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CHAPTER 2

JUSTIFICATION OF THE FEASIBILITY OF USING A2 MILK IN THE PRODUCTION OF SOME DAIRY PRODUCTS

ABSTRACT

The prospects of using A2 cow's milk in the production of dairy products were considered and analyzed. The object of research is the method of enriching A2 milk and the technology of hard cheeses made from raw milk from cows with different β-casein genotypes (A1A1, A1A2, A2A2). When digesting A2 milk, β-casomorphin-7 is not formed, which negatively affects the physiology of the gastrointestinal tract, cardiovascular, nervous and endocrine systems. It is recommended to use milk to which biologically active substances have been added, therefore the issue of its enrichment is relevant. In industrial conditions, artificial vitamin and mineral complexes are used to enrich milk. Taking into account that natural and synthetic substances act differently on the human body, it is proposed to use processed derivatives of carrots (Daucus carota) as a natural food additive. The use of carrot powders improves the amino acid spectrum of milk. The mass concentration of amino acids in A2 milk enriched with carrot peel powder was 4.87 g/100 g. The highest concentration, g/100 g, of glutamic acid (0.84), proline (0.50), aspartic acid (0.42), leucine (0.41), valine (0.35) was found. It was established that the consumption of 200 g of such milk provides the daily needs of the body in essential amino acids: threonine, leucine and phenylalanine (by 16 %), methionine (by 4 %), isoleucine (by 14 %), lysine (by 18 %), valine (by 20 %). It was established that A2 milk, enriched with powder from whole carrot roots, has a higher content of carotenoids (0.1068 mg/100 ml), providing the body's need for them by 1.4 %. Such milk can be an additional source of vitamin A produced in the human body. It is recommended to use powder from whole carrot roots for the enrichment of A2 milk in industrial conditions. Physicochemical indicators and cheeseability of milk of cows with different genotypes were studied. The content of fat, protein and solids in the milk of cows with the β-casein genotype A2A2 were slightly higher compared to A1A1 and A1A2. A comprehensive study of the quality indicators of hard cheese samples showed that the type of β-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 %). The amino acid profile of cheese from the milk of cows with the β-casein A1A2 and A2A2 genotype 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 β-casein genotype A1A2 was higher (mean value 13.1 %) than with A1A1 and A2A2. The obtained results are of practical importance, since it is possible to take into account how changes in the β-casein genotype in raw milk can affect the yield of cheese and, therefore, the profitability of production.

KEYWORDS

A2 milk, hard cheeses, enriched A2 milk, carrot powder, milk carotenoids, milk amino acids, β-casein A2, biological value, cheese yield, milk proteins.

CHAPTER 2

Milk is the first product in the diet of all mammals [1]. It is an affordable, popular and nutritious food product that contains a variety of important macronutrients. Milk contains about 3.2 % proteins, 4.8 % lactose and 3.5 % fat [2]. Every day, billions of people around the world consume milk and milk products, which play a key role in healthy nutrition and human development throughout life. Dairy products make up about 25–30 % of the average human diet.

At the same time, there is a large number of people who categorically refuse to drink milk. This is due to many factors, including the negative effects on the body when consuming milk and dairy products [3].

Adverse reactions to cow's milk are mainly described as intolerance to the lactose disaccharide contained in milk [4]. Lactose intolerance [5] and an allergy to dairy products are recorded in 65 % of the population. Eating dairy products that lead to symptoms of lactose intolerance usually results in temporary symptoms without harming the gastrointestinal tract.

According to the World Allergy Organization (WAO), 6–8 % of children under the age of 3 have food allergies, and 4.9 % of children have an allergy to cow's milk protein [6]. This food allergy is manifested by a wide range of clinical syndromes due to immunological reactions to cow's milk proteins [7]. Cow's milk allergy is an allergic reaction to the protein contained in cow's milk [8]. Allergy to cow's milk mainly occurs in childhood and often outgrows with age, although 15–20 % of allergic children become persistently allergic with elevated levels of immunoglobulin E (IgE).

Milk and milk products provide 18.1 % of the total daily protein requirement. Milk proteins are a heterogeneous group of polymeric compounds that have a wide range of different molecular structures and properties. About 30 % of the total protein contained in cow's milk is β-casein. It can be in different forms: A1A1, A2A2, or in the form of a combination of A1A2. The only difference between these two variants of β-casein (β-CN) is that the same position contains different amino acids. Type A1 contains histidine, which promotes enzymatic hydrolysis, and type A2 contains proline, which prevents proteolytic cleavage [9, 10]. The A2 variant is present in the milk of many mammals, including humans, goats, sheep and cows, while the A1 variant is present only in cattle.

Some studies have found that a key polymorphism in the β-casein protein may contribute to the association between cow's milk and human health [11–13]. Cow's milk protein is one of the most common allergens encountered by young children. Significant allergens are casein proteins (alpha-s1-, alpha-s2-, beta- and kappa-casein) and whey proteins (alpha-lactalbumin and beta-lactoglobulin).

It is known that milk allergens retain their biological activity even after boiling, pasteurization, ultra-high-temperature processing and evaporation for the production of dry infant formulas. The latest recommendations issued by the World Allergy Organization state that goat, sheep and buffalo milk should not be used as a substitute for children with an allergy to cow's milk.

Therefore, it is necessary to look for other ways to solve the problem of intolerance to milk and dairy products.

2.1 CHARACTERISTICS OF A2 MILK

In recent years, a new type of cow's milk has appeared on the market – "Milk A2". A2 milk contains only the A2 variant of the beta-casein protein. The history of A2 milk began not so long ago. At the beginning of the 20th century, in 2000, scientists from New Zealand investigated the value of A2 milk. It was New Zealand that patented A2 milk and began to take the first steps in spreading information about its benefits. However, for the first time A2 milk entered the market in Australia. It was there that other dairy products from A2 milk – cream, baby formula, ice cream, yogurt, etc. – were developed for the first time. In 2003, the production of A2 milk also started in the United States with considerable success.

A little later, in 2011, Great Britain joined the countries listed above. There, A2 milk was produced with the aim of extending the territory of the kingdom and Ireland. The first batches of A2 infant formula were sent to China in 2013.

Cows with the A2A2 gene produce only A2 milk. Cow breeds such as Jerseys, Guernseys, Normans and Brown Swiss have a higher percentage of the A2 gene than Holsteins. It takes many generations to form an A2 herd. In Ukraine, breeds in which the A2A2 genotype predominates include Lebedyn breed (**Fig. 2.1**).

The breed was bred to improve local peasant cattle (Ukrainian gray) and create hybrids of the Swiss breed. Milk from cows of the Lebedyn breed has an optimal ratio of milk fat and protein. Individuals differ in long lactation. Milk is a high-quality product with excellent taste properties.

Sales of A2 milk are growing rapidly, and Market Watch notes that the market is expected to grow by an average of 22 % by 2025. In addition to natural A2 milk, Nestle and A2 Milk companies began to specially manufacture A2 baby food, which is trusted by global experts and consumers in Australia, England, the USA and China.

Fig. 2.1 A cow of the «Lebedyn» breed

A2 milk and A2 dairy products are characterized by the fact that they do not contain the A1 variant of β-casein. A2 milk is natural cow's milk, which has a number of advantages in several respects, namely:

– milk containing only A2 β-casein has the potential to promote the production of the antioxidant glutathione in human blood plasma;

– mixtures based on A2 milk help to relieve children's colic and normalize metabolism;

– milk and dairy products A2 do not cause symptoms of neurological and mental disorders characteristic of opioids contained in food;

– when consuming A2 milk, people with lactose intolerance have fewer symptoms of gastrointestinal disorders;

– milk proteins A2A2 also increase the expression of the opioid receptor much less, compared to milk proteins A1A1;

– in the urine of patients with autism and schizophrenia, when consuming only A2A2 milk, the level of BCM-7 content decreases;

– consumption of A2A2 milk helps to prevent the risk of developing type 1 diabetes.

During the enzymatic hydrolysis of milk A1, the peptide β-casomorphin-7 (BCM-7) is formed, which is a known agonist of μ-opioid receptors. Beta-casomorphins are opioid molecules. The opioid system and its associated signaling pathways, feedback mechanisms, and physiological cascades are highly conserved across mammalian species, where it plays a diverse range of roles and interacts with many other important physiological systems.

Its impact on human health, including effects on the nervous and hormonal systems, early development, lactation, response to environmental stimuli, mother-child bonding, etc., is an extremely important topic in medicine and science and is still the subject of significant research and development.

It can directly affect the physiology of the gastrointestinal tract, as well as other parts of the body, for example, the cardiovascular, nervous and endocrine systems [14]. Lactose intolerance is often considered the cause of such disorders. However, there is increasing evidence that beta-casein A1A1 is also associated with cow's milk intolerance [15].

There are scientifically proven facts that consumption of milk with β-casein variant A2A2 prevents cardiovascular diseases [16] and prevents type 1 diabetes [17]. A2 milk contributes to the formation of less severe symptoms of autism and schizophrenia [18].

The negative impact of milk with the β-casein A1 genotype on human health is also highlighted in works [19–21]. The relationship between the β-casein A1 variant of milk and various diseases, neurological disorders such as schizophrenia, autism and sudden infant death syndrome has been shown. The link between neurological effects and BCM-7 depends on its ability to cross the blood-brain barrier and act as an opioid peptide. Its interaction with opioid receptors causes exorphin activity in the brain, which unfolds, for example, as a change in behavior, an analgesic effect, etc. An additional neurological outcome that was often investigated was the analgesic effect. Most studies compared this effect with other BCM fragments and investigated the opioid pathway to achieve the analgesic effect, including binding properties (e.g., opioid receptor affinity).

However, according to the authors [22], there is no convincing evidence that β-casein A1 in milk has an adverse effect on humans. This statement is also confirmed in [23]. 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 direction is necessary.

Consumption of A2 has some functional advantages compared to A1, which are directly related to the effect of BCM-7 on human physiology. These functional properties are well established through extensive research over the past several decades.

Currently, most milk sold on the market contains a mixture of A1 and A2 β-casein, which can be obtained from heterozygous A1A2 cows or a mixture of milk from homozygous A1A1 and A2A2 animals.

The study of sensory quality, color and composition of A2 milk compared to A1 milk showed that different genotypes do not affect the smell, taste or general perception of milk. However, some differences were found in the color. The color parameters of A2 milk were closer to the gold standard color, which made it more attractive to consumers without artificial food colors. In dairy products made from milk from cows with the A2A2 genotype, the amino acid sequence of the protein is preserved regardless of the methods of its processing.

Milk must be obtained from healthy cows of genotype A2, in which no infectious diseases have been detected and which are under veterinary supervision. Milk must be produced in compliance with established hygienic requirements for the production of raw milk suitable for human consumption. Technical conditions for raw milk A2 have been developed A2 whole milk TU U 01.4-00447853-014:2022.

According to organoleptic indicators, milk must meet the requirements listed in **Table 2.1**.

Table 2.1 Organoleptic criteria of raw A2 milk

Name of indicators	Characteristic
Taste and smell	Clean, characteristic of fresh milk, without extraneous taste and smell
Color	From white to light cream
Consistence	A homogeneous liquid without flakes of protein and sediment

In terms of physical and chemical parameters, milk must meet the requirements listed in **Table 2.2**.

Table 2.2 Physical and chemical criteria of raw A2 milk

Name of indicators	Normalized values for varieties	
	extra	higher
Density (at a temperature of 20 °C), kg/m^3 not less than	1028.0	1027.0
Mass fraction of dry substances, %	≥12.0	≥11.8
Acidity		
°T	from 16 to 18.0	
pH	from 6.72 to 6.61	
Purity group, not lower than	I	
Freezing point, °C, not higher than	minus 0.520	
The temperature of milk during reception, °C, is not higher than	8	

After milking, the milk must be cleaned and cooled to a temperature no higher than 6 °C. For milk that will be processed at the enterprise no later than 2 hours after milking, the temperature is not set. Milk received at the processing plant is quickly cooled to a temperature not higher than 6 °C and stored at this temperature until processing.

According to biochemical indicators, milk must meet the requirements listed in **Table 2.3**.

Table 2.3 Biochemical criteria of raw A2 milk

Name of indicators	Normalized values for varieties	
	extra	higher
Allele C (A2) of the beta-casein gene (CSN2)	Present	
Allele C (A1) of the beta-casein gene (CSN2)	Missing	

Determination of allelic variants of the beta-casein gene (CSN2) should be carried out once a month, and if necessary, unscheduled. The producer of milk raw materials must guarantee that

this raw material is obtained from genotyped allele C (A2) of the beta-casein gene (CSN2), identified and registered animals, as well as the absence of inhibitors and adulterating substances.

Determination of biochemical (genetic) indicators of milk is carried out using molecular biological analysis – allele recognition analysis to determine the genotype of samples. The basis of the analysis is polymerase chain reaction (PCR) in real time. The analysis consists in quantitative detection of the fluorescent signal.

This type of analysis makes it possible to recognize the allelic polymorphism rs43703011 of the β-casein gene (CSN2) with the aim of further dividing cows by genotypes (A1A1, A1A2 and A2A2).

The purpose of allele recognition is to classify unknown samples as follows:

– homozygotes (samples containing only allele 1);

– homozygotes (samples containing only allele 2);

– homozygotes (samples containing allele 1 and allele 2).

When recognizing alleles, fluorescent dye-labeled probes specific for each allele are included in the PCR process.

The probes contain different fluorescent reporter dyes to detect the amplification of each allele.

According to microbiological indicators, milk must meet the requirements listed in **Table 2.4**.

Table 2.4 Microbiological criteria of raw milk

Name of indicators	Normalized values for varieties	
	extra	higher
Number of mesophilic aerobic and facultatively anaerobic microorganisms (KMAFAM), thousand CFU/cm^3	≤100	≤300
Number of somatic cells, thousands/cm^3	≤400	≤400

According to safety indicators, A2 milk must meet the requirements specified in **Table 2.5**.

For the production of A2 whole milk, whole raw commercial milk is used, obtained from cows with the A2A2 genotype, when purchased from dairy farms, collective agricultural enterprises, private and farm households, regardless of the form of ownership and types of activity.

Considering that A2 milk and A2 milk products have a higher value in the world compared to conventional milk products, increasing the production of A2 milk products will contribute to increasing the profitability of the dairy sector.

The purpose of the study is to develop a method of enriching A2 milk with amino acids, carotenoids and to determine the influence of the protein composition of raw milk on the yield of hard cheese and its nutrient content. This will make it possible to increase the biological value of A2 milk and reduce the manifestations of allergic symptoms when consuming it, as well as selectively select dairy breeds of cows suitable for cheese production according to their protein composition.

Table 2.5 Safety indicators of milk

Name of indicators	Normalized values for varieties
The content of toxic elements, mg/kg, no more than:	
the culprit	0.1 (0.05)
cadmium	0.03 (0.02)
arsenic	0.06
mercury	0.005
copper	1.0
zinc	5.0
Pesticides, mg/kg, no more than:	
hCG (gamma isomer)	0.05
Hexachloran	0.05
Antibiotics, no more than:	
Tetracycline, units/g	0.01
Streptomycin, units/g	0.01
Penililine, units/g	0.5
Content of radionuclides, Bq/kg, no more than:	
cesium Cs^{137}	100
strontium Sr^{90}	20
The content of mycotoxins, mg/kg, not more than:	
aflatoxin B_1	0.001
aflatoxin M_1	0.0005
Nitrates, mg/kg, not more than	10
Hormonal drugs mg/kg, no more than:	
diethylstilbestrol	Not allowed
estradiol-17	0.0002

To solve the set goal, the following tasks should be performed:

– analyze the amino acid composition of A2 milk enriched with carrot processing products;

– determine the content of carotenoids in A2 milk enriched with carrot root powders with and without skins;

– develop a scheme of A2 milk technology using waste-free use of dry carrot roots (without removing the skin);

– investigate the physical and chemical parameters of raw milk from cows with different genotypes for β-casein (A1A1, A1A2, A2A2);

– calculate and compare the yield of hard cheese from the milk of cows with different genotypes according to β-casein;

– investigate the organoleptic and physico-chemical parameters of samples of hard cheeses made from the milk of cows with different β-casein genotypes;

– establish the amino acid profile of hard cheeses from the milk of cows with different genotypes according to β-casein.

The object of research is the method of enriching A2 milk and the technology of hard cheeses made from raw milk from cows with different β-casein genotypes (A1A1, A1A2, A2A2).

Subjects of research: physicochemical indicators of raw milk from cows with different genotypes for β-casein (A1A1, A1A2, A2A2); yield of hard cheese from this milk and its quality indicators.

Research hypothesis: consumption of milk containing only β-casein A2 will reduce allergic manifestations; the use of a natural food additive to enrich milk with useful nutrients will contribute to their better assimilation by the body; 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 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.

2.2 METHOD OF INCREASING THE BIOLOGICAL VALUE OF A2 MILK

Milk is an attractive product for fortification because it has a high nutritional density in a small volume and relatively low price.

The biological value of milk can also be improved by enriching it with functional nutrients. Dairy products enriched with trace elements usually contain calcium, phosphorus, magnesium, iron, zinc, copper, manganese, selenium, iodine, chromium, molybdenum and cobalt. In addition, milk is enriched with vitamins A, D, C, E and K and biotin, pantothenic or folic acids [24].

It has been established that milk enriched with micronutrients can be an effective means of reducing anemia in children under three years of age in developing countries [25]. Most of these biologically active substances are manufactured artificially in industrial conditions. They help to adjust the chemical composition of the product, but are not used by the body in the same way as their natural counterparts. In addition, their excessive use can be harmful.

The addition of linseed oil, phytosterols, and polydextrose had a positive effect on the physicochemical and organoleptic properties of milk. Such milk was well stored at refrigeration temperature for 1 week, having almost unchanged organoleptic, physicochemical and microbiological properties [26]. But the use of vegetable fats increases the fat content of milk, which does not meet the needs of some consumers.

Milk enrichment allows not only to increase the biological value, but also to increase its added value. The work [27] shows the results of research on the decrease in demand for plain, pure milk and the growing tendency to increase the consumption of flavored milk of medium and high fat content. However, artificial flavors should not be used in baby food.

Enriching milk with vitamin A and giving it certain organoleptic properties is possible due to the use of carotenoids. It is known that carotenoids have antioxidant properties, provitamin A activity, immune, endocrine and metabolic activity, play a role in cell cycle regulation [28].

Therefore, milk enriched with carrot carotenoids has better storage capacity. Apparently, this is due to the fact that β-carotene slows down microbiological processes. A natural source of carotenoids is carrot (Daucus carota), 35 % of the carotenoids of dry carrots are converted into vitamin A in the living organism. However, there are no industrial technologies for using carrots to enrich milk.

In industrial conditions, the vitamin complex FT 041081EU, which contains 12 important vitamins (A, D, E, C, Bc, B1, B2, B6, B12, PP, B5, biotin) and mineral FT 042836EU, is used in industrial conditions to enrich dairy products. which includes Fe, Zn and I [29]. It is not yet clear how successfully synthetic biologically active substances are assimilated and used by the body.

All this allows to state that the existing milk enrichment technologies mainly involve the use of synthetically created vitamin and mineral complexes. There is practically no assortment of milk with the use of natural food additives. Despite the fact that natural vitamins are absorbed much better than synthetic ones [30].

The biological value of milk is also determined by its amino acid composition, since amino acids participate in the biosynthesis of cells, which is very important for the vital activity of the human body [31]. It is advisable to increase the concentration of amino acids in milk due to natural additives.

Therefore, the development of a method of enriching milk containing β-casein A2 with natural food additives is an urgent issue.

A technique for enriching A2 milk with carrot powders has been developed (**Fig. 2.2**). Initially, it is recommended to conduct a molecular biological analysis of the milk of various cows to determine the form of β-casein. It is recommended to separate cows with the A2A2 genotype from animals with other genotypes in order not to conduct molecular biological analysis of each batch of milk. For the purpose of periodic monitoring, it is advisable to analyze the milk 1 time per month.

Determined milk β-casein in milk samples used in the experiments by a molecular biological method, which is based on a real-time polymerase chain reaction using the 7500 Fast Real-time System (Applied Biosystems) test system. This type of analysis makes it possible to recognize the allelic polymorphism rs43703011 of the β-casein gene (CSN2) by genotypes (A1A1, A1A2, and A2A2). Alleles were recognized using fluorescent probes (Taq Man) specific to each allele, marked with dye. Taq Man Universal PCR Master Mix reaction mixture, electronic dispensers with adapter, and mechanical variable volume dispensers (20–200) μL, (200–1000) μl were used.

After assessing the quality of milk, it matures in tanks within 24 hours. After the aging process, the milk is heated and separated. The cream selected during the separation process is cooled

and stored for no more than 6 hours. Some of it is used to normalize milk and dairy products, and some is used for the production of butter.

The normalized mixture is cleaned and homogenized at a pressure of 18–20 MPa. A homogenized milk mixture enriched with carrot powder is sent for pasteurization. Pasteurization of the mixture is carried out for 10–15 minutes at a temperature of 70–75 °C. Pasteurized milk is filtered and subject to ultra-pasteurization at a temperature of 150 °C for 5 seconds. Pasteurized milk is quickly cooled to 0–4 °C, packaged and sent for storage. It is recommended to use carrot powder made from whole roots (10 % by weight of milk) since the peels contain a large number of useful nutrients.

Carrot pulp is recommended to be used in the production of fermented milk products as a food additive. Enriched milk is pasteurized at a temperature of 90–95 °C for 15–20 s. After which it is cooled, packaged in consumer containers, and sent for storage. A feature of the developed technique (**Fig. 2.2**), involving the enrichment of A2 milk with carrot powder, is the waste-free processing of vegetables, in particular carrots; carrot pulp is proposed to be used as a food additive for the production of fermented milk products. The use of the proposed technique in production will expand the range of dairy products for functional purposes, in particular A2 products. This will partially solve the problem of ensuring the demand for medium-fat flavored milk.

The study used whole (fat content of 3.85) and skimmed A2 milk, obtained from cows at the vivarium of Sumy National Agrarian University. In addition, industrial samples of A2 milk made by Ichnianskyi Milk Powder and Butter Plant PJSC with a standardized mass fraction (2.5 %) of fat were used.

5 milk samples were analyzed (**Fig. 2.3**):

– whole A2 milk with a fat mass fraction of 3.85 % (Control);

– whole A2 milk (3.85 %) with the addition of 10 % powders made from peeled carrots (Sample 1);

– whole A2 milk (3.85 % fat) with the addition of 10 % powders made from carrot peels (Sample 2);

– skimmed A2 milk with the addition of 10 % powders made from carrot peels (Sample 3);

– industrial sample of A2 milk (2.5 % fat) with the addition of 10 % raw carrots (Sample 4).

For the manufacture of carrot powders in the laboratory, let's use carrots of the variety Shantane. Thoroughly washed root vegetables were disinfected with chlorine dioxide, rinsed with clean running water, peeled, and cut into slices (2 mm thick). Slices were dried at 50–60 °C for 2 hours in a 1.8 kW infrared laboratory dryer. After drying, the material was crushed in a disk mill LZM-1 and sifted through a brass sieve No. 015. Only a fraction less than 0.15 mm in size was used to enrich milk. In the same way, carrot peels were processed into powders.

Powders and fresh carrots, crushed into mush, were introduced into milk and thoroughly mixed for 30 minutes. Next, the enriched milk mixture was heated to a temperature of 70–75 °C and filtered. As filter partitions, filters for milk strainer FARMA (The Netherlands) with a diameter of 95 mm were used. Filtered enriched milk was pasteurized (t=90–95 °C, τ=15–20 s). Pasteurized milk was cooled to 20 °C and analyzed.

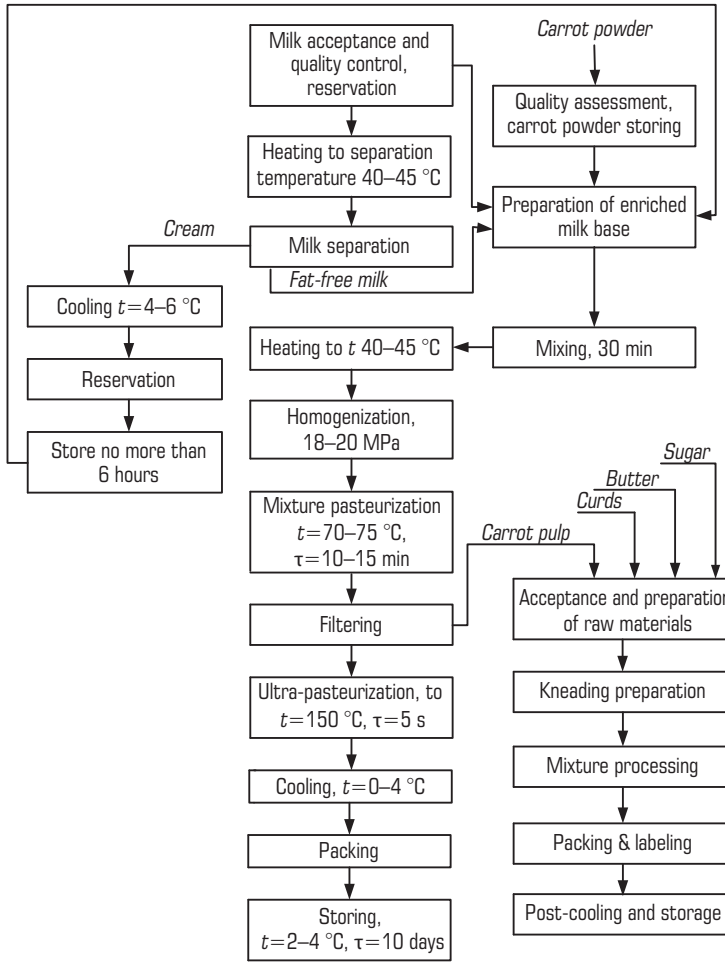

Fig. 2.2 Technological scheme of production of enriched A2 milk enriched with powdered carrot powder from unpeeled carrots (with peel)

It is possible to determine amino acids in the milk by the HPLC method using a liquid chromatograph Agilent 1200 (USA) by diode-matrix detection with a wavelength of 280 nm. The chromatographic division was the same and was carried out on column C18 at a temperature of 16 °C. Acetonitrile and acetate buffer (pH 6.0) were used as the mobile phase in the gradient elution mode

with an eluent flow rate of 1.0 ml/min. 18 amino acids were recognized in milk, including 7 essential ones (threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) (**Table 2.6**).

Fig. 2.3 Research samples of milk A2

The highest mass concentration of amino acids (4.87 g/100 g) was in whole A2 milk enriched with carrot powder made from carrot peels (Sample 2). It is worth noting that the concentration of amino acids in A2 milk without additives was 2.59 g/100 g. Most of all was found in Sample 2, g/100 g: glutamic acid (0.84), proline (0.50), aspartic acid (0.42), leucine (0.41), valine (0.35).

Sample 3, made on the basis of skimmed milk and carrot powder from the peels, also had a high content of mass concentration of most amino acids, compared to milk without additives (Control).

The amino acid composition of Sample 1 was slightly better than Control as there was an increase in the mass concentration of some essential amino acids. Namely, g/100 g: glutamic acid by 0.16; aspartic acid by 0.13; lysine by 0.09; valine, arginine, phenylalanine by 0.07; threonine, leucine, and isoleucine by 0.06; tyrosine by 0.05; serine and proline by 0.04; glycine and histidine by 0.02.

When using fresh carrots to enrich milk (Sample 4), compared to Sample 1 and Control, an increase in the mass concentration of proline by 0.05 and 0.09 g/100 g was observed, respectively. The concentration of aspartic acid in this sample by 0.15 g/100 g was higher than in milk without additives.

In particular, in the composition of milk enriched with carrot powder from carrot peels, a greater amount of glutamic and aspartic acid, proline, leucine and valine was found, compared with control. In addition, in the composition of prototypes of A2 milk, there is also an increase in the content of proline, leucine, and valine, that is, amino acids that ensure the assimilation of the protein complex. The use of 200 g of milk enriched with carrot powders will provide part of the daily need for essential amino acids (**Table 2.6**).

The daily requirement for threonine, leucine, and phenylalanine is provided by 16 %, methionine – by 4 %, isoleucine – by 14 %, lysine – by 18 %, valine – by 20 %. This fully coincides with the conclusions of many researchers. It is known that the following amino acids are indispensable for cows: Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val. It is possible to identify 7 essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine.

Table 2.6 Mass fraction of amino acids in prototypes of A2 milk

Amino acid	Norm per day	Control	Sample 1	Sample 2	Sample 3	Sample 4
	g	g/100 g				
Aspartic acid	3	0.19	0.32	0.42	0.39	0.34
% of daily need		6	11	14	13	11
Threonine	2–3	0.09	0.15	0.21	0.20	0.17
% of daily need		4	6	8	8	7
serine	3	0.17	0.21	0.24	0.24	0.18
% of daily need		6	7	8	8	6
Glutamic acid	5	0.53	0.69	0.84	0.76	0.71
% of daily need		11	14	17	15	14
Proline	5	0.27	0.31	0.50	0.42	0.36
% of daily need		5	6	10	8	7
Glycine	0.3	0.04	0.06	0.08	0.07	0.07
% of daily need		13	20	27	23	23
Alanine	3	0.10	0.12	0.17	0.16	0.11
% of daily need		3	4	6	5	4
Cystine	2–3	0.01	0.02	0.03	–	0.01
% of daily need		0.4	0.8	1.2	–	0.4
Valine	3–4	0.16	0.23	0.35	0.31	0.24
% of daily need		5	7	10	9	7
Methionine	2–4	0.01	0.01	0.06	0.07	0.05
% of daily need		0.3	0.3	2	2	2
Isoleucine	3–4	0.13	0.19	0.26	0.23	0.20
% of daily need		4	5	7	7	6
Leucine	4–6	0.28	0.34	0.41	0.37	0.35
% of daily need		6	7	8	7	7
Tyrosine	1	0.11	0.16	0.20	0.19	0.16
% of daily need		11	16	20	19	16
Phenylalanine	2–4	0.13	0.20	0.25	0.22	0.19
% of daily need		4	7	8	7	6
Histidine	2	0.08	0.10	0.14	0.14	0.09
% of daily need		4	5	7	7	5
Lysine	3–5	0.21	0.30	0.34	0.32	0.27
% of daily need		5	8	9	8	7
Arginine	5	0.08	0.15	0.23	0.17	0.11
% of daily need		2	3	5	3	2

According to the proposed research hypothesis, the addition of carrot powder to A2 milk will help increase its carotenoid content. Such assumptions were made based on the results

of the analysis of information on the chemical composition of carrot powder and the effect of certain processing methods on the preservation of carotenoids. When homogenizing the milk-carrot mixture, carotenoids, which are part of the carrot powder, diffuse into the milk. This is evidenced by the change in its color. Thus, the product is enriched with natural pigments that are precursors of vitamin A and have antioxidant properties.

The organoleptic parameters of enriched milk A2 were determined and compared with the control sample. The appearance, consistency, color, taste and smell were determined organoleptically. The results of the study are presented in the form of a profile (**Fig. 2.4**).

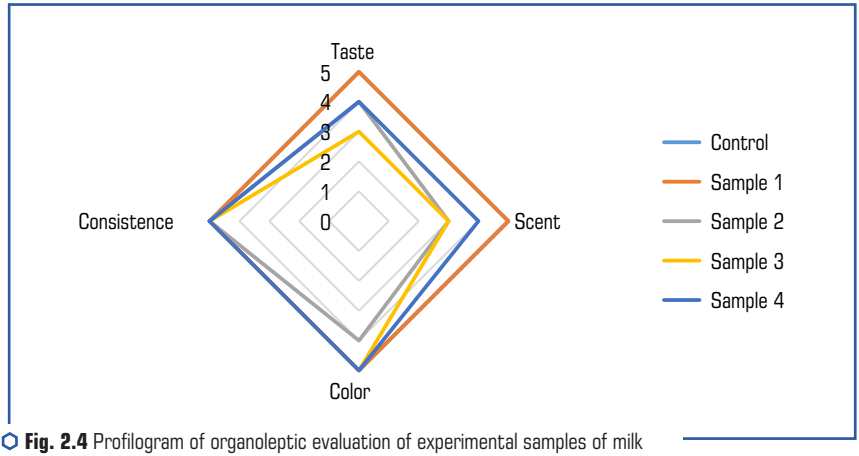

Fig. 2.4 Profilogram of organoleptic evaluation of experimental samples of milk

Unlike the Control sample and Sample 1, Samples 2 and 3 had a pronounced carrot odor. A slight smell of carrots was also observed in Sample 4. The results showed that when using carrot powders made from carrot peels, Samples 2 and 3 acquired a vegetable taste, which negatively affected the overall sensory properties of the product. However, the color of Samples 2 and 3 was very pronounced, creamy, similar to curdled milk. This color may indicate a higher concentration of carotenoids in A2 milk. The study showed that when using whole milk, the concentration of carotenoids in it was higher than when using skimmed milk. Considering that the samples enriched with carrot powder made from peels and raw carrots had a carrot smell, it is recommended to use 10 % carrot powder made from peeled root crops to enrich A2 milk. Some physicochemical indicators of the quality of milk A2 (Sample 1) enriched with 10 % carrot powder were analyzed. The results are presented in **Table 2.7**. The mass fraction of fat was determined by the gravimetric method. The mass fraction of protein is determined by the formol titration method, which is based on the neutralization of the carboxyl groups of monoaminodicarboxylic acids of proteins with sodium hydroxide solution.

The results of the study showed that the mass fraction of protein in enriched milk slightly increased (by 0.03 %).

● **Table 2.7** Results of the analysis of physicochemical parameters of A2 milk enriched with carotenoids ($n=3$, $p \leq 0.05$)

Name of indicators	Control	Sample 1
Mass fraction of fat, %	3.85±0.05	3.85±0.05
Mass fraction of protein, %	2.85±0.05	2.88±0.05

The mass fraction of carotenoids was determined by the chemical method. The batch of 15 g was placed in a round-bottom flask with a return refrigerator and hydrolyzed for 30 minutes in an alkaline-alcohol medium with constant boiling of the mixture and stirring. To prevent oxidation, 150 mg of ascorbic acid was added to the mixture. At the end of hydrolysis, the mixture was cooled and quantitatively transferred to the distribution watering can, adding distilled water. Extraction of non-washable substances from the mixture was carried out 3 times with diet ether, in portions of 50 ml. For further purification after evaporation of the solvent up to 20–30 ml, re-saponification was carried out. To do this, an equal volume of a 5 % alcohol solution of potassium hydroxide was added to the extract and reheated with a return refrigerator for 30 minutes in a water bath at the boiling point of the mixture.

The cooled solution was transferred to the distribution watering can, adding a small amount of distilled water to the separation of the layers. The top layer was washed 5–8 times with distilled water until a neutral reaction according to phenolphthalein, dried by adding anhydrous sodium sulfate, and evaporated on a rotary evaporator at a temperature of 30 °C to a volume of 3–5 ml. Further purification of carotene was carried out on a column with aluminum oxide containing 5 % water, using a chromatographic mixture of hexane-acetone (98:2) and a slight vacuum. Elution was carried out until the eluate became completely transparent, which was checked spectrophotometrically, using pure hexane as a control.

The eluate was evaporated dry on the rotary evaporator, the residue was dissolved in hexane, quantitatively transferred to a measuring flask with a volume of 25 ml, and the optical density of the solution was determined at a wavelength of 452 nm. The calculation of the mass fraction of carotene in the product was carried out taking into account the return coefficient. Previously, it was defined as the ratio of the amount of the carotene standard eluted from the column to the amount applied for chromatography, as well as the extinction coefficient E 1 % 1 cm=2500. Studies were conducted in several sequences, calculating the average result.

The results of determining the content of carotenoids in the studied samples are illustrated in **Fig. 2.5**.

The highest concentration of carotenoids was observed in Sample 2 (0.2435 mg/100 ml). Sample 1, which was found to be the most acceptable for consumption of all fortified A2 milk samples, contained 0.1068 mg/100 ml of carotenoids, which was significantly higher than that of the raw carrot-fortified sample and the Control sample.

The recommended rate of consumption of carotenoids, with an established physiological effect on the body – 15 mg per day, was used to evaluate the obtained research results.

Consumption of 200 g of milk enriched with carrot powder provides the body's need for carotenoids by 1.4 %.

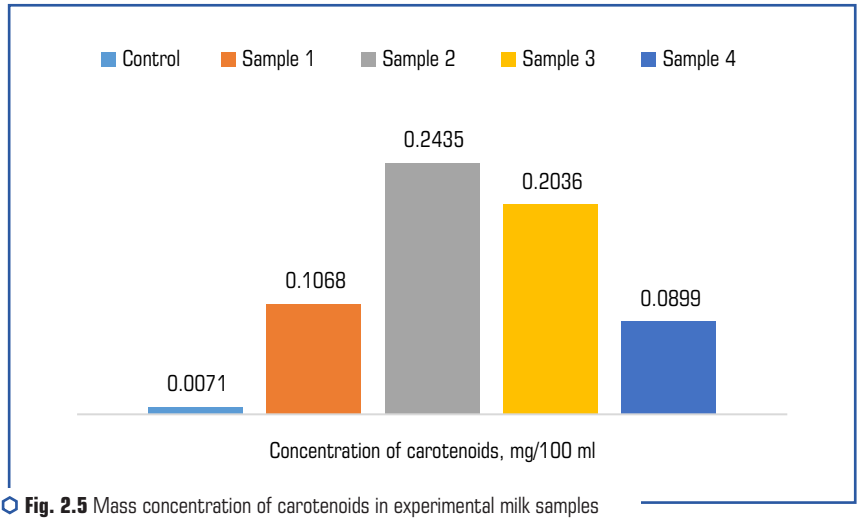

Fig. 2.5 Mass concentration of carotenoids in experimental milk samples

It is economically advantageous for the production of carrot powders to use carrots that are not peeled. At the same time, considerable attention should be paid to the preparation of raw materials for processing (washing, disinfection, and rinsing).

2.3 THE RAW SUITABILITY OF A2 MILK FOR CHEESE PRODUCTION

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 β-casein composition of proteins is an important selection feature that affects the technical properties of milk [32, 33].

The growing proportion of cows with the A2A2 genotype in many countries has led to an increase in the mass production of "A2 milk" [34] 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 β-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 β-casein genotypes, to rationally use raw milk in production.

Work [35] reports the results of a marketing study 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 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 [36], 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 β-casein A1 and A2 on the coagulation properties of milk remains unsolved.

This is the approach used in work [37]. The authors investigated the potential effects of β-casein genotypes A1 and A2 in percentage on cheese production. It was shown that with an increase in the relative content of β-casein A2 (>50 % of the total volume of milk), the yield of cheese decreased significantly; samples with β-casein A2 content below 75 % were characterized by a high content of nutrients. Thus, it was demonstrated that the amount of A1 milk ≥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 [13] 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 β-κ-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 [38]. It was shown that the milk of Holstein-Friesian cows with the β-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 β-casein A2A2. An option to overcome the relevant difficulties may be selective breeding of cows with the β-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 to state that it is appropriate to conduct a study on determining the complex effect of β-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.

The aim 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 aim, the following objectives were set:

– to investigate the physicochemical parameters of raw milk from cows with different genotypes for β-casein (A1A1, A1A2, A2A2);

– to calculate and compare the yield of hard cheese from the milk of cows with different genotypes according to β-casein;

– to investigate the organoleptic and physical-chemical parameters of samples of hard cheeses made from the milk of cows with different β-casein genotypes;

– to establish the amino acid profile of hard cheeses from the milk of cows with different genotypes according to β-casein.

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

Research subjects: physicochemical parameters of raw milk from cows with different genotypes for β-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.

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 β-casein genotypes (A1A1, A1A2 and A2A2). Raw milk was examined for quality indicators according to DSTU 3662:2018. The density of milk was measured by the aerometric method according to DSTU 6082:2009. Acidity (pH) of milk samples was determined by the potentiometric method according to DSTU 8550:2015. The mass fraction of dry substances in milk 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. The mass fraction of fat was determined by the acid method (Gerber method) according to DSTU ISO 2446:2019.

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 2.8**.

● **Table 2.8** Physicochemical parameters of raw milk samples with different genotypes ($n=3$, $p\leq0.05$)

Sample No.	Genotype of β-casein	Acidity, units pH	Density, kg/m³	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 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. The results of our studies into the physical and chemical parameters of milk samples (**Table 2.3**) did not reveal significant differences in the acidity and density of cow's milk with different variations of β-casein.

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.

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.

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 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 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 (Caglificio 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.

Fig. 2.6 shows the appearance of samples of hard cheese made from the milk of cows with different genotypes (A1A1, A1A2, A2A2).

Cheese samples was examined for quality indicators according to DSTU 6003:2008. Acidity (pH) of cheese samples was determined by the potentiometric method according to DSTU 8550:2015. The mass fraction of dry substances in 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 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 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. 2.7**).

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.

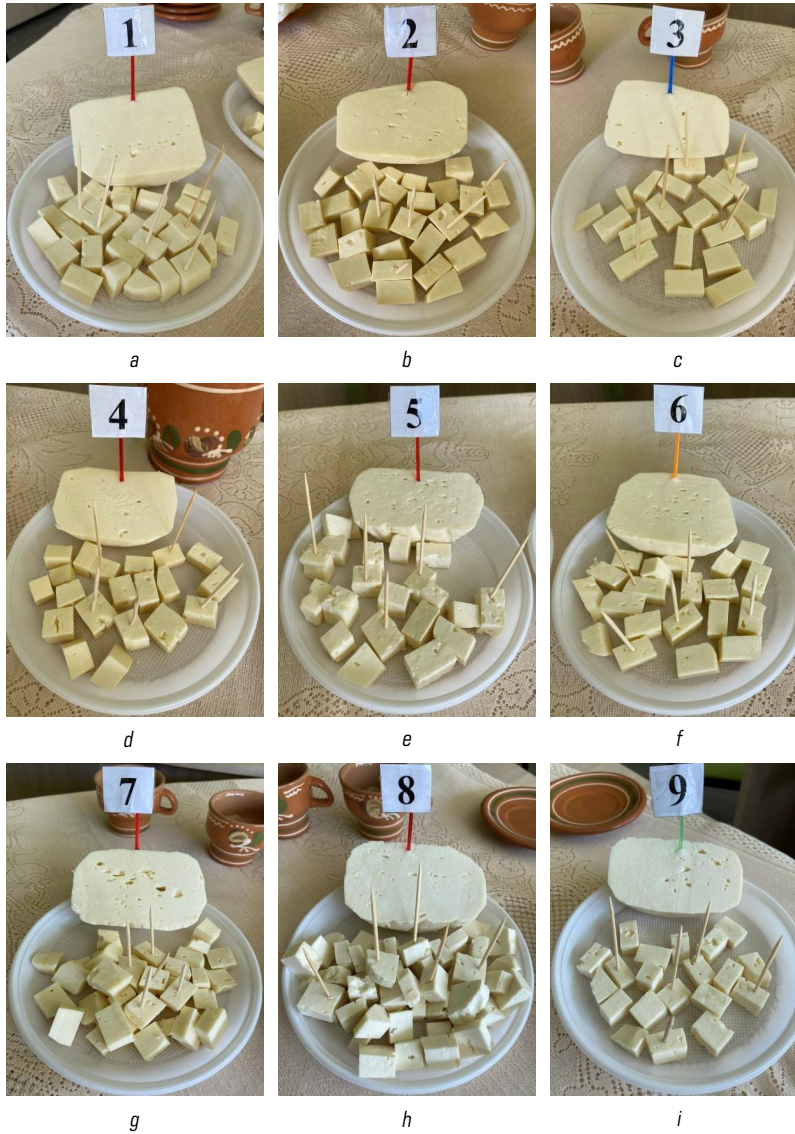

Fig. 2.6 Physical appearance of hard cheese samples: *a* – sample No. 1; *b* – sample No. 2; *c* – sample No. 3; *d* – sample No. 4; *e* – sample No. 5; *f* – sample No. 6; *g* – sample No. 7; *h* – sample No. 8; *i* – sample No. 9

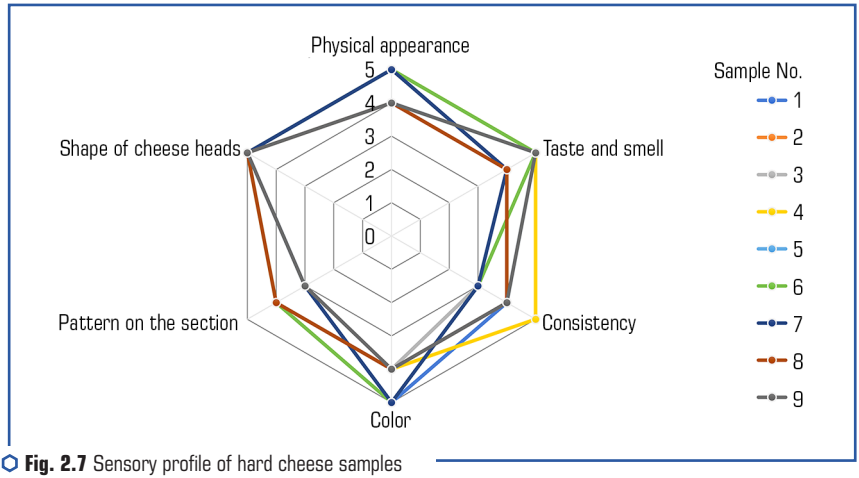

Fig. 2.7 Sensory profile of hard cheese samples

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.

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 the organoleptic analysis (**Fig. 2.7**) showed that variations in the β-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 physical and chemical indicators of samples of hard cheese from milk with different genotypes are given in **Table 2.9**.

Changes in the β-casein genotype in cows had a noticeable effect on the chemical composition of cheese after 30 days of ripening (**Table 2.9**). 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.

● **Table 2.9** 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 β-casein	Acidity, units pH	Density, kg/m^3	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

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" (Czech Republic, Prague). 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, let's 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.

The averaged results are presented on a chart (**Fig. 2.8**). 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/g). 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.

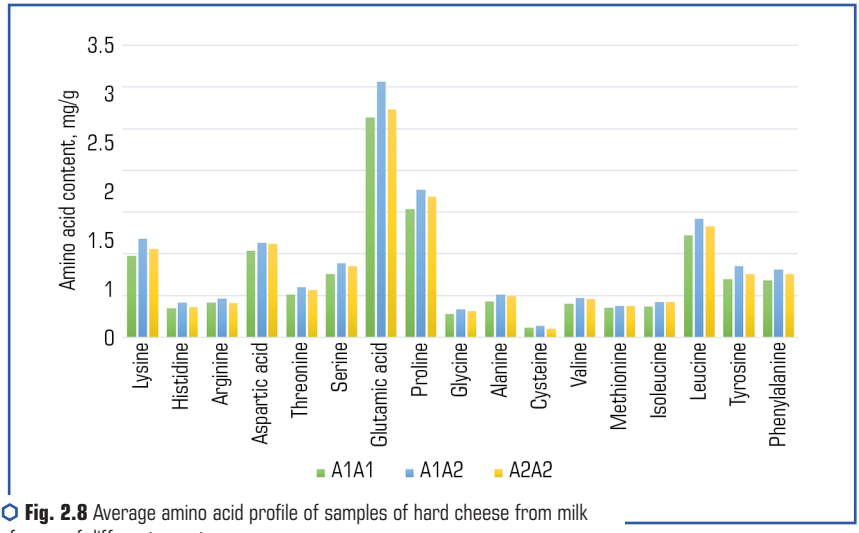

Fig. 2.8 Average amino acid profile of samples of hard cheese from milk of cows of different genotypes

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/g), 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.

The results of the amino acid profiles of the experimental samples of hard cheese showed that the β-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 [39], 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.

The yield of hard cheese from the studied milk samples of cows with different genotypes (A1A1, A1A2, A2A2) was calculated according to the following formula:

$$B = \frac{m_{cheese}}{m_{milk}} \cdot 100\ \%,$$

where B – the yield of cheese, %; m_{cheese} – mass of cheese (30 days after production), kg; m_{milk} – mass of milk, kg.

The results are shown on a histogram (**Fig. 2.9**).

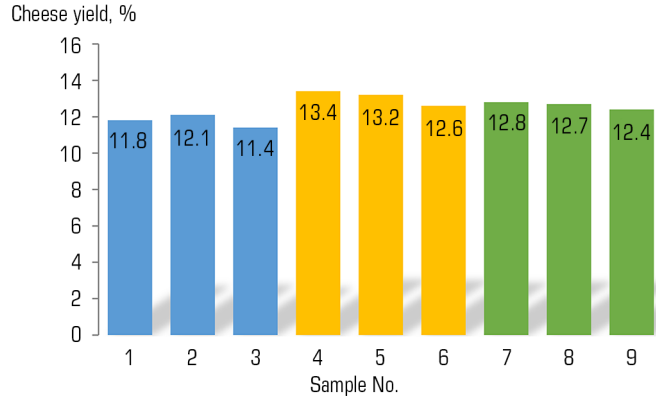

Fig. 2.9 Yield of hard cheese from the milk of cows with different genotypes

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

The calculated yield of cheese (**Fig. 2.9**) showed that the yield of cheese from milk with β-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.

Comprehensive studies have shown that the β-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 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.

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 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 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.

CONCLUSIONS

1. The use of carrot powders makes it possible to increase the concentration of amino acids in A2 milk by 2.28 g/100 g, compared to the control. In prototypes of milk, the largest number of amino acids (glutamic, aspartic acids, leucine, valine) was found, which have a positive effect on the maintenance of vital body functions.

2. Research results showed that the highest concentration of carotenoids (0.1068 mg/100 ml) was observed in prototypes of milk enriched with powder from whole carrot roots. This indicates that the enrichment of A2 milk with carrot powder is an additional source of vitamin A, produced in the human body.

3. An industrial technique has been developed to increase the biological value of A2 milk with carrot powder. The expediency of using the developed technique is to use waste-free processing of raw materials. Carrot powders, under industrial conditions of dairy enterprises of Ukraine, can be added to A2 dairy raw materials.

4. Our research established that the physicochemical parameters of raw milk of cows with different genotypes of β-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 β-casein genotype A2A2 were slightly higher compared to A1A1 and A1A2.

5. 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 β-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 %).

6. The amino acid profile of cheese from the milk of cows with the β-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.

7. Calculations of the yield of cheese showed that the yield of cheese from milk of cows with β-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.

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