cottage cheese samples shows that the genetic variation of  $\beta$ -casein does not affect the sensory properties of the final product. However, the moisture, protein and fat content in cheese from cows with the A1A1 genotype is on average 72.27%, 9.77% and 15.47%, respectively. In samples of cheese from cows with the A1A2 genotype, the average moisture content is 67.17%, protein – 18.30%, fat – 14.37%. For cheese from cows with the A2A2 genotype, the average moisture content is 67.47%, protein – 15.30%, fat – 15.40%.

Analysis of the average milk component content in the structure of cottage cheese shows that in the process of producing cheese from samples of A1A1 genotype milk, protein losses of 23.86% and fat losses of 0.62% are observed. For cheese samples made from milk of the A1A2 genotype, the average level of protein loss is 23.88% and fat loss is 0.30%. At the same time, in samples obtained from milk of the A2A2 genotype, protein loss is recorded at the level of 22.64% and fat loss is 0.50%. It is found that the efficiency of cheese production from cows' milk with the A2A2 genotype is the highest and on average is 141.26%,

which exceeds the similar indicators for milk A1A1 by 13.18% and A1A2 by 2.21%.

#### Conflict of interest

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship or other nature, which could affect the study and its results presented in this article.

#### Financing

The study was conducted without financial support.

#### Data availability

The manuscript has no linked data.

#### Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies in the creation of the current work.

#### References

1. Bilyi, V., Merzlov, S., Narizhnyy, S., Mashkin, Y., Merzlova, G. (2022). Amino Acid Composition of Whey and Cottage Cheese Under Various Rennet Enzymes. *Scientific Horizons*, 24 (9), 19–25. [https://doi.org/10.48077/scihor24\(9\).2021.19-25](https://doi.org/10.48077/scihor24(9).2021.19-25)
2. Verdier-Metz, I., Coulon, J.-B., Pradel, P. (2001). Relationship between milk fat and protein contents and cheese yield. *Animal Research*, 50 (5), 365–371. <https://doi.org/10.1051/animres.2001138>
3. Ladyka, V., Pavlenko, Y., Sklyarenko, Y. (2021).  $\beta$ -casein gene polymorphism use in terms of brown dairy cattle preservation. *Archivos de Zootecnia*, 70 (269), 88–94. doi: <https://doi.org/10.21071/azv70i269.5422>
4. Zbik, K., Onopriuk, A., Górska-Horczyczak, E., Wierzbicka, A. (2024). Trends and Opportunities in the Dairy Industry: A2 Milk and Processing Methods. *Applied Sciences*, 14 (15), 6513. <https://doi.org/10.3390/app14156513>
5. Banerjee, S. (2018). A2 milk: the unknown story about a milk protein. *Acta Scientific Nutritional Health*, 2 (3), 28–31.
6. Ladyka, V., Bolgova, N., Synenko, T., Skliarenko, Y., Vechorka, V. (2023). Determining the influence of raw milk protein composition on the yield of cheese and its nutrient content. *Eastern-European Journal of Enterprise Technologies*, 6 (11 (126)), 33–41. <https://doi.org/10.15587/1729-4061.2023.292063>
7. Ladyka, V., Bolhova, N., Synenko, T., Skliarenko, Y., Vechorka, V. (2024). Determining the influence of raw milk  $\beta$ -casein polymorphism on the efficiency of making soft cheese. *Eastern-European Journal of Enterprise Technologies*, 5 (11 (131)), 33–42. <https://doi.org/10.15587/1729-4061.2024.311236>
8. Heck, J. M. L., Schennink, A., van Valenberg, H. J. F., Bovenhuis, H., Visker, M. H. P. W., van Arendonk, J. A. M., van Hooijdonk, A. C. M. (2009). Effects of milk protein variants on the protein composition of bovine milk. *Journal of Dairy Science*, 92 (3), 1192–1202. <https://doi.org/10.3168/jds.2008-1208>
9. Farrell, H. M., Jimenez-Flores, R., Bleck, G. T., Brown, E. M., Butler, J. E., Creamer, L. K. et al. (2004). Nomenclature of the Proteins of Cows' Milk—Sixth Revision. *Journal of Dairy Science*, 87 (6), 1641–1674. [https://doi.org/10.3168/jds.s0022-0302\(04\)73319-6](https://doi.org/10.3168/jds.s0022-0302(04)73319-6)
10. 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>
11. Brooke-Taylor, S., Dwyer, K., Woodford, K., Kost, N. (2017). Systematic Review of the Gastrointestinal Effects of A1 Compared with A2  $\beta$ -Casein. *Advances in Nutrition*, 8 (5), 739–748. <https://doi.org/10.3945/an.116.013953>
12. Kay, S.-I. S., Delgado, S., Mittal, J., Eshraghi, R. S., Mittal, R., Eshraghi, A. A. (2021). Beneficial Effects of Milk Having A2  $\beta$ -Casein Protein: Myth or Reality? *The Journal of Nutrition*, 151 (5), 1061–1072. <https://doi.org/10.1093/jn/nxaa454>
13. Vigolo, V., Franzoi, M., Cendron, F., Salvadore, G., Penasa, M., Cassandro, M., De Marchi, M. (2022). Characterization of the genetic polymorphism linked to the  $\beta$ -casein A1/A2 alleles using different molecular and biochemical methods. *Journal of Dairy Science*, 105 (11), 8946–8955. <https://doi.org/10.3168/jds.2022-22136>
14. Sumner, A., Di Frangia, F., Ajmone Marsan, P., De Noni, I., Malacarne, M. (2020). Occurrence, biological properties and potential effects on human health of  $\beta$ -casomorphin 7: Current knowledge and concerns. *Critical Reviews in Food Science and Nutrition*, 60 (21), 3705–3723. <https://doi.org/10.1080/10408398.2019.1707157>

15. Jeong, H., Park, Y.-S., Yoon, S.-S. (2023). A2 milk consumption and its health benefits: an update. *Food Science and Biotechnology*, 33 (3), 491–503. <https://doi.org/10.1007/s10068-023-01428-5>

16. Cieślinska, A., Fiedorowicz, E., Zwierzchowski, G., Kordulewska, N., Jarłowska, B., Kostyra, E. (2019). Genetic Polymorphism of  $\beta$ -Casein Gene in Polish Red Cattle – Preliminary Study of A1 and A2 Frequency in Genetic Conservation Herd. *Animals*, 9 (6), 377. <https://doi.org/10.3390/ani9060377>

17. Dantas, A., Kumar, H., Prudencio, E. S., de Avila, L. B., Orellana-Palma, P., Dosoky, N. S. et al. (2023). An approach on detection, quantification, technological properties, and trends market of A2 cow milk. *Food Research International*, 167, 112690. <https://doi.org/10.1016/j.foodres.2023.112690>

18. Gai, N., Uniacke-Lowe, T., O'Regan, J., Goulding, D. A., Kelly, A. L. (2023). Influence of  $\beta$ -casein genotype on physicochemical properties and functionality of bovine milk. *Journal of Dairy Science*, 106 (12), 8357–8367. <https://doi.org/10.3168/jds.2023-23687>

19. Cipolat-Gotet, C., Cecchinato, A., De Marchi, M., Bittante, G. (2013). Factors affecting variation of different measures of cheese yield and milk nutrient recovery from an individual model cheese-manufacturing process. *Journal of Dairy Science*, 96 (12), 7952–7965. <https://doi.org/10.3168/jds.2012-6516>

20. Stocco, G., Cipolat-Gotet, C., Gasparotto, V., Cecchinato, A., Bittante, G. (2018). Breed of cow and herd productivity affect milk nutrient recovery in curd, and cheese yield, efficiency and daily production. *Animal*, 12 (2), 434–444. <https://doi.org/10.1017/s1751731117001471>

21. Bisutti, V., Pegolo, S., Giannuzzi, D., Mota, L. F. M., Vanzin, A., Toscano, A. et al. (2022). The  $\beta$ -casein (CSN2) A2 allelic variant alters milk protein profile and slightly worsens coagulation properties in Holstein cows. *Journal of Dairy Science*, 105 (5), 3794–3809. <https://doi.org/10.3168/jds.2021-21537>

22. Niero, G., Franzoi, M., Manuelian, C. L., Visentin, G., Penasa, M., De Marchi, M. (2021). Protein profile of cow milk from multibreed herds and its relationship with milk coagulation properties. *Italian Journal of Animal Science*, 20 (1), 2232–2242. <https://doi.org/10.1080/1828051x.2021.1996288>

23. Auldist, M. J., Johnston, K. A., White, N. J., Fitzsimons, W. P., Boland, M. J. (2004). A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *Journal of Dairy Research*, 71 (1), 51–57. <https://doi.org/10.1017/s0022029903006575>

24. Vigolo, V., Franzoi, M., Penasa, M., De Marchi, M. (2022).  $\beta$ -Casein variants differently affect bulk milk mineral content, protein composition, and technological traits. *International Dairy Journal*, 124, 105221. <https://doi.org/10.1016/j.idairyj.2021.105221>

25. Marko, R., Uros, G., Branislav, V., Milan, M., Danijela, K., Vlado, T., Zoran, S. (2020). Beta-Casein Gene Polymorphism in Serbian Holstein-Friesian Cows and Its Relationship with Milk Production Traits. *Acta Veterinaria*, 70 (4), 497–510. <https://doi.org/10.2478/acve-2020-0037>

26. Ladyka, V., Bolhova, N., Huba, S., Sokolenko, V., Skliarenko, Y. (2024). Investigation of the influence of milk protein genotype on the process of fermentation of milk curds by mesophilic lactic acid streptococci. *Scientific Horizons*, 27 (8), 113–121. <https://doi.org/10.48077/scihor8.2024.113>

**Volodymyr Ladyka**, Doctor of Agricultural Sciences, Professor, Academician of National Academy of Agrarian Sciences of Ukraine, Department of Technology of Production and Processing of Animal Products and Cinology, Sumy National Agrarian University, Sumy, Ukraine, ORCID: <https://orcid.org/0000-0001-6748-7616>

✉ **Tetiana Synenko**, PhD, Department of Technology and Food Safety, Sumy National Agrarian University, Sumy, Ukraine, e-mail: [t.p.synenko@gmail.com](mailto:t.p.synenko@gmail.com), ORCID: <https://orcid.org/0000-0002-5300-5142>

**Nataliia Bolhova**, PhD, Associate Professor, Department of Food Technology and Safety, Sumy National Agrarian University, Sumy, Ukraine, ORCID: <https://orcid.org/0000-0002-0201-0769>

**Yuriy Skliarenko**, Doctor of Agricultural Sciences, Laboratory of Animal Husbandry and Fodder Production, Institute of Agriculture of the Northeast of the National Academy of Sciences, Gorden, Ukraine, ORCID: <https://orcid.org/0000-0002-6579-2382>

**Viktoria Vechorka**, Doctor of Agricultural Sciences, Associate Professor, Department of Animal Genetics, Breeding and Biotechnology, Sumy National Agrarian University, Sumy, Ukraine, ORCID: <https://orcid.org/0000-0003-4956-2074>

✉ Corresponding author