

*Cheese production is a complex process that is influenced by many factors: protein:fat ratio, acidity, and type of rennet. An option for improving the profitability of the cheese industry is the genetic selection of dairy cows to produce milk with good rennet protein coagulation. The object of the study is the technology of cheeses made from milk from cows with different  $\beta$ -casein genotypes (A1A1, A1A2, A2A2). The subject of the study is the physical-chemical parameters of milk from cows with different genotypes for  $\beta$ -casein; yield of cheese from this milk and its quality indicators. Samples of Gouda cheese were produced according to traditional technology. The research established that the quality indicators of milk samples are typical for fresh cow's milk. The content of fat, protein, and dry matter in the milk of cows with the  $\beta$ -casein genotype A2A2 were slightly higher compared to A1A1 and A1A2. The study of the quality indicators of the cheese samples showed that the type of  $\beta$ -casein did not affect the organoleptic properties of the cheese. However, according to the content of the main chemical components, cheeses made from A1A2 milk had a higher content of dry matter and protein (61,6 % and 19,2 % on average, respectively) and a lower fat content (37.2 %). The amino acid profile of cheese from milk of cows with  $\beta$ -casein A1A2 and A2A2 genotypes showed a higher total content of amino acids – 14.89 mg/g and 13.84 mg/g, respectively. Calculations of cheese yield showed that cheese yield from milk of cows with  $\beta$ -casein genotype A1A2 was higher (mean value 13.1 %) than with A1A1 and A2A2. The obtained results are of practical importance, as it is possible to take into account how changes in the  $\beta$ -casein genotype in milk can affect the yield of cheese, and therefore, the profitability of production*

**Keywords:** cheese yield, milk proteins,  $\beta$ -casein, A2 milk, nutrients

# DETERMINING THE INFLUENCE OF RAW MILK PROTEIN COMPOSITION ON THE YIELD OF CHEESE AND ITS NUTRIENT CONTENT

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## 1. Introduction

Cheese production accounts for the largest share of raw milk produced in the world (more than 75 %). The raw suitability of milk depends on the protein:fat ratio and the qualitative composition of casein. The  $\beta$ -casein composition of proteins is an important selection feature that affects the technical properties of milk [1, 2].

The growing proportion of cows with the A2A2 genotype in many countries has led to an increase in the mass production of “A2 milk” [3] and the need to expand research into the use of this milk in the production of dairy products, especially hard cheese.

Scientific research on this topic is important to understand the potential effect of  $\beta$ -casein A2 on cheesemaking and the relationship between genetic polymorphism and cheesemaking characteristics of raw materials. The results of such studies are needed in practice because they will make it possible to predict the yield of cheese from the milk of cows with different  $\beta$ -casein genotypes, to rationally use raw milk in production.

## 2. Literature review and problem statement

Paper [4] reports the results of studies into the influence of CSN2–CSN3  $\beta$ -,  $\kappa$ -casein, and  $\beta$ -lactoglobulin genotypes on indicators of milk productivity, protein fraction content and milk quality of Simmental cows. It was shown that the CSN2–CSN3 genotype plays an important role for the variation in the content of protein fractions and protein composition in milk. It is emphasized that genetic variations of the 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, the cited work did not investigate the influence of other genotypes of the main milk proteins, which also affect the indicators of milk productivity of cows. The solution to this problem is the expansion of the database of genetic types of milk proteins with their further research.

Work [5] describes the changes in the range of milk proteins, which arose as a result of recent research on milk proteins. It has been shown that  $\beta$ -casein is one of the most common fractions of milk protein and makes up to 45 % of the

total casein of cow's milk, with several genetic variants: A1, A2, A3, B, C, D, E, F, G, H1, H2, and I. However, variants A1 and A2  $\beta$ -casein are most often found in cattle populations [6].

Detailed characteristics of  $\beta$ -casein A1 and A2 genotypes are presented in works [7, 8]. It was shown that the difference in the genetic variability of  $\beta$ -casein lies in the single-nucleotide polymorphism of the CSN2 gene, which changes the coding of amino acid 67 in the polypeptide chain from proline (Pro67 for  $\beta$ -casein A2) to histidine (His67 for  $\beta$ -casein A1). However, the functional and technical properties of such milk have not been investigated.

In [9] it was shown that milk with  $\beta$ -casein A2 genotype releases a much smaller amount of bioactive opioid peptide  $\beta$ -casomorphin 7 during digestion, i.e., has a less harmful effect on human health, compared to A1 milk. The negative impact of milk with the  $\beta$ -casein A1 genotype on human health is also highlighted in works [10–12]. A relationship between the  $\beta$ -casein A1 variant of milk and various diseases such as type 1 diabetes, neurological disorders such as schizophrenia, autism, and sudden infant death syndrome has been shown. However, according to the authors of [13], there is no convincing evidence that  $\beta$ -casein A1 in milk has an adverse effect on humans. This statement is also confirmed in [14]. Due to the ambiguous results of research on the effects of A1 and A2 milk on human health, it is impossible to clearly assess the functional significance of A2 milk, so further research in this area is necessary.

Work [15] reports the results of a marketing study on consumer preferences for A2 dairy products and evaluates the effect of  $\beta$ -casein A2 on the sensory characteristics of soft cheeses. It was shown that cheeses from the milk of cows with the  $\beta$ -casein genotype A2 were characterized by a creamier texture with a delicate structure compared to cheeses from A1 milk. However, consumers did not notice significant sensory differences in the products. The authors also indicated that consumers do not know about the usefulness of A2 milk. The reason may be insufficient awareness of this issue. The solution to this problem may be the development of marketing strategies to promote the benefits associated with A2 milk.

In cheese making, the key factors affecting the profitability of production are the amount and content of protein in raw milk. Monitoring all the relationships between the quality of raw materials and cheese production, such as the yield of cheese and the preservation of milk components in the cheese mass, is an important step to determine the efficiency of the entire technological process.

In [16], the results of the study into the determination of factors affecting the coagulation properties of cow, sheep, and goat milk are given. It has been shown that the amount and ratio of milk protein fractions strongly influence the coagulation properties of milk. The authors emphasize that genetic variations in milk proteins, especially casein, affect both the amount and proportions of different proteins in milk. But the question of the influence of genetic variations of  $\beta$ -casein A1 and A2 on the coagulation properties of milk remains unsolved.

This is the approach used in work [17]. The authors investigated the potential effects of  $\beta$ -casein genotypes A1 and A2 in percentage on cheese production. It was shown that with an increase in the relative content of  $\beta$ -casein A2 (>50 % of the total volume of milk), the yield of cheese decreased significantly; samples with  $\beta$ -casein A2 content below 75 % were characterized by a high content of nutrients. Thus, it was demonstrated that the amount of A1 milk  $\geq 75$  % in the milk mixture for cheese production has a beneficial effect on increasing the profitability of production. However, the authors

did not take into account the fact that milk can come to milk processing enterprises in different quantities from cows with the specified genetic variations, which are difficult to control.

Work [18] reports the results of a study on determining the formation of a clot under the action of the rennet enzyme chymosin in the milk of cows of the Swedish red and white dairy breed. It was shown that milk from cows with  $\beta$ - $\kappa$ -casein genotypes A1A2 and A2A2 coagulated worse with the formation of a weak clot compared to A1A1 milk. The result may be a lower yield of cheese during production. Similar results are highlighted in [19]. It was shown that the milk of Holstein-Friesian cows with the  $\beta$ -casein genotype A2A2 had a lower cheese yield compared to A1A1 and A1A2. The reason for this may be poor rennet coagulation of milk with  $\beta$ -casein A2A2. An option to overcome the relevant difficulties may be selective breeding of cows with the  $\beta$ -casein A1A1/A1A2 genotype.

However, the works reviewed above do not take into account that the production of cheese, namely the rennet coagulation of milk, is a complex process that is influenced by many factors: protein:fat ratio, acidity, type of rennet enzyme, etc. An option for improving the profitability of the cheese industry is the genetic selection of dairy cows to obtain milk with good rennet protein coagulation. An indispensable characteristic is the preservation/restoration of nutrients in the finished cheese.

All this allows us to state that it is appropriate to conduct a study on determining the complex effect of  $\beta$ -casein A1/A2 polymorphism and the chemical composition of milk on the production of cheese by evaluating the yield, content of nutrients, and chemical composition of cheese.

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### 3. The aim and objectives of the study

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The purpose of this study is to determine the influence of the protein composition of raw milk on the yield of hard cheese and the content of nutrients in it. This will make it possible to selectively select dairy breeds of cows according to their protein composition suitable for cheese production.

To achieve the goal, the following tasks were set:

- to investigate the physicochemical parameters of raw milk from cows with different genotypes for  $\beta$ -casein (A1A1, A1A2, A2A2);
- to calculate and compare the yield of hard cheese from the milk of cows with different genotypes according to  $\beta$ -casein;
- to investigate the organoleptic and physical-chemical parameters of samples of hard cheeses made from the milk of cows with different  $\beta$ -casein genotypes;
- to establish the amino acid profile of hard cheeses from the milk of cows with different genotypes according to  $\beta$ -casein.

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### 4. The study materials and methods

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#### 4.1. The object and hypothesis of the study

The object of our research is the technology of hard cheeses made from raw milk from cows with different  $\beta$ -casein genotypes (A1A1, A1A2, A2A2).

Research subjects: physicochemical parameters of raw milk from cows with different genotypes for  $\beta$ -casein (A1A1, A1A2, A2A2); yield of hard cheese from this milk and its quality indicators.

Research hypothesis assumes that the technological properties of raw milk depend on several factors, including genetic

variations of proteins. The positive functional properties of A2 milk, the increase in the proportion of cows with the A2A2 genotype determine the expansion of the assortment of dairy products, in particular cheeses. It is assumed that the study into the influence of the protein composition of raw milk on the yield of hard cheese and the content of nutrients in it will make it possible to selectively select dairy breeds of cows suitable for the production of cheese according to their protein composition.

#### 4. 2. Researched raw materials and finished products used in the experiment

A commercial herd of the Ukrainian black-spotted dairy breed in the Sumy region was chosen for the study. In this study, 10 kg of milk was collected during morning milking from nine cows with different  $\beta$ -casein genotypes (A1A1, A1A2 and A2A2). The investigated samples of hard cheese "Gouda" were produced from whole milk according to traditional technology in accordance with the requirements of DSTU 6003:2008 "Hard cheeses. General technical conditions". Nine samples of cheese from cow's milk of different genotypes were prepared in parallel.

10 kg of raw milk was used to make cheese. Pasteurization, leavening, fermentation, and subsequent formation of cheese grains were carried out at a laboratory cheese factory.

The process of manufacturing samples of hard cheese "Gouda" under laboratory conditions consists of the following stages: milk purified from mechanical impurities is pasteurized at a temperature of (72–75) °C with a holding time of 20 seconds. In milk cooled to a temperature of (36±1) °C, dry leaven of direct application is added in the amount recommended by the manufacturer. Sourdough consists of mixed cultures of microorganisms – *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis var. Diacetylactis* ("Dalton", Italy). Next, a calcium chloride solution (at the rate of 20–40 g per 100 kg of mixture) and rennet enzyme "Albamax 600" (100 % chymosin) are added (Caglifacio Clerici, Italy). The mixture is fermented at a temperature of (36±1) °C until a dense clot is formed. Next, the clot is cut, the cheese grain is processed (kneading, second heating at a temperature of (39±1) °C, drying of the cheese grain). The formed cheese heads are pressed, then salted in brine (salt concentration, 18–20 %; temperature, 10–14 °C). The cheese is dried at a temperature of (10–12) °C for 4 hours. The dried cheese heads are covered with a protective coating "Polisved" and sent for ripening at a temperature of (12±2) °C for 30 days. Ripened cheese is stored in a refrigerator at a temperature of (6±2) °C.

#### 4. 3. Methodology for determining the quality indicators of samples

Quality assessment of milk and cheese samples was carried out according to generally accepted procedures.

Raw milk was examined for quality indicators according to DSTU 3662:2018, cheese samples – according to DSTU 6003:2008.

The density of milk was measured by the aerometric method according to DSTU 6082:2009. Acidity (pH) of milk and cheese samples was determined by the potentiometric method according to DSTU 8550:2015.

The mass fraction of dry substances in milk and cheese samples was determined by drying to a constant value of the indicator according to DSTU 8552:2015. The mass fraction of protein was determined by the Kjeldahl method according to DSTU ISO 8968-1:2005, DSTU 5038:2008. The mass

fraction of fat was determined by the acid method (Gerber method) according to DSTU ISO 2446:2019.

Organoleptic indicators of cheese samples were determined according to DSTU 6003:2008, with recommendations described in the international standard ISO 22935-2:2023.

The analysis of amino acids in cheese samples was carried out by the method of ion-exchange liquid column chromatography using the automatic amino acid analyzer "T 339" (Prague, Czech Republic). The following procedure was used: a weighed sample (with a protein content of about 2 mg) is mixed to the bottom of a test tube, 0.5 ml of distilled water and 0.5 ml of concentrated hydrochloric acid are added. The tube is cooled in a mixture of dry ice with acetone or liquid nitrogen. After the contents of the test tube freeze, air is pumped out of it using a vacuum pump to prevent oxidation of amino acids as a result of hydrolysis. Then the test tube is sealed and placed for 24 hours in a thermostat with a constant temperature (106±1) °C. At the end of hydrolysis, the test tube is opened, having previously cooled to room temperature. The contents are quantitatively transferred into a glass beaker and placed in a vacuum desiccator over granulated caustic sodium. Then air is removed from the desiccator using a water pump. After drying the sample, we add 3–4 ml of deionized water to the cuvette and repeat the drying procedure. The sample prepared in this way is dissolved in 0.3N lithium citrate buffer (pH 2.2) and applied to the ion exchange column of the amino acid analyzer.

Threefold repeatability of studies was used in the conduct of research. The obtained experimental data are represented in units of the international SI system.

The yield of hard cheese from the studied milk samples was calculated according to the following formula (1):

$$B = \frac{m_{cheese}}{m_{milk}} \cdot 100 \%, \quad (1)$$

where  $B$  is the yield of cheese, %;

$m_{cheese}$  – mass of cheese (30 days after production), kg;

$m_{milk}$  – mass of milk, kg.

Mathematical and statistical treatment of our results was carried out on a computer using MS Excel 2016 software. The determined value of the reliability of the deviation ( $p$ ) does not exceed 0.05, which indicates that the value of the accuracy indicator ( $P$ ) of the results is more than 0.95.

### 5. Results of studying the dependence of the content of nutrients and the yield of hard cheese on the protein composition of raw milk

#### 5. 1. Results of investigating the physical and chemical parameters of raw milk

The results of determining the physicochemical parameters of test samples of cow's milk with different variations of  $\beta$ -casein, A1A1, A1A2, A2A2, are given in Table 1.

The results of our study into the physical and chemical indicators of milk samples are typical for fresh cow's milk and meet the requirements of DSTU 3662:2018.

According to the results, the average value of dry matter content in milk samples from cows with the A1A1 genotype is 12.54 %, while the ratio of protein to fat content is in the range of 0.61...0.73.

In milk samples from cows with the A1A2 genotype, the average value of dry matter content is 12.41 %, and the ratio of protein to fat content is within 0.69...0.8.

Table 1

Physicochemical parameters of raw milk samples with different genotypes ( $n=3, p \leq 0.05$ )

Sample No.	Genotype of $\beta$ -casein	Acidity, units pH	Density, kg/m <sup>3</sup>	Mass share of dry matter, %	Mass fraction of protein, %	Mass fraction of fat, %
1	A1A1	6.58±0.01	1026.0±1.0	12.54±0.02	2.93±0.1	4.34±0.01
2		6.55±0.01	1027.0±1.0	12.43±0.02	2.96±0.1	4.02±0.01
3		6.62±0.01	1026.0±1.0	12.65±0.02	2.85±0.1	4.66±0.01
4	A1A2	6.52±0.01	1026.0±1.0	12.47±0.02	2.95±0.1	4.26±0.01
5		6.56±0.01	1027.0±1.0	12.42±0.02	3.04±0.1	3.79±0.01
6		6.51±0.01	1027.0±1.0	12.45±0.02	2.93±0.1	3.97±0.01
7	A2A2	6.64±0.01	1026.0±1.0	12.24±0.02	2.97±0.1	4.66±0.01
8		6.65±0.01	1026.0±1.0	12.78±0.02	2.89±0.1	4.65±0.01
9		6.69±0.01	1025.0±1.0	13.08±0.02	2.88±0.1	5.07±0.01

The content of solids in milk samples from cows with genotype A2A2 is on average 12.93 %, and the ratio of protein content to fat is in the range from 0.56 to 0.63.

According to formula (1), the yield of cheese (%) from the milk of cows with different genotypes (A1A1, A1A2, A2A2) was calculated. The results are shown on a histogram (Fig. 2).

**5. 2. Determination of the yield of hard cheese from the milk of cows with different genotypes according to  $\beta$ -casein**

Fig. 1 shows the appearance of samples of hard cheese made from the milk of cows with different genotypes (A1A1, A1A2, A2A2).

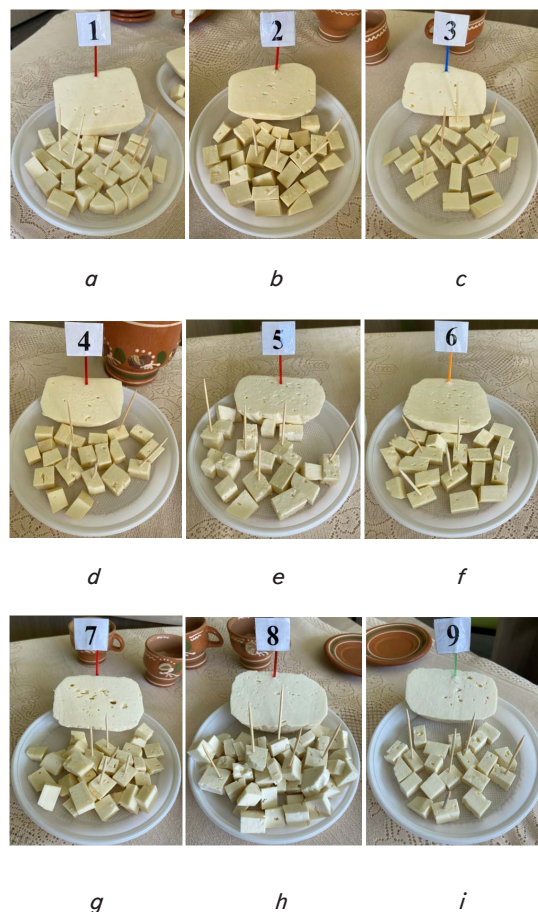


Fig. 1. Physical appearance of hard cheese samples: a – sample No. 1; b – sample No. 2; c – sample No. 3; d – sample No. 4; e – sample No. 5; f – sample No. 6; g – sample No. 7; h – sample No. 8; i – sample No. 9

**Cheese yield, %**

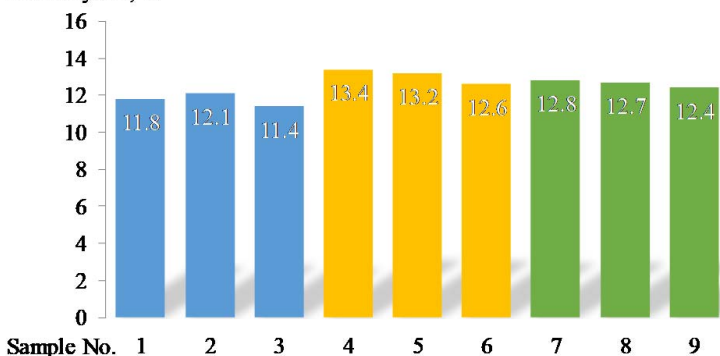


Fig. 2. Yield of hard cheese from the milk of cows with different genotypes

The averaged results showed the yield of cheese from A1A1 milk of 11.8 %; A1A2 – 13.1 %; A2A2 – 12.6 %.

**5. 3. Results of investigating the organoleptic and physicochemical parameters of samples of hard cheeses made from the milk of cows with different genotypes according to  $\beta$ -casein**

The results of the sensory analysis of the general characteristics of hard cheese (appearance, taste and smell, consistency, color, pattern on the section, shape of heads) by the expert group are represented in the form of a profilogram (Fig. 3).

According to the received sensory analysis profiles, the samples of hard cheeses from the milk of cows with the A1A1 genotype have an average appearance rating of 5.0 points. The taste and smell of the cheeses were rated at 4.0 points, the consistency – 3.7 points, the color – 4.3 points, the cut pattern – 3.7 points, the shape of the cheese heads – 5.0 points. At the same time, cheeses are characterized by experts as cheeses with a nice oval shape; with a good taste, but a weak aroma; with satisfactory consistency and uniform color; with an uneven arrangement of cells on the section.

Samples of hard cheeses from the milk of cows with the A1A2 genotype were evaluated on average by their appearance – 4.7 points. Taste and smell of cheeses – 5.0 points, consistency – 4.0 points, color – 4.3 points, cross-section pattern – 4.0 points, shape of cheese heads – 5.0 points. Samples of cheeses have a good appearance; with excellent taste and smell; with a good consistency; uniform color and location of the cells on the section.

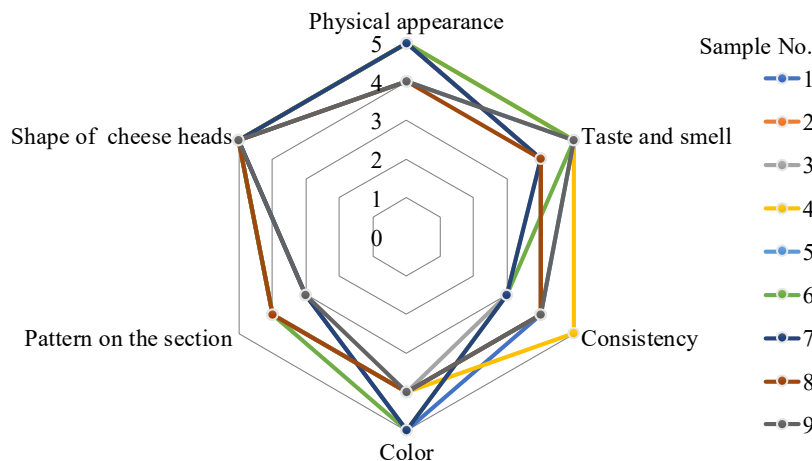


Fig. 3. Sensory profile of hard cheese samples

Samples of hard cheeses from the milk of cows with the A2A2 genotype have an average appearance rating of 4.3 points. Taste and smell of cheeses – 4.3 points, consistency – 3.7 points, color – 4.3 points, cross-section pattern – 3.3 points, shape of cheese heads – 5 points. Cheeses are characterized as satisfactory in appearance; with a good taste, but a weak aroma; with satisfactory consistency and uniform color; with an uneven slit-like arrangement of the cells on the section.

The results of physical and chemical indicators of samples of hard cheese from milk with different genotypes are given in Table 2.

The composition of cheeses from the milk of cows with the  $\beta$ -casein A1A1 genotype after 30 days of ripening averaged 21.5 %, 36.1 %, and 61.9 % of protein, fat, and dry matter, respectively.

Samples of cheeses from milk – A1A2 according to the average chemical composition were characterized by the content of protein – 27.1 %, fat – 33.9 %, and the total content of dry substances – 64.6 %.

Samples of A2A2 milk cheeses contained an average of 19.2 % protein, 37.2 % fat, and the dry matter content was 61.6 %.

Table 2

Physicochemical indicators of samples of hard cheese from the milk of cows with different genotypes ( $n=3, p \leq 0.05$ )

Sample No.	Genotype of $\beta$ -casein	Acidity, units pH	Density, kg/m <sup>3</sup>	Mass share of dry matter, %	Mass fraction of protein, %
1	A1A1	5.13±0.01	61.7±0.02	21.1±0.1	36.1±0.01
2		5.13±0.01	62.8±0.02	22.4±0.1	35.8±0.01
3		5.17±0.01	61.3±0.02	20.9±0.1	36.3±0.01
4	A1A2	5.35±0.01	65.4±0.02	29.8±0.1	30.7±0.01
5		5.37±0.01	64.4±0.02	28.4±0.1	35.6±0.01
6		5.36±0.01	63.9±0.02	23.2±0.1	35.4±0.01
7	A2A2	5.23±0.01	62.5±0.02	19.8±0.1	37.2±0.01
8		5.24±0.01	61.6±0.02	18.7±0.1	38.1±0.01
9		5.26±0.01	60.8±0.02	19.1±0.1	36.3±0.01

**5. 4. Results of investigating the amino acid profile of hard cheeses from the milk of cows with different genotypes according to  $\beta$ -casein**

The amino acid profile of the studied samples of hard cheese was analyzed chromatographically. The averaged results are presented on a chart (Fig. 4).

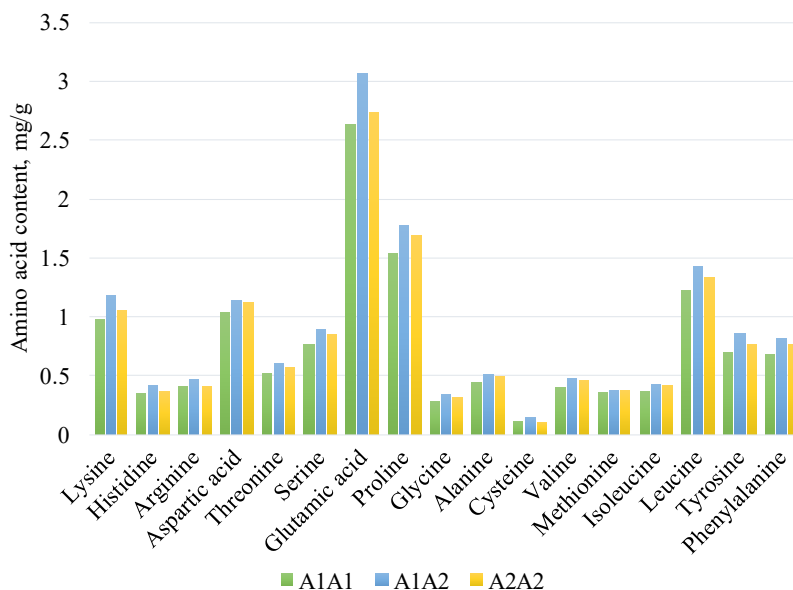


Fig. 4. Average amino acid profile of samples of hard cheese from milk of cows of different genotypes

The study revealed 17 amino acid residues in hard cheese samples. Milk cheese samples A1A1 are characterized by a high content of essential amino acids, such as leucine (0.814...1.639 mg/g), lysine (0.672...1.295 mg/g), phenylalanine (0.451...0.914 mg/g), threonine (0.353...0.688 mg/g), histidine (0.232...0.467 mg/g), valine (0.276...0.543 mg). And there are also substitute amino acids, in particular, a high content of glutamic acid (2.193...3.098 mg/g), aspartic acid (0.741...1.335 mg/g), proline (0.929...2.092 mg/g), serine (0.524...0.986 mg/g), tyrosine (0.447...0.925 mg/g), and others.

Milk cheese samples A1A2 contain a high content of essential amino acids, such as leucine (1.174...1.724 mg/g), lysine (1.036...1.359 mg/g), phenylalanine (0.721...0.931 mg/g), threonine (0.547...0.7 mg/g), histidine (0.387...0.464 mg/g), valine (0.411...0.574 mg). And there are also substitute amino acids, in particular, a high content of glutamic acid (2.895...3.398 mg/g), proline (1.276...2.256 mg/g), aspartic acid (1.035...1.33 mg/g), serine (0.867...1.005 mg/g), tyrosine (0.804...0.965 mg/g), and others.

A high content of essential amino acids was found in samples of A2A2 milk cheese: leucine (1.206...1.542 mg/g), lysine (0.931...1.225 mg/g), phenylalanine (0.693...0.861 mg/g), threonine (0.499...0.647 mg/g), valine (0.389...0.517 mg), isoleucine (0.355...0.489 mg/g). As well as replacement amino acids, in particular, a high content of glutamic acid (2.573...2.894 mg/g), aspartic acid (1.015...1.238 mg/g), proline (1.449...1.994 mg/g), serine (0.776...0.934 mg/g), tyrosine (0.676...0.862 mg/g), and others.

## 6. Discussion of results of determining the dependence of the content of nutrients and the yield of hard cheese on the protein composition of raw milk

The results of our studies into the physical and chemical parameters of milk samples (Table 1) did not reveal significant differences in the acidity and density of cow's milk with different variations of  $\beta$ -casein. The study of the content of fat, protein, and total solids in milk with  $\beta$ -casein genotypes showed that these values were slightly higher in the A2A2 genotype compared to A1A1 and A1A2.

The results of the obtained physicochemical indicators of milk samples are consistent with the data of other scientists [20–23], which show that  $\beta$ -casein genotype variations do not have a significant effect on the general physicochemical properties of milk (fat and protein content).

However, as a result of the study, it was established that milk samples with the A1A2 genotype had a higher level of protein:fat ratio (on average equal to 0.74), compared to milk samples with the A1A1 genotype (protein:fat – 0.67) and A2A2 (protein:fat – 0.61).

It is well known that the ratio of protein and fat in milk affects the yield and quality of cheese. For example, a protein to fat ratio of 0.7:0.8 will most likely result in a higher cheese yield. The authors of [24] practically established that a high fat content in milk negatively affects the quality of cheese (moisture content increases), but at the same time, the yield of cheese increases. Conversely, when the protein content increases, the quality of cheese increases, but the yield of cheese decreases.

The above data are consistent with our study. The calculated yield of cheese (Fig. 2) showed that the yield of cheese from milk with  $\beta$ -casein genotype A1A2 was significantly

higher (average value 13.1 %). This is related to the chemical composition and the optimal value of the protein:fat ratio (on average 0.74) in the original milk samples.

Formula (1), used in this study to calculate cheese yield, does not take into account other factors, such as the initial composition of milk, so a full comparison of the results with literature data is not possible. However, the results obtained in this study differ from the data reported by scientists [25], cheeses made from milk – A1A1 and A2A2 showed a similar yield and a higher yield than from A1A2.

The results of the organoleptic analysis (Fig. 3) showed that variations in the  $\beta$ -casein genotype do not significantly affect the sensory characteristics of the cheese. Cheese samples made from milk from cows with genotypes A1A1 and A2A2 have significantly worse texture, taste and aroma compared to samples made from A1A2 milk.

The results of the organoleptic analysis of the obtained samples of hard cheeses are consistent with the data of scientists [15] who prove that milk with the  $\beta$ -casein genotype A2A2 does not affect the general sensory characteristics of the finished product, which was also confirmed by the analysis of consumer preferences.

In [1] it was found that  $\beta$ -casein with the A2A2 genotype forms a more porous clot consistency, which leads to lower clot strength compared to  $\beta$ -casein A1A1.

Scientists [26] note that A2A2 milk is less suitable for cheese production due to its low ability to coagulate under the action of rennet, and therefore cheese with poorer sensory acceptability.

In [27] it was found that the A1A2 genotype was associated with higher cheese hardness compared to A2A2.

Changes in the  $\beta$ -casein genotype in cows had a noticeable effect on the chemical composition of cheese (Table 3). The most noticeable were significant increases in the content of dry matter and protein in cheese samples from A1A2 milk (on average, 61.6 % and 19.2 %, respectively) and a decrease in fat content (37.2 %). Samples of cheese from A1A1 or A2A2 milk, on the contrary, were characterized by increased moisture (lower content of dry substances) and fat.

However, the average values in this study correspond to those reported in the works of scientists [1, 28]. Higher percentages of the main components of cheese (content of protein and fat, dry matter) are shown in the work of scientists [29].

The amino acid composition of cheese determines its biological value [30]. The results of the amino acid profiles of the experimental samples of hard cheese showed that the  $\beta$ -casein A2A2 genotype influenced the increase of the total content of amino acids in the finished cheese. In particular, A1A2 and A2A2 milk cheese samples had amino acid contents of 14.89 and 13.84 mg/g protein, respectively, which is relatively higher than that of A1A1 milk cheese (12.82 mg/g protein). Such results are explained by the difference in the amino acid profile of the original milk. According to data [31], A1A2 milk has a significantly higher content of essential amino acids (histidine, lysine, isoleucine, methionine, and valine) and conditionally essential amino acids (proline, serine, and tyrosine), as well as replaceable aspartic acid. A2A2 milk has a significantly higher leucine content compared to A1A1 and A1A2 milk.

Comprehensive studies have shown that the  $\beta$ -casein genotype of cows has a significant effect on the nutrient content and yield of hard cheese from their milk. The information analysis clearly confirms that the consumption of

cow's milk with  $\beta$ -casein A2 leads to an overall improvement in the condition of the gastrointestinal tract and a reduction in the intestinal discomfort associated with milk. However, significant differences in technological properties can be observed between A2 and A1 milk, and A2 milk has worse cheese-making properties.

The main limitations of the study are the analysis of raw milk from a commercial herd of the Ukrainian black-spotted dairy breed in the Sumy region. Methods of raising, keeping, and feeding Ukrainian cows (as a result of the composition of raw milk) may differ from countries with other climatic and cultural differences. However, the characteristics of raw milk based on genetic variations of milk proteins and cheese made from such milk can be applied to other countries.

The disadvantage of this study is that the study of the influence of the protein composition of raw milk on the yield of cheese was made only by the rennet coagulation method, using the example of Gouda hard cheese. Further research should investigate the cheese yield of several different technologies and methods of protein coagulation.

Our conclusions are of practical importance, as it can be taken into account that changes in the genotype of  $\beta$ -casein in raw milk can affect the yield of cheese and, therefore, the profitability of production. When conducting further research, special attention should be paid to the selection of rennet, the interaction of the variation of milk proteins with rennet and establishing the transition of protein substances into the serum. Incorrectly selected rennet, or its low quality, can reduce the practical value of the results.

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## 7. Conclusions

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1. Our research established that the physicochemical parameters of raw milk of cows with different genotypes of  $\beta$ -casein (A1A1, A1A2, A2A2) are typical for fresh cow's milk and meet the requirements of regulatory documents. The content of fat, protein, and solids in the milk of cows with the  $\beta$ -casein genotype A2A2 were slightly higher compared to A1A1 and A1A2.

2. Calculations of the yield of cheese showed that the yield of cheese from milk of cows with  $\beta$ -casein genotype A1A2 was higher (average value 13.1 %) than with A1A1 and A2A2. These results are interrelated with the chemical composition of milk and the optimal protein:fat ratio in the original milk samples.

3. A comprehensive study of the quality indicators of samples of hard cheeses made from the milk of cows with different genotypes showed that the type of  $\beta$ -casein did not affect the sensory characteristics of the cheese. However, according to the content of the main chemical components, cheeses made from A1A2 milk had a higher content of dry matter and protein (on average, 61.6 % and 19.2 %, respectively) and a lower content of fat (37.2 %).

4. The amino acid profile of cheese from the milk of cows with the  $\beta$ -casein A1A2 and A2A2 genotype in raw milk showed a higher total content of amino acids – 14.89 mg/g and 13.84 mg/g, respectively.

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## Conflicts of interest

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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 and the results reported in this paper.

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## Data availability

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All data are available in the main text of the manuscript.

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## Use of artificial intelligence

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

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