One of the ways to increase the profitability of the cheese industry is the genetic selection of dairy cows to obtain milk with excellent cheese-capacity characteristics. The object of this study is the technology of fresh soft cheeses made by the acid-rennet and thermo-acid method from the milk of cows with different β-casein genotypes (A1A1, A1A2, A2A2). The subjects of research are the physicochemical indicators of raw milk from cows with different genotypes for β-casein (A1A1, A1A2, A2A2); as well as the yield of soft cheeses. The study has established that the physicochemical parameters of milk from cows with different β-casein genotypes are typical for fresh cow's milk. The study showed that with the acid-rennet method, the composition of cheeses from A1A1 milk was 51.60 %, 21.63 %, and 23.62 % of moisture, protein, and fat, respectively. A1A2 milk cheeses contained 50.70 % moisture, 20.96 % protein, and 25.12 % fat. A2A2 milk cheeses consisted of 52.50 % moisture, 20.70 % protein, and 23.71 % fat. With the thermo-acid method, cheeses from A1A1 milk were characterized by the moisture content of 55.13 %, proteins – 23.31 %, and fat – 20.21 %. A1A2 milk cheeses contained 58.13 %, 22.62 %, and 17.98 % of moisture, protein, and fat, respectively. A2A2 milk cheeses consisted of 54.03 % moisture, 22.33 % protein, and 22.25 % fat. The calculation of the production efficiency of soft cheeses from the milk of cows with different genotypes according to β-casein with the acid-rennet method of production is 119.3 % on average, which is more compared to milk A1A2 (by 4 %) and A2A2 (by 7 %). With the thermo-acidic method, the efficiency of cheese production from A2A2 milk is 107.5 %, which is higher compared to A1A2 milk (by 9 %) and A1A1 (by 5 %). The conclusions show that changes in the β-casein genotype in raw milk can affect the yield and quality of cheese, and therefore, the profitability of production

Keywords: soft cheese, cheese yield, quality, acid-rennet technique, thermo-acid technique, β-casein, A2 milk

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DETERMINING THE INFLUENCE OF RAW MILK Β-CASEIN POLYMORPHISM ON THE EFFICIENCY OF MAKING SOFT CHEESE

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1. Introduction

Cheese production is a worldwide practice and is especially widespread in European countries, in particular in Ukraine. According to FAO data [1], it is predicted that by 2032 global milk processing and production of various dairy products, especially cheese, should increase by 30 %.

Cheese is considered a high-quality food product due to its high biological value and nutritional composition obtained by fermentation, acid or thermo-acid coagulation of proteins, ultrafiltration, with/without ripening of cheese mass [2].

The composition of cheese depends on many factors, including the species and breed of the cattle, the stage of lactation, the protein:fat ratio in the milk. The β-casein composition of proteins is an important selection feature that affects the technical properties of milk [3].

The benefits of “A2 milk” for the human body compared to “A1 milk” were investigated and confirmed in works [4, 5]. The practical advantages of “A2 milk” are also confirmed by the increase in its production in the dairy sector and the presence of companies in the dairy industry seeking to produce milk containing β-casein A2. Accordingly, there is a need to expand research on the use of “A2 milk” in the production of dairy products, especially cheese.

Therefore, studies on determining the influence of β-casein polymorphism of raw milk on its cheese-making characteristics are relevant. They will make it possible in practice to rationally select the technology for cheese production and predict its yield taking into account data on the β-casein genotype, and therefore rationally use raw milk for production.

2. Literature review and problem statement

β-casein plays an important role during cheese making due to its positive correlation with actual cheese yield [6]. β-Casein constitutes more than 35 % of total milk protein and has 15 different genetic variants, 7 of which have been identified, mainly in European cattle breeds (A1, A2, A3, B, C, I, and E) [7].

It was reported in [8] that the difference in the genetic variability of A1 and A2 β-casein is in a single-nucleotide polymorphism of the CSN2 gene, which changes the coding of amino acid 67 in the polypeptide chain from proline (Pro67 for β-casein A2) to histidine (His67 for β-casein A1). In [9], it is emphasized that due to the difference in the amino acid residue, the two variants of β-casein form different structural units of enzymatic cleavage during digestion, correspondingly leading to different possible effects on human health. However, the functional and technical properties of such milk have not been investigated.

Information analysis [10, 11] reveals that "A2 milk" continues to gain popularity in the dairy sector and the share of cows with the A2 genotype has been growing significantly over the past 25 years. Therefore, research on determining the technological properties of raw milk from cows with the β-casein A2 genotype is promising. First of all, it is necessary to understand the potential consequences of using "A2 milk" in technology with the transformation/removal of the main components of milk (protein), in particular, cheese technology.

In [12], the influence of the protein composition of raw milk on the yield of hard cheese and its content of nutrients was considered. It was determined that the type of β-casein did not affect the sensory characteristics of hard 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 %). Calculations of cheese yield showed that cheese yield from milk of cows with β-casein genotype A1A2 was higher (mean value 13.1 %) than from A1A1 and A2A2. The disadvantage of the cited work is that the study on the influence of the protein composition of raw milk on the yield of cheese was performed only by the rennet coagulation method, using the example of Gouda hard cheese. Accordingly, further research should investigate the cheese yield of several different technologies and methods of protein coagulation.

This is the approach used in [13]. The authors investigated the coagulation properties of milk with β-casein genotype A2A2 in comparison with control milk (a mixture of genotypes A2A1/A1A1/A2A2). It was established that the time of acid and rennet coagulation did not change in the studied milk samples. However, A2 milk showed higher acid gel density and cheese firmness than control milk. Potential yield and syneresis were higher in "milk A2", corresponding to higher cheese hardness. The results show that the use of the acid-rennet technique of coagulation in the production of milk products with the β-casein genotype A2A2 allows obtaining high-quality products without significant differences compared to control milk. However, according to the authors of work [14], "A2 milk" has a longer acid gel formation time and lower gel hardness compared to "A1 milk".

Work [15] reports the results of a marketing research on consumer preferences for A2 dairy products and evaluates the effect of β-casein A2 on the sensory characteristics of soft cheeses. It was shown that cheeses from the milk of cows with the β-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 works [16, 17] it was investigated that the type of β-casein genotype affects the yield of cheeses obtained by the rennet method of protein coagulation. It was shown that milk with β-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. Accordingly, 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.

Polymorphisms of milk proteins affecting the composition of milk and its coagulation properties in both the enzymatic and acidic methods in the production of cream cheese, sour cream, and butter are described in [18]. However, in information sources there are almost no data on the influence of β-casein genotypes on the coagulation properties of milk protein in the production of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of protein coagulation.

In the production of cheese, an important factor affecting the profitability of production is the high-quality composition of proteins in raw milk. In addition, monitoring all relationships between the quality of raw materials and cheese production, such as the yield of cheese and the preservation of dairy components in the cheese mass, is an important stage in determining the efficiency of the entire technological process. One of the ways to increase the profitability of the cheese industry is the genetic selection of dairy cows to obtain milk with high coagulating properties of protein.

All this allows us to state that it is appropriate to carry out a study on determining the integrated effect of β-casein A1/A2 polymorphism and the chemical composition of milk on the production of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of protein coagulation.

3. The aim and objectives of the study

The purpose of our study is to determine the influence of β-casein polymorphism of raw milk on the efficiency of fresh soft cheese production. This will make it possible to selectively choose 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 physical-chemical parameters of raw milk from cows with different genotypes for β-casein (A1A1, A1A2, A2A2);

– to investigate and compare the organoleptic and physical-chemical indicators of samples of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of coagulation of proteins from the milk of cows with different genotypes according to β-casein;

– to calculate the efficiency of production of soft cheeses from the milk of cows with different genotypes according to β-casein and to compare the yield of cheeses made by acid-rennet and thermo-acid techniques of protein coagulation.

4. The study materials and methods

4. 1. The object and hypothesis of the study

The object of our research is the technology of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of protein coagulation from the milk of cows with different β-casein genotypes (A1A1, A1A2, A2A2).

The subjects of research are the physicochemical indicators of raw milk from cows with different genotypes for β-casein (A1A1, A1A2, A2A2); the yield of cheeses made by acid-rennet and thermo-acid techniques of coagulation of proteins from this milk and its quality indicators.

Our 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 of the influence of the protein composition of raw milk on the quality and yield of cheeses produced by different technologies could provide an opportunity to selectively choose dairy breeds of cows according to the protein composition suitable for cheese production.

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. Milk samples were taken in March–April 2024. In this study, 10 kg of milk was collected during morning milking from nine cows with different β-casein genotypes (A1A1, A1A2 and A2A2). Immediately after milking, the milk samples were transported to the university laboratory, immediately cooled to a temperature of (4±2) °C in cold water and stored in a cold chamber ((4±2) °C) for no more than 6 hours.

4. 2. 1. Production of cheese by the acid-rennet technique of protein coagulation

The studied samples of soft fresh cheese "Brynza" were produced from whole milk by the acid-rennet technique of coagulation of proteins according to the conventional technology in accordance with the requirements of DSTU 7065:2009 "Brynza. General technical conditions". Nine samples of cheese from cow's milk of different genotypes were prepared in parallel. In research, cheese samples are marked as K1...K9 according to the raw milk sample number.

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 making samples of fresh cheese "Brynza" under laboratory conditions consists of the following stages: milk purified from mechanical impurities is pasteurized at a temperature of (72±1) °C with a holding time of 20 seconds. In the milk cooled to a temperature of (33±1) °C, bacterial preparations are added directly, in the amount recommended by the manufacturer (2.8–4.0 g per 100 kg of milk mixture). The leavening preparation consists of mixed cultures of microorganisms – *Lactobacillus lactis; Lactococcus lactis subsp. cremoris; Lactobacillus bulgaricus; Streptococcus thermophilus* (mesophilic-thermophilic starter WhiteDaily 80, manufactured by Chr. Hansen, Denmark). Next, a calcium chloride solution is added (at the rate of 10 g per 100 kg of milk mixture). The prepared rennet solution is added in the amount recommended by the manufacturer (2.9–3.5 ml per 100 kg of milk mixture). 100 % chymosin "Albamax 600" (Caglificio Clerici, Italy) was used in the study. After adding all the necessary components, the milk mixture is thoroughly mixed for 10–12 minutes and left alone until a clot forms. Settling of the milk mixture is carried out at a temperature of (33±1) °C, controlling the pH change to a value of 5.3 units. Next, the clot is cut (into cubes of 1×1×1 cm size, for 5–7 minutes), cheese grain is processed (kneading for 10–15 minutes). Then they leave it alone for 12–15 minutes, after which 60–80 % of the whey is removed. The formed cheese layer is cut and laid out in forms. Cheese heads are self-pressed (12 hours) and pressed under minimal load (2–3 hours). Then they are salted in brine (salt concentration 18–20 %, temperature 10–14 °C) for 4 hours. Next, the cheese heads are packed in vacuum bags and stored until the moment of research, but no longer than 10 days at a temperature of (6±2) °C.

4. 2. 2. Production of cheese by thermo-acid technique of protein coagulation

The studied samples of soft fresh cheese of the "Adygei" type were produced from whole milk by the thermo-acid technique of protein coagulation according to conventional technology in accordance with the requirements of DSTU 4395:2005 "Soft cheeses. General technical conditions". In the research, Adygei cheese was produced by curdling milk proteins under the influence of temperature and the introduction of a 5 % solution of citric acid. Nine samples of cheese from cow's milk of different genotypes were prepared in parallel. In research, cheese samples are marked as T1...T9 according to the raw milk sample number.

10 kg of raw milk was used to make cheese. Heat treatment, acidification (introduction of citric acid solution), and the subsequent formation of cheese grains were carried out at a laboratory cheese factory.

A 5 % solution of citric acid (at the rate of 0.5–1 l of solution per 100 kg of mixture) was added to the prepared pasteurized milk at a temperature of (95±1) °C, carefully pouring it in small portions along the wall of the container. The clot in the form of flakes formed on the surface was kept at a temperature of (95±1) °C for 5–7 minutes. After that, the cheese grains were salted with table salt of the "Extra" class in the amount of 1–2 % of the cheese mass. The curd grain was carefully transferred into molds and left to cool and self-press for 1.5–2 hours. During this time, the cheese was turned over twice, slightly shaking the mold. Next, the cheese heads were packed in vacuum bags and stored until the moment of research, but no longer than 10 days at a temperature of (6±2) °C.

4. 3. Methodology for determining 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 7065:2009, DSTU 4395:2005.

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 7065:2009, DSTU 4395:2005, with recommendations described in the international standard ISO 22935-2:2023.

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

The efficiency of cheese production was determined in accordance with the recommendations described in [19] by determining the recovery of milk components in the cheese mass and the ratio of the experimental cheese yield to the theoretical one.

The recovery of milk components (dry matter, protein, fat) in the curd mass was calculated according to formula (1):

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

where $R_{solids,\ protein,\ fat}$ – recovery of milk components, namely dry matter, protein and fat, in cheese, %;

weight of cheese$_{solids,\ protein,\ fat}$ – mass of dry substances, protein, and fat in cheese, g;

weight of milk$_{solids,\ protein,\ fat}$ – weight of milk solids, protein, and fat, g.

The efficiency of cheese production was calculated according to formula (2):

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

where E is the efficiency of cheese production, %;

C – yield of experimental cheese, %;

Th_C – theoretical yield of cheese, %.

The experimental yield of cheese from the studied milk samples was calculated according to formula (3):

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

where C is the yield of cheese, %;

m_{cheese} – mass of fresh cheese, kg;

m_{milk} – mass of milk, kg.

The theoretical yield of cheese was calculated according to formula (4):

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

where Th_C is the theoretical yield of cheese, %;

%fat, %protein – mass fraction of fat, protein in milk, %;

%W – mass fraction of moisture in cheese, %.

Mathematical and statistical processing of the results was carried out on a computer using Excel software from the Microsoft Office 2016 package (Microsoft Corporation, Washington, USA). 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 research on the influence of β-casein polymorphism of raw milk on the production process of soft cheese

5. 1. Results of studies of physicochemical parameters of raw milk samples

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

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

According to our results, the average value of dry matter content in milk samples from cows with the A1A1 genotype is 12.83 %, while the ratio of protein to fat content is within 0.75…0.96.

In milk samples from cows with the A1A2 genotype, the average value of dry matter content is 12.72 %, and the ratio of protein to fat content is within 0.77…1.3.

Table 1

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

Sample No.	Genotype of β-casein	Acidity, units pH	Density, kg/m^3	Mass fraction of solids, %	Mass fraction of protein, %	Mass fraction of fat, %
1	A1A1	6.76±0.01	1027.0±1.0	12.98±0.01	3.03±0.01	4.02±0.01
2		6.74±0.01	1027.0±1.0	12.69±0.01	2.91±0.01	3.59±0.01
3		6.72±0.01	1028.0±1.0	12.84±0.01	2.95±0.01	3.05±0.01
4	A1A2	6.77±0.01	1028.0±1.0	12.88±0.01	2.97±0.01	3.27±0.01
5		6.84±0.01	1025.0±1.0	12.42±0.01	2.82±0.01	3.65±0.01
6		6.82±0.01	1028.0±1.0	12.88±0.01	2.94±0.01	3.75±0.01
7	A2A2	6.79±0.01	1027.0±1.0	12.83±0.01	2.96±0.01	3.46±0.01
8		6.77±0.01	1025.0±1.0	12.40±0.01	2.82±0.01	4.10±0.01
9		6.79±0.01	1027.0±1.0	12.56±0.01	2.86±0.01	3.79±0.01

The content of solids in milk samples from cows with genotype A2A2 is on average 12.59 %, and the ratio of protein to fat content is in the range from 0.68 to 1.02.

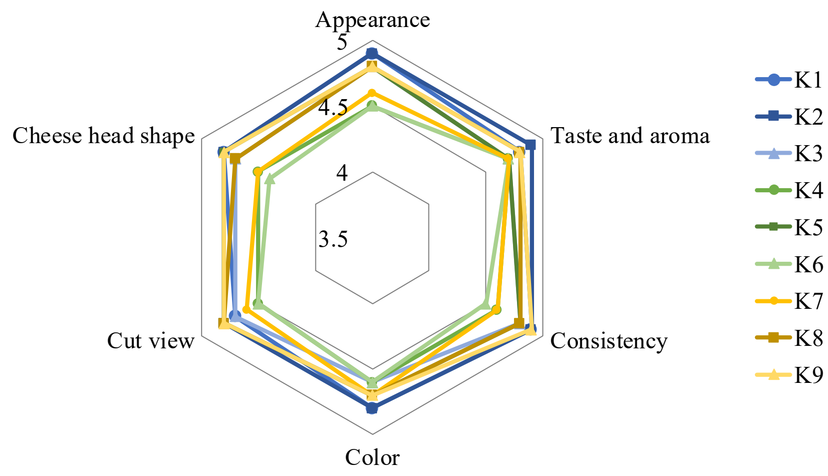
5. 2. Results of studying the quality indicators of cheeses from milk with different genotypes according to β-casein

The results of the sensory analysis of the general characteristics of fresh soft cheeses (appearance, taste and smell, consistency, color, pattern on the section, shape of heads) by the expert group are represented in the form of profilograms (Fig. 1).

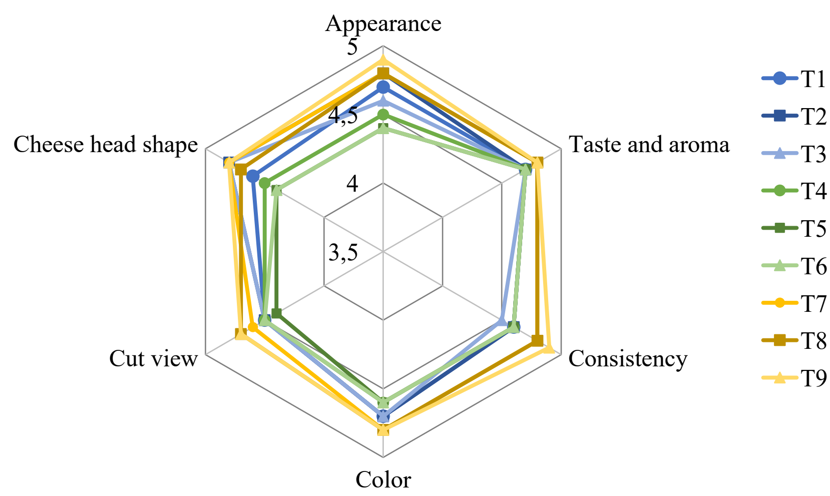
According to the obtained sensory analysis profiles, the samples of soft cheeses made by the acid-rennet technique from the milk of cows with the A1A1 genotype have an average appearance rating of 4.9 points. The taste and smell of the cheeses were rated at 4.8 points, the consistency – 4.9 points, the color – 4.7 points, the cut view and shape of the cheese heads – 4.7 points. At the same time, cheeses are characterized by experts as oval-shaped cheeses; with a good taste, pronounced sour-milk aroma; with a satisfactory consistency and uniform color. Samples of cheeses made from

A1A2 milk are rated 4.6 points on average for their appearance. The taste and smell of the cheeses – 4.7 points, the consistency and color – 4.6 points, the cross-section and shape of the cheese heads – 4.6 points. Samples of cheeses have a good appearance; with excellent taste and smell; uniform color on the cut and delicate consistency. Samples of cheeses from A2A2 milk have an average appearance rating of 4.7 points. The average evaluations of the taste and smell of cheeses, consistency, color, and appearance on the cut are 4.7 points, the shape of the cheese heads – 4.6 points. Cheeses are characterized as satisfactory in appearance; with a good taste, but a weak aroma; with a satisfactory delicate consistency and uniform color.

Samples of soft cheeses made by thermo-acid technique from milk of cows with genotype A1A1 have an average evaluation of appearance, taste, and smell – 4.7 points. Consistency and appearance on the cut were rated at 4.5 points on average, color and shape of the heads at 4.7 points. The tasting panel noted that the cheeses have an excessively delicate consistency but with a pleasant creamy taste and sour-milk aroma. Samples of cheeses from A1A2 milk were rated 4.5 points on average for appearance, 4.7 points for taste and smell. The consistency and color were rated at 4.6 points, the appearance and shape of the cheese heads – at 4.4 points. The cheeses are characterized by a pleasant sour-milk taste, but they have an unsatisfactory consistency. Samples of cheeses from A2A2 milk have an average rating of 4.8 points for appearance and taste and smell. Consistency and color are also rated at 4.8 points. The appearance on the section – 4.6 points, the shape of the cheese heads – 4.7 points. Samples of soft cheeses were characterized by experts as cheeses with an attractive appearance, a satisfactory taste, and a pleasant sour-milk aroma. The consistency is satisfactory, typical of this group of cheeses.



a



b

Fig. 1. Sensory profile of samples of fresh soft cheeses from the milk of cows with different genotypes according to β-casein produced:
a – by the acid-rennet technique of protein coagulation;
b – thermo-acid technique of protein coagulation

The results of physical-chemical indicators of samples of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of coagulation of proteins from the milk of cows with different genotypes according to β-casein are given in Table 2.

Table 2

Physical-chemical indicators of samples of fresh soft cheeses made by acid-rennet and thermo-acid techniques of protein coagulation from milk of cows with different genotypes according to β-casein (n=3, p≤0.05)

Production technique	Genotype of β-casein	Cheese sample	Moisture content, %	Mass fraction of protein, %	Mass fraction of fat, %	Acidity, units pH
1	2	3	4	5	6	7
Acid-rennet	A1A1	K1	52.41±0.01	21.82±0.01	22.71±0.01	5.23±0.01
		K2	51.55±0.01	20.95±0.01	24.45±0.01	5.24±0.01
		K3	50.94±0.01	22.21±0.01	23.71±0.01	5.24±0.01
	A1A2	K4	50.46±0.01	21.45±0.01	24.98±0.01	5.25±0.01
		K5	51.15±0.01	20.35±0.01	25.42±0.01	5.24±0.01
		K6	50.62±0.01	21.24±0.01	24.99±0.01	5.25±0.01
	A2A2	K7	52.22±0.01	21.38±0.01	23.39±0.01	5.21±0.01
		K8	52.75±0.01	20.35±0.01	23.93±0.01	5.22±0.01
		K9	52.67±0.01	20.55±0.01	23.82±0.01	5.22±0.01

Continuation of Table 2

1	2	3	4	5	6	7
Thermo-acidic	A1A1	T1	55.50±0.01	23.44±0.01	19.73±0.01	5.88±0.01
		T2	55.12±0.01	22.55±0.01	21.00±0.01	5.89±0.01
		T3	54.80±0.01	23.95±0.01	19.89±0.01	5.90±0.01
	A1A2	T4	58.45±0.01	23.09±0.01	17.26±0.01	5.91±0.01
		T5	57.91±0.01	21.90±0.01	18.93±0.01	5.88±0.01
		T6	58.14±0.01	22.87±0.01	17.77±0.01	5.91±0.01
	A2A2	T7	54.15±0.01	22.87±0.01	21.54±0.01	5.88±0.01
		T8	53.85±0.01	21.90±0.01	22.91±0.01	5.88±0.01
		T9	54.20±0.01	22.12±0.01	22.31±0.01	5.90±0.01

With the acid-rennet technique of protein coagulation, the composition of cheeses from A1A1 milk was on average 51.60 %, 21.63 %, and 23.62 % of moisture, protein, and fat, respectively. Samples of cheeses from A1A2 milk on average contained 50.70 % moisture, 20.96 % protein, and 25.12 % fat. And samples of cheeses from A2A2 milk on average consisted of 52.50 % moisture, 20.70 % protein, and 23.71 % fat.

With the thermo-acid technique of protein coagulation, cheeses from A1A1 milk were characterized by an average moisture content of 55.13 %, protein content of 23.31 %, and fat content of 20.21 %. Samples of cheeses from A1A2 milk on average contained 58.13 %, 22.62 %, and 17.98 % of moisture, protein, and fat, respectively. And the composition of cheese samples from A2A2 milk on average consisted of 54.03 % moisture, 22.33 % protein, and 22.25 % fat.

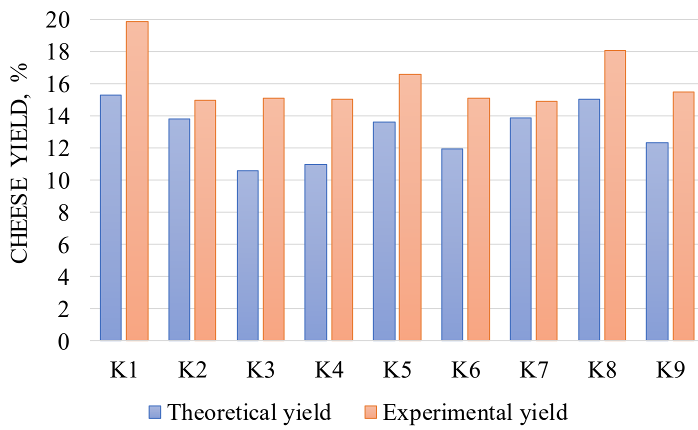
5. 3. Determining the efficiency of cheese production from the milk of cows with different genotypes in terms of β-casein

According to formulas (3), (4), the yield of cheese (%) from the milk of cows with different genotypes (A1A1, A1A2, A2A2) was calculated. Our results regarding the yield of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of coagulation of proteins from the milk of cows with different genotypes according to β-casein are represented in the histogram (Fig. 2).

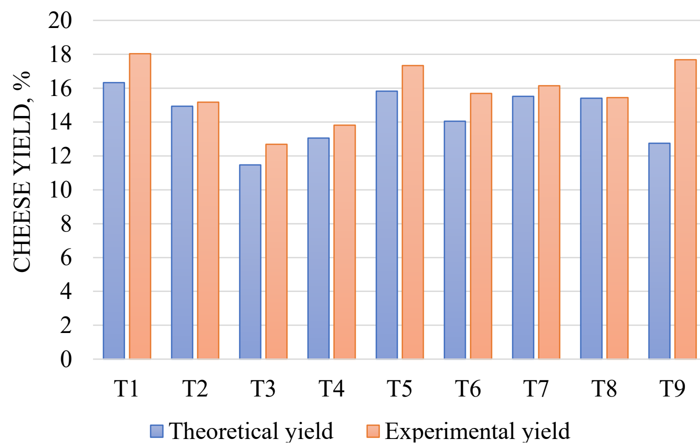
The averaged results showed the experimental yield of cheese with the acid-rennet technique of coagulation of proteins from milk A1A1 – 16.64 %; with A1A2 – 15.67 %; with A2A2 – 16.14 %.

With the thermo-acid technique of protein coagulation, the experimental yield of cheese is 15.29 % from A1A1 milk, 15.61 % from A1A2 milk, and 16.42 % from A2A2 milk.

Estimated data on the efficiency of production of soft cheeses from the milk of cows with different genotypes in terms of β-casein, namely the recovery of the main components of milk in cheese and the ratio of the experimental yield of cheese to the theoretical one, are given in Table 3.



a



b

Fig. 2. The yield of fresh soft cheeses from the milk of cows with different genotypes in terms of β-casein produced: *a* – by the acid-rennet technique of protein coagulation; *b* – by the thermo-acid technique of protein coagulation

The average values of the recovery of milk components in cheese showed that with the acid-rennet technique of cheese production, 26.99 % of protein and 32.56 % of fat were lost in A1A1 milk samples. In samples of cheeses from A1A2 milk, the average loss of protein is 27.95 % and fat – 29.11 %, and from A2A2 milk – 28.32 % protein and 37.15 % fat.

With the thermo-acid technique of protein coagulation, the average values of recovery of A1A1 milk components in cheese showed that there is a loss of 21.32 % of protein and 42.40 % of fat. In samples of cheeses from A1A2 milk, the average loss of protein is 22.25 %, fat – 49.31 %. During the production of cheeses from A2A2 milk, an average of 22.65 % of protein and 41.14 % of fat is lost.

Table 3

Efficiency of production of fresh soft cheeses made by acid-rennet and thermo-acid techniques of coagulation of proteins from milk of cows with different genotypes in terms of β-casein

Production technique	Genotype of β-casein	Cheese sample	Recovery of milk components in cheese, *R*, %		Cheese production efficiency, %
			Protein	Fat	
Acid-rennet	A1A1	K1	71.95	56.48	130.2
		K2	71.82	68.10	108.2
		K3	75.25	77.73	119.6
	A1A2	K4	72.05	76.38	115.9
		K5	71.99	69.65	121.6
		K6	72.11	66.64	108.2
	A2A2	K7	71.96	67.61	107.7
		K8	71.99	58.35	119.9
		K9	71.68	62.85	106.9
Thermo-acidic	A1A1	T1	77.34	49.08	110.4
		T2	77.50	58.50	101.7
		T3	81.20	65.21	92.5
	A1A2	T4	77.75	52.79	89.2
		T5	77.67	51.87	109.5
		T6	77.81	47.38	95.3
	A2A2	T7	77.64	62.26	104.1
		T8	77.67	55.88	100.2
		T9	77.34	58.86	118.2

6. Discussion of results based on determining the influence of the β-casein polymorphism of raw milk on the production process of soft cheese

The results of research on 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 β-casein. The results of the average content of solids in milk samples showed that the value fluctuates at the level of (12.8±0.2) % for all variations of β-casein genotypes. The study of the protein content in milk revealed that the indicated indicators were slightly higher (on average by 0.1 %) in the A1A1 genotype compared to A1A2 and A2A2. Conversely, the value of fat content in milk with the A2A2 genotype was higher compared to the other two β-casein genotypes.

The results of the physical and chemical parameters of the milk samples are consistent with the data reported by other scientists [20–24]. It was shown that β-casein genotype variations do not have a significant effect on the general physicochemical properties of milk (acidity, dry matter, fat, and protein content).

As a result of our study, it was established that milk samples with the A1A1 genotype had a higher protein:fat ratio (on average equal to 0.84), compared to milk samples with the A1A2 genotype (protein:fat – 0.82) and A2A2 (protein:fat – 0.76).

The protein content and its fractional composition play a decisive role in cheese production – from the formation of curds to the high-quality ripening of cheese. This statement is confirmed in work [7], since cheese consists of a paracasein network in which fat globules and part of the soluble phase of milk are trapped, the concentration of casein in milk is positively correlated with the amount of cheese produced per unit of milk (percentage of cheese yield). In addition, scientists emphasize that the physicochemical characteristics and structural properties of milk casein micelles (average size, proportion of caseins, concentration of colloidal calcium phosphate, genetic variants of casein) affect the rheological properties of the resulting cheese. As a result, the ability of cheese to retain milk components (mainly water, protein, and fat) in cheese (efficiency of cheese production).

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 [23] practically found that a high fat content in milk negatively affects the quality of cheese (the 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 experimental yield of cheese (Fig. 2) showed that the yield of cheese from milk with β-casein genotype A1A1 and A2A2 produced by the acid-rennet technique was significantly higher (average value 16.6 and 16.4 %). This is related to the chemical composition (higher fat content) in the original milk samples. The influence of the protein:fat ratio in milk samples with the β-casein genotype A2A2 (average value 0.76) on the yield of cheese was also experimentally confirmed when produced by the thermos-acidic method (average value of cheese yield 16.4 %).

The results reported in this study are consistent with data from scientists in [24]: cheeses made from milk – A1A1 and A2A2 showed a similar yield and a higher yield than from A1A2.

The results of organoleptic analysis (Fig. 1) showed that β-casein genotype variations do not significantly affect the sensory characteristics of soft cheese produced by acid-rennet or thermo-acid technique of protein coagulation. In all cases, the produced cheese was of a delicate consistency, moderately dense typical for this group of cheeses. However, the results do not agree with the previous results described in [13]. This is explained by the fact that genetic variants of casein affect the rheological properties of the resulting cheese, namely the ability to retain water in the cheese. Accordingly, in the production of fresh soft cheeses, a moist, tender consistency is desirable. Conversely, in the production of hard aged cheeses, the retention of excess moisture in the cheese dough has a negative effect on the sensory parameters of the finished product.

The results of the organoleptic analysis of the obtained cheese samples are consistent with the data in [15], which prove that milk with the β-casein A2A2 genotype does not affect the general sensory characteristics of the finished product, which was also confirmed by the analysis of consumer preferences.

The results obtained in the work also agree with the data in [3, 25]. The authors found that β-casein with the A2A2 genotype forms a more porous clot consistency, which leads to lower clot strength compared to β-casein A1A1.

Changes in the β-casein genotype in cows and the method of protein coagulation had a noticeable effect on the chemical composition of cheese (Table 2). The most noticeable was a significant increase in moisture content in samples of cheese from A2A2 milk produced by the acid-rennet technique (52.5 % on average, respectively). Samples of cheese from A1A1 or A2A2 milk, on the contrary, were characterized by a slight decrease in moisture content (50.7...51.6 %) and an increase in protein content (20.9...21.6 %).

On the other hand, when cheese was produced by the thermo-acid technique of coagulation of proteins, cheese samples from A2A2 milk on average had a lower moisture content (54.0 %) compared to samples from A1A1 (55.1 %) and A1A2 milk (58.1 %). The protein content in cheese samples from A1A2 and A2A2 milk was on average 22.3...22.6 %, slightly less than from A1A1 milk (23.3 %).

However, the average values in this study correspond to those reported in [26]. The higher percentages of the main components of cheese (protein and fat content) are shown in [27].

The yield of cheese also depends on the recovery of certain milk components in the cheese mass, which determine the overall efficiency of cheese production. In work [7] it is emphasized that β-casein is a protein fraction of milk with a high influence on the actual yield of cheese and is associated with the recovery of protein and solids in the finished product.

The results of determining the recovery of milk components in the curd mass according to formula (1) showed that with the acid-rennet technique of cheese production in all samples, the recovery of protein is on average 71.8...73.0 %, fat is slightly less – 62.9...70, 8 %. At the same time, the efficiency of cheese production from the milk of cows with the A1A1 β-casein genotype is 119.3 % on average, which is higher than that of A1A2 milk (by 4 %) and A2A2 (by 7 %) (Table 3).

With the thermo-acid technique of protein coagulation, the average values of protein recovery in the samples are much higher – 77.5...78.6 %. Conversely, the value of fat recovery in cheese is lower (50.6...58.9 %) compared to the acid-rennet technique of cheese production. The efficiency of cheese production from A2A2 milk in this case is 107.5 % on average, which is higher compared to A1A2 milk (by 9 %) and A1A1 (by 5 %) (Table 3).

The results are consistent with the data from [16]. The authors claim that it is inappropriate to use milk with the A2A2 β-casein genotype for the production of raw milk, as it is associated with the deterioration of the recovery of milk components in the cheese and, as a result, a less efficient production process.

Also, the adverse effect of the β-casein A2 genotype on the coagulation properties of milk is described in [20]. The scientists claim that the worse coagulation properties of A2 milk are mainly due to lower hydrophobicity, lower Ca and P content, and larger micelle size. Also, the difference in the primary structure of β-casein A1 and A2 leads to different behavior in different environments, which affects the technical and functional properties of milk.

Comprehensive studies have shown that the β-casein polymorphism of raw milk has a significant impact on the efficiency of cheese production. The information analysis clearly confirms that the consumption of cow's milk with β-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 when using the acid-rennet technique of coagulation. However, the opposite dependences are observed when curdling protein fractions by the thermoacidic method – the actual yield of cheese is greater.

The main limitations of our study are the analysis of raw milk from a commercial herd of the Ukrainian black and white dairy breed in the Sumy region (Ukraine). Methods of raising, keeping, and feeding Ukrainian cows (as a result of the composition of raw milk) may differ in 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 work is the study of the effect of β-casein polymorphism of raw milk on the production efficiency of soft cheese made by classical technologies (acid-rennet and thermo-acid techniques), from native raw materials without standardization for fat content. In further research, the cheese-making properties of the protein fractions of milk raw materials under the influence of various coagulating agents (temperature, enzyme preparations) should be investigated, excluding the influence of the fat part.

Our conclusions are of practical importance since it can be taken into account that changes in the genotype of β-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 fermented cultures in the production of acid or acid-rennet cheese. It is also necessary to pay attention to the selection of temperature regimes when processing dairy raw materials since improperly performed heat treatment affects the protein structure and can reduce the practical value of the results.

7. Conclusions

1. Our research established that the physicochemical parameters of raw milk of cows with different genotypes of β-casein (A1A1, A1A2, A2A2) are typical of fresh cow's milk and meet the requirements of regulatory documents. The results of the average content of solids in milk samples showed that the value fluctuates at the level of (12.8±0.2) % for all variations of β-casein genotypes. The study of the protein content in milk showed that the indicated indicators

were slightly higher (on average by 0.1 %) in the A1A1 genotype compared to A1A2 and A2A2. Conversely, the value of fat content in milk with the A2A2 genotype was higher compared to the other two β-casein genotypes.

2. A comprehensive study of the quality indicators of samples of fresh soft cheeses made by the acid-rennet and thermo-acid techniques of coagulation of proteins from the milk of cows with different genotypes showed that the type of β-casein did not affect the sensory characteristics of the cheese. However, with the acid-rennet technique of protein coagulation, the composition of cheeses from A1A1 milk on average was 51.60 %, 21.63 %, and 23.62 % of moisture, protein, and fat, respectively. Samples of cheeses from A1A2 milk on average contained 50.70 % moisture, 20.96 % protein, and 25.12 % fat. And samples of cheeses from A2A2 milk on average consisted of 52.50 % moisture, 20.70 % protein, and 23.71 % fat.

With the thermo-acid technique of protein coagulation, cheeses from A1A1 milk were characterized by an average moisture content of 55.13 %, protein content of 23.31 %, and fat content of 20.21 %. Samples of cheeses from A1A2 milk on average contained 58.13 %, 22.62 %, and 17.98 % of moisture, protein, and fat, respectively. And the composition of cheese samples from A2A2 milk on average consisted of 54.03 % moisture, 22.33 % protein, and 22.25 % fat.

3. Our calculations of the efficiency of the production of soft cheeses from the milk of cows with different genotypes in terms of β-casein showed that the average values of recovery of milk components in cheese with the acid-rennet technique of cheese production in samples from A1A1 milk lose 26.99 % of protein and 32.56 % fat. In samples of cheeses from A1A2 milk, the average loss of protein is 27.95 % and fat – 29.11 %, and from A2A2 milk – 28.32 % protein and 37.15 % fat. At the same time, the efficiency of cheese production from the milk of cows with the β-casein genotype A1A1 is on average 119.3 %, which is higher than that of A1A2 milk (by 4 %) and A2A2 (by 7 %).

With the thermo-acid technique of protein coagulation, the average values of recovery of A1A1 milk components in cheese showed that there is a loss of 21.32 % of protein and 42.40 % of fat. In samples of cheeses from A1A2 milk, the average loss of protein is 22.25 %, fat – 49.31 %. During the production of cheeses from A2A2 milk, an average of 22.65 % of protein and 41.14 % of fat is lost. The efficiency of cheese production from A2A2 milk in this case is 107.5 % on average, which is higher compared to A1A2 milk (by 9 %) and A1A1 (by 5 %).

Significant differences in technological properties can be observed between A2 and A1 milk, and A2 milk has worse cheese-making properties when using the acid-rennet technique of coagulation. However, the opposite dependences are observed when curdling protein fractions by the thermoacidic method – the actual yield of cheese is greater.

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, and the results reported in this paper.

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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.

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