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Study of the influence of the variation of raw milk having the kappa-casein gene genotype on the production of soft cheese

Ladyka, V.[1], Skliarenko, Y.[1,2]*, Bolhova, N.[1], Vechorka, V.[1], Synenko, T.[1], Pavlenko, Y.[1], Chernyavska, T.[1] and Shylo, V.[1]

[1]Sumy National Agrarian University, Sumy, Ukraine; [2]Institute of Agriculture in the Northeast of the National Academy of Agrarian Sciences of Ukraine, Sumy, Ukraine.

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Abstract It was established that under the condition of using the acid-rennet method of cheese production, protein losses were greater in the production of milk from cows of genotype AA and AB AA and AB (27.9 and 27.8%). Greater fat losses were found in milk samples from cows of the AB genotype (39.6%). The greatest losses of protein (22.6%) and fat (49.8%) were obtained under the condition of using the thermo-acid method of coagulation of proteins in milk samples from cows of the AA genotype. The superiority of milk samples from cows with AA and BB genotypes was established based on the ratio of actual and estimated cheese yield.

Keywords: Genotype, Soft cheese, Physico-chemical indicators, Sensory profile, Production efficiency

Introduction

A significant part of milk in most countries of the world processed into cheese (Cassandro *et al.,* 2008). Thus, in Israel, almost a third of the produced milk processed into hard cheeses, and the other third into soft cheeses (Lavon *et al.,* 2024). In Switzerland, almost 50% of the obtained milk processed into cheese (Guggisberg *et al.,* 2024).

Economically important elements of cheese production are milk coagulation time, coagulation quality, cheese yield (Ikonen *et al.,* 1999). The breed of cattle, the content of somatic cells in milk, the biochemical composition of milk and others have a significant influence on these properties of milk (Bittante *et al.,* 2012; Guinee *et al.,* 2006; Leitner *et al.,* 2019; Stocco *et al.,* 2018).

* **Corresponding Author:** Skliarenko, Y.; **Email:** Sklyrenko9753@ukr.net

Genetic variants of kappa-casein have a significant impact on the technological properties of milk. It has been proven that milk from cows with BB and AB genotypes for this locus has a higher coagulation rate and cheese hardness (Di Gregorio *et al.,* 2017). The economic condition of researching the genotypes of animals based on the kappa-casein gene lies in its influence on the process of milk coagulation and the quality of the obtained processed products - cheese and yogurt. It was also established that animals with genotype BB have a higher protein content in milk compared to milk from animals with genotypes AB and AA (Pandey *et al.,* 2024). In cheese production, milk obtained from cows with the BB genotype generated higher profit compared to milk from cows with the AA genotype. This is explained by the higher cheese yield and the shorter coagulation process time. (Lavon *et al.,* 2024).

The dependence of the milk technological properties on the kappa-casein gene genotype determines the carrying out of selection measures to increase the frequency of the B allele in dairy cattle populations. In the 90s of the last century, the share of the B allele in the population of the Holstein breed was only 0.17%, but it has significantly increased over the past twenty years (Lavon *et al.,* 2024). Populations of Ukrainian dairy cattle differ significantly in the frequency of kappa-casein gene genotypes. The lowest frequency of the BB genotype among local cattle is characteristic of black and white cattle obtained from breeders of the Holstein breed (Ladyka *et al.,* 2022; Ladyka *et al.,* 2023; Malikova *et al.,* 2024). Most of the research on the effect of the kappa-casein gene on the technological properties of milk aimed at research its influence on the production of hard cheeses. The influence of genotype on the production of soft cheese remains almost unexplored. This determined the purpose of our research. The purpose of the research was to determine the cheese making properties of raw milk from cows with different kappa-casein gene genotypes using the example of soft cheese production.

Materials and methods

The experimental location

Experimental studies were carried out at a breeding farm for breeding black and spotted cattle, which is located in the Sumy region of Ukraine (State Enterprise Experimental Farm of the Institute of Agriculture of Northern East of National Academy of Agrarian Sciences of Ukraine).

Genetic research

The determination of kappa-casein gene polymorphism was carried out in the genetics laboratory of the Institute of Physiology named after O.O. Bogomolets of the National Academy of Sciences of Ukraine using molecular biological analysis to identify alleles via real-time polymerase chain reaction (PCR).

Monovets (Sarstedt, Germany) were used to collect blood samples, followed by freezing to -20ºC. In order to obtain DNA for further genotyping, a kit for the purification of genomic DNA Monarch® New England BioLab (USA) was used. We will use primers to amplify the gene fragment: 5'-GAAATCCCTACCATCAATACC-3 '; 5'-CCATCTACGCTAGTTTAGATG-3 '.

To restrict the kappa-casein gene, we use the Hinf1 restrictase. After restriction, fragments of 113, 91, and 49 bp were detected in animals with genotype AA; 224, 113, 91, and 49 bp in animals with genotype AB; and 224 and 49 bp in animals with genotype BB. Electrophoretic separation of DNA restriction fragments was carried out in accordance with the methodological guidelines (Ladyka *et al*., 2022).

The principle of group formation

Three groups of cows (three heads in each) with the following kappa-casein gene genotypes: Group I – AA; II group - AB; III group - BB. were formed to provide the research.

Methodology for determining the qualityraw materials and finished products

10 kg of milk was taken from each animal during morning milking. After cooling the milk to a temperature of 4°C, the milk was delivered to the laboratory of the Sumy National Agrarian University within an hour.

For the production of Brynza cheese samples (using the acid-rennet method), traditional technology complying with the recommendations of DSTU 7065:2009 (National Standards of Ukraine) was used. Three samples were made from the milk of cows (10 liters each) that had genotypes AA, AB and BB according to the studied gene.

The stages of cheese production included: filtration of milk, pasteurization of milk for 2 seconds at a temperature of 72 °C. After that, bacterial preparations (mesophilic-thermophilic starter WhiteDaily 80, manufacturer Chr. Hansen, Denmark) are added to the cooled milk (33°C) in the amount of 2.8-4.0 g per 100 kg, $CaCl_2$ is additionally added in the amount of 10 g per 100 kg and rennet (100% chymosin "Albamax 600", Caglificio Clerici, Italy)) in the amount 2.9-3.5 ml per 100 kg.

Subsequently, the mixture was stirred for 12 minutes and then left until a curd formed.

The coagulation conditions of the formed mixture were: temperature 33°C and pH 5.3 units.

For 5 minutes, the formed curd was cut into 1×1×1 cm cubes, after which it was stirred for 10 minutes.

After a 12-minute settling period, up to 80% of the whey was drained, the formed curd mass was cut, and self-pressing in molds was carried out for 12 hours. Then, additional pressing was carried out for 3 hours. The cheese heads were salted in brine for 4 hours (20% NaCl at 14°C). For storage, the cheese was vacuum-packed. Storage conditions were up to 10 days at a temperature of (6±2) °C.

Samples of soft Adyghe-type cheese were produced using the thermo-acid method in accordance with DSTU 4395:2005 “Soft cheeses. General specifications.” Three samples were produced from milk obtained from cows (10 L each) with the AA, AB, and BB genotypes for the studied gene.

The cheese production stages included milk pasteurization at 95°C and acidification (1 L of a 5% citric acid solution per 100 kg of milk).

The formed curd mass was held for 7 minutes at 95°C and salted (NaCl at 2% of the resulting mass). The cooled mass was transferred into molds for self-pressing, which lasted 2 hours, during which it was turned twice. For storage, the cheese was vacuum-packed. Storage conditions were up to 10 days at a temperature of (6±2) °C.

To evaluate raw milk and received cheese samples, generally accepted methods were used in accordance with State Standards 3662:2018, 7065:2009, 4395:2005:

- aerometric method;
- potentiometric method;
- the Kjeldahl method;
- Gerber's method.

The international standard ISO 22935-2:2023 was used to describe organoleptic indicators.

The research was carried out in triplicate.

To determine the efficiency of cheese production, the following formulas were used (Stocco *et al*., 2018):

$$R_{dry\ matter,\ protein,\ fat} = \frac{Weight\ of\ Cheese_{dry\ matter, protein, fat}}{Weight\ of\ milk_{dry\ matter, protein, fat}} * 100\% \quad (1)$$

where R dry matter, protein, fat – recovery of milk components in a cheese, namely dry matter, protein and fat, %;

weight of cheese dry matter, protein, fat – dry matter protein and fat, mass in a cheese, g; weight of milk dry matter, protein, fat –dry matter, protein and fat mass in milk, g.

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

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

where E – cheese production efficiency, %;

C – experimental cheese yield, %;

ThC – theoretical cheese yield, %.

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

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

where C – chese yeild, %;

m_{cheese} – fresh cheese mass, kg;

m_{milk} – milk mass, kg.

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

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

where ThC – theoretical cheese yield, %;

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

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

Statistical processing of research results

Microsoft Excel from the Microsoft Office 2016 suite was used for statistical processing of the obtained results (Microsoft Corporation, Washington, USA). The reliable deviation (p) calculated by us has a level lower than 0.05. Accordingly, the value of the accuracy index (P) exceeds 0.95.

Results

As a result, of research on the physical and chemical properties of milk, we found that there was no significant difference between the experimental groups, except for the fat content in milk (Table 1).

Table 1. Characteristics of raw milk from cows with different kappa-casein gene genotypes (n=3, $p \leq 0.05$)

Indicators	**Experimental groups (M ± m)**		
	I	II	III
Acidity, units pH	6.81±0.02	6.75±0.01	6.74±0.02
Density, kg/m3	1027.0±1.00	1026.3±0.67	1027.7±0.33
Mass fraction of dry matter, %	12.7±0.15	12.6±0.09	12.8±0.01
Mass fraction of protein, %	2.91±0.05	2.88±0.03	2.96±0.01

The first experiment, using the acid-rennet method of proteins coagulation, milk samples from cows with genotype AB (experimental group II) and BB (experimental group III) differed by higher average moisture content in cheese. The higher average content of protein in milk differed from cows with genotype BB, fat – AA (experimental group I). No significant difference found in terms of acidity.

The second experiment, using the thermos-acid method of proteins coagulation, the higher average moisture content is characteristic of samples of cheese made from milk of cows with the AA genotype (experimental group I). The average protein content was dominated by samples from cows with the BB genotype (experimental group III), fat – AB (experimental group II). The studied samples did not differ much in terms of acidity (Table 2).

Table 2. Dependence of physical and chemical parameters of soft cheese on the genotype of cows according to the kappa-casein gene (n=3, $p \leq 0.05$)

Indicators	**Experimental groups (M±m)**					
	acid-rennet method			thermo-acid method		
	I	II	III	I	II	III
Moisture content, %	50.7±0.21	51.9±0.40	51.4±0.43	58.2±0.16	54.3±0.42	54.4±0.22
Protein content, %	21.0±0.34	20.8±0.20	21.9±0.28	22.6±0.37	22.3±0.22	23.6±0.36
Fat content, %	25.1±0.15	24.1±0.17	23.5±0.11	17.9±0.50	22.3±0.64	20.9±0.55
Acidity, units pH	5.25±0.01	5.23±0.01	5.22±0.01	5.90±0.01	5.89±0.01	5.89±0.01

The expert group conducted a sensory analysis of soft cheeses. The following characteristics were examined: type, taste, aroma, texture, colour and pattern on the cross-section, shape (Figure 1).

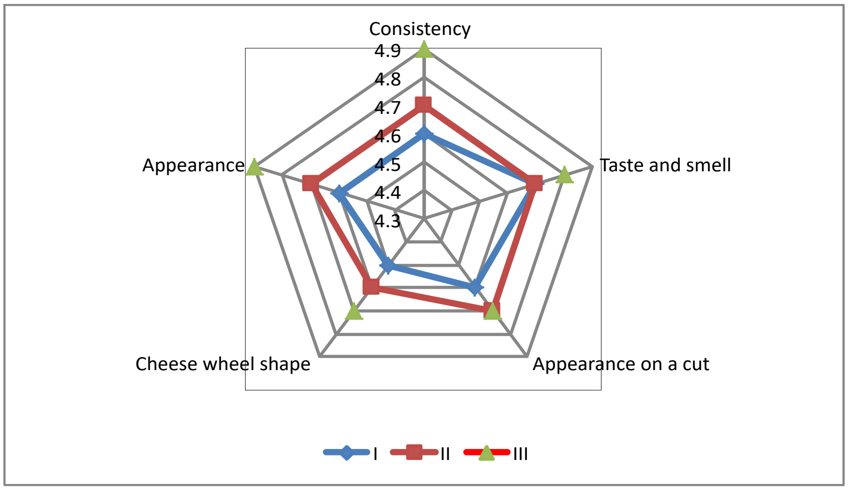

Figure 1. Sensory profile of soft cheese samples from the milk of cows with different kappa-casein genotypes produced by the acid-rennet method of protein coagulation

Samples of soft cheeses produced using the acid-rennet method from cow's milk with different genotypes for the studied gene showed certain differences in sensory characteristics.

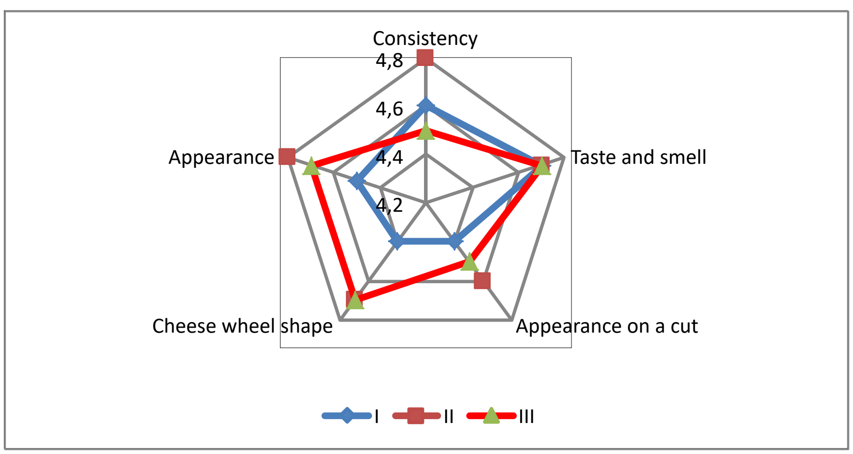

Figure 2. Sensory profile of soft cheeses samples from the milk of cows with different kappa-casein genotypes produced by the thermo-acid method of proteins coagulation

The highest average scores for appearance, taste, aroma, and texture were obtained by samples of group III. Samples of the first experimental group had the lowest average sensory scores. Samples of the second experimental group showed intermediate values.

Samples of soft cheeses produced using the thermo-acid method from the studied groups did not differ in their average scores for taste and aroma. For the other sensory characteristics, samples of the second experimental group obtained the highest average scores. Samples of the first experimental group received lower average scores for the appearance of the cut surface, the shape of the cheese heads, and overall external appearance. In terms of texture, the lowest average scores were obtained by samples of the third experimental group (Figure 2).

According to the research results and the corresponding calculations, it was established that the higher average yield of cheese is characteristic of samples from cows with genotype AB (acid-rennet method of protein coagulation; thermo-acid method of protein coagulation) and AA (thermo-acid method of protein coagulation). Although it should be noted that the difference with samples from cows with the BB genotype was within 0.3-0.9% and 0.4-0.5%, respectively (Figure 3).

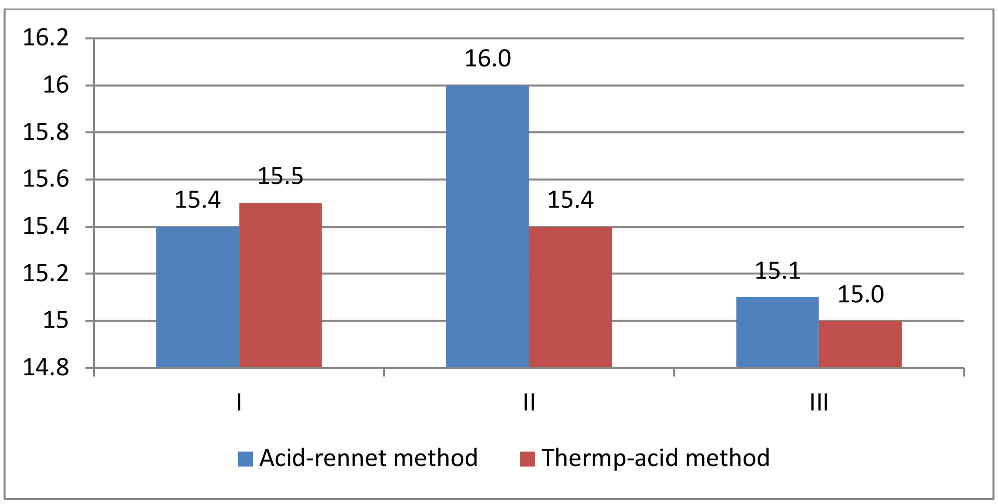

Figure 3. Dependence of yield of soft cheeses from milk of cows with different kappa-casein gene genotypes

The theoretical cheese yield calculation corresponded to the trends of experimental studies (Figure 4).

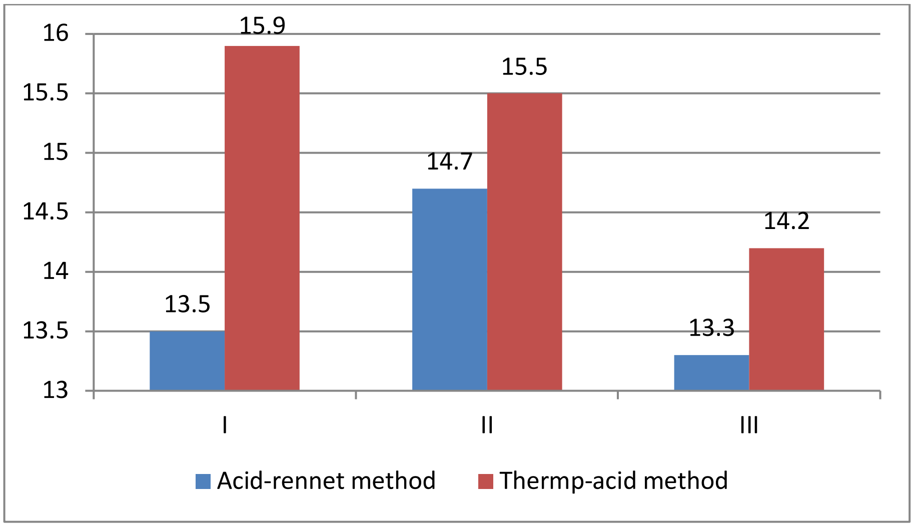

Figure 4. Theoretical output of soft cheeses produced from the milk of cows with different kappa-casein genotypes

The results of the first experiment indicated a greater loss of protein during the acid-rennet method of cheese production in milk samples from cows with genotype AA (experimental group I) and AB (experimental group II) (27.9 and 27.8%), fat - AB (experimental group II) (39.6%) (Table 3).

Table 3. Dependence of indicators of the efficiency of soft cheese production on the genotype of cows according to the kappa-casein gene

Indicators	**Experimental groups**					
	acid-rennet method			acid-rennet method		
	I	II	III	I	II	III
Recovery of milk protein in cheese, R,%	72.1±0.04	72.2±0.06	74.0±1.10	77.7±0.04	77.4±0.06	79.7±1.19
Recovery of milk fat in cheese, R,%	70.5±2.88	60.4±3.25	70.7±3.37	50.2±1.67	55.9±0.87	62.9±0.98
Cheese production efficiency	114.0±3.88	109.0±3.90	113.0±3.98	97.1±6.01	99.5±0.50	105.5±3.87

Discussion

Samples obtained from cows with the homozygous BB genotype are characterized by a higher protein content in milk. The obtained results are fully consistent with the results of other researchers (Pandey *et al*., 2024). The lower yield of cheese from milk from cows with the BB genotype compared to milk from animals with the AA and AB genotypes does not agree with the results of other studies (Lavon *et al*., 2024).

Our results regarding the recovery of milk components indicate the influence of the κ-casein gene on cheese production, which is consistent with the findings of other researchers (Lavon *et al*., 2024).

Our results indicate that the milk meets the requirements of the national standard, regardless of the genotype for the kappa-casein gene.

It was established that when using the acid-rennet method of protein coagulation, samples of milk from cows with AB and BB genotypes differed by higher average moisture content in cheese, higher average protein content - BB, and higher average fat content - AA. It was established that when using the thermo-acid method of coagulation of proteins, cheese samples made from milk of cows with genotype AA are distinguished by a higher average moisture content in cheese samples, average protein content - BB, and average fat content – AB. Experts determined that the obtained samples of soft cheeses had a good appearance and a characteristic fermented milk taste and aroma. The consistency is satisfactory; this parameter is typical for this group of cheeses. The research results indicate greater losses of protein during the acid-rennet method of cheese production in milk samples from cows with genotype AA and AB (27.9 and 27.8%), fat - AB (39.6%). The largest protein losses during the thermos-acid method of proteins coagulation in milk samples from cows with genotype AA (22.6%), fat - AA (49.8%). Samples of milk from cows with AA and BB genotypes characterized by a better ratio of experimental cheese yield to theoretical yield in the acid-rennet method of cheese production, and BB in the thermo-acid method of production.

Acknowledgements

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Conflicts of interest

The authors declare no conflict of interest.

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