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DEVISING A TECHNIQUE FOR IMPROVING THE BIOLOGICAL VALUE OF A2 MILK BY ADDING CARROT POWDER

This paper considers the development of a technique to increase the biological value of milk and reduce the manifestations of allergic symptoms when it is consumed. The object of the study is a technique for enriching A2 milk. When digesting A2 milk, β-casomorphine-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 are added, so the question of its enrichment is relevant. Under industrial conditions, artificial vitamin and mineral complexes are used to enrich milk. Given that natural and synthetic substances have different effects on the human body, it is proposed to use carrot processing derivatives (Daucus carota) as a natural enrichment additive. The use of carrot powders improves the amino acid spectrum of milk. The mass concentration of amino acids in A2 milk enriched with powder from carrot peels was 4.87 g/100 g. The highest concentration found was, g/100 g: glutamic acid (0.84), proline (0.50), aspartic acid (0.42), leucine (0.41), and valine (0.35). It was established that the use 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 found 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 under industrial conditions

Keywords: A2 milk, carrot powder, milk carotenoids, milk amino acids, β-casein A2, biological value

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1. Introduction

Milk is a mandatory product in the daily diet of children and the main source of protein intake in their bodies. The Cabinet of Ministers of Ukraine (Resolution No. 305 of March 24, 2021) recommends consuming milk and dairy products, to which vitamins and minerals are added [1]. However, milk consumption can cause allergies. Allergy to cow's milk is an allergic reaction to the protein contained in cow's milk [2].

Cow's milk protein is one of the most common allergens faced by young children. Significant allergens are casein proteins (alpha-s1-, alpha-s2-, beta- and kappa-casein) and whey proteins (alpha-lactalbumin and beta-lactoglobulin) [3]. According to the World Allergy Organization (WAO), 6–8 % of children under 3 years of age have food allergies, 4.9 % of them react to cow's milk protein [4]. This food allergy is manifested by a wide range of clinical syndromes due to immunological reactions to cow's milk proteins [5].

Therefore, addressing the issue aimed at preventing the occurrence of allergic reactions in children and adults by enriching cow's milk with useful nutrients is a timely and relevant task.

2. Literature review and problem statement

About 30 % of the total protein contained in cow's milk is β-casein. It can be in various 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. In type A1 – histidine, which contributes to enzymatic hydrolysis, and in type A2 – proline, which prevents the course of this process [6].

In the enzymatic hydrolysis of milk A1, a peptide β-casomorphine-7 (BCM-7) is formed, which is a well-known agonist of μ-opioid receptors. It can directly affect the physiology of the gastrointestinal tract, as well as other systems, for example, the cardiovascular, nervous, and endocrine [7]. Often, the cause of such disorders is considered lactose intolerance. However, there is growing evidence that beta-casein A1A1 is also associated with intolerance to cow's milk [8].

There are scientifically proven facts that the consumption of milk with a fractional composition of β-casein type A2A2 prevents cardiovascular diseases [9] and prevents the occurrence of type 1 diabetes [10]. A2 milk contributes to the formation of less severe symptoms of autism and schizophrenia [11]. A variant to reduce the manifestations of allergic symptoms may be the consumption of milk containing only β-casein type A2A2.

Today, A2 milk and dairy products are widely popular in the markets of Australia, New Zealand, China, the USA, Great Britain, Russia, and Canada. In these countries, this milk has long been known. Currently, the price of A2 milk on world markets differs from the price of regular milk by more than twice. There are only two enterprises producing A2 milk in Ukraine: Sumy Cheese Factory «O'BEREG», PRAT «Ichnyansky Plant of Powdered Milk and Butter». The range of milk at these enterprises is limited to A2 milk, and lactose-free A2 milk.

Milk enrichment technologies make it possible to improve its biological value. 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, K, and biotin, pantothenic or folic acids [12]. It has been established that milk enriched with micronutrients can be an effective means of reducing anemia of children under three years of age in developing countries [13]. Most of these biologically active substances are made artificially under industrial conditions. They help adjust the chemical composition of the product but are not used by the body like 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 a cooling temperature for 1 week, having almost unchanged organoleptic, physical-chemical, and microbiological properties [14]. However, the use of vegetable fats increases the fat content of milk, which does not meet the needs of some consumers.

Enrichment of milk makes it possible not only to improve its biological value but also to increase its added value. Paper [15] reports the results of research on reducing the demand for simple, pure milk and the growing trend towards increasing the consumption of flavored milk of medium and high fat content. However, artificial flavors should not be used in food products for children.

Enrichment of milk with vitamin A and giving it certain organoleptic properties is possible through the use of carotenoids. It is known that carotenoids have antioxidant properties, provitamin activity A, immune, endocrine, and metabolic activity, and play a role in the regulation of the cell cycle [16].

Therefore, milk enriched with carrot carotenoids has a better storage capacity. Obviously, this is due to the fact that β-carotene slows down microbiological processes [17]. A natural source of carotenoids is carrots (*Daucus carota*) [18], 35 % of carotenoids of dry carrots in a living organism are converted to vitamin A [19]. However, there are no industrial technologies for using carrots to enrich milk.

Under industrial conditions, the vitamin complex FT 041081EU is used to enrich dairy products, which contains 12 important vitamins (A, D, E, C, Bs, B1, B2, B6, B12, PP, B5, biotin) and mineral FT 042836EU, which includes Fe, Zn, and I [20]. It is not yet clear how successfully synthetic biologically active substances are absorbed and used by the body.

All this suggests that the current technologies for milk enrichment mainly involve the use of synthetically created vitamin-mineral complexes. There is practically no assortment of milk using natural food additives despite the fact that natural vitamins are absorbed much better than synthetic ones [21].

The biological value of milk is also determined by its amino acid composition since amino acids are involved in cell biosynthesis, which is very important for the vital activity of the human body [22]. It is advisable to increase the concentration of amino acids in milk by natural additives.

Given the small range of A2 dairy products in the market and the lack of milk enriched with natural additives, it is possible to solve the problem by offering a new technique. It is important that this technique is rational and unified while the technologies developed on its basis are waste-free.

3. The aim and objectives of the study

The aim of this study is to devise a technique for enriching A2 milk with amino acids and carotenoids. This will make it possible to increase its biological value and reduce the manifestations of allergic symptoms when it is used.

To accomplish the aim, the following tasks have been set:

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

– to determine the content of carotenoids in A2 milk enriched with powders from carrot roots with and without peels;

– to develop a technological scheme of A2 milk using the waste-free utilization of dry carrot roots (without removing the peel).

4. The study materials and methods

The object of this study is a technique of enriching A2 milk. The main hypothesis of the study assumes that the use of milk, which contains only β-casein A2, will reduce allergic manifestations. The use of a natural dietary supplement for the enrichment of milk with useful nutrients will contribute to their better absorption by the body. The assumptions adopted in the study are aimed at developing a rational technique to increase the biological value of milk.

The study used whole (fat content of 3.8) and skimmed A2 milk, obtained from cows at the vivarium of Sumy National Agrarian University. In addition, industrial samples of A2 milk made by TOV «Ichnyansky plant of powdered milk and butter» with a standardized mass fraction (2.5 %) of fat were used.

We 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.

5 milk samples were analyzed:

– 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, we used 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.

We determined 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 [23].

It is known that the intensity of carotenoid transition and color gain increases in fat-vegetable mixtures [24]. Taking into account this fact and based on previous results, three milk samples were prepared in the next stage.

As a control, A2 milk of industrial production from TOV «Ichnyansky plant of powdered milk and butter» (fat mass fraction 2.5 %) was used.

Sample 1 was made on the basis of the same milk from a 1 % carotene-containing additive. For the manufacture of a carotene-containing additive, a powder made from whole carrot root vegetables was mixed with fish oil in a ratio of 1:1 and infused for a day. After that, the suspension was divided by filtration into liquid and solid phases. The liquid phase was used as an additive to enrich milk.

Sample 2 was made on the basis of the same milk as previous samples. For enrichment, 10 % powder from whole carrot root crops produced by the technique described above was added.

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.

5. Results of investigating the feasibility of using carrot powders for milk enrichment

5. 1. Results of investigating the amino acid spectrum of milk

18 amino acids were recognized in milk, including 7 essential ones (threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) (Table 1).

Table 1

Mass fraction of amino acids in prototypes of A2 milk

Amino acid	Norm per day	Con-trol	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

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.

5. 2. Results of investigating the content of carotenoids in fortified milk

The results of determining the content of carotenoids in the studied samples are illustrated in Fig. 1.

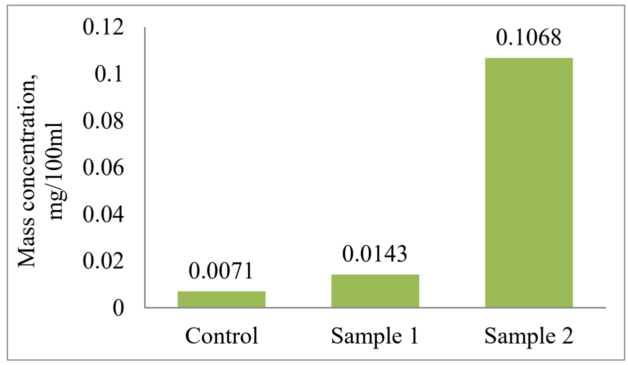

Fig. 1. Mass concentration of carotenoids

The highest concentration of carotenoids was observed in sample 2 (0.1068 mg/100 ml). The addition of a fish oil-based herbal supplement to milk also showed no significant increase in carotenoids (0.0143 mg/100 ml). It is worth noting that the use of the same amount of A2 milk without additives provides the body's need for carotenoids only by 0.09 %.

5. 3. Devising a technique for enriching milk with carrot powder

A technique for enriching A2 milk with carrot powders has been developed (Fig. 3). 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.

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 [25].

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.

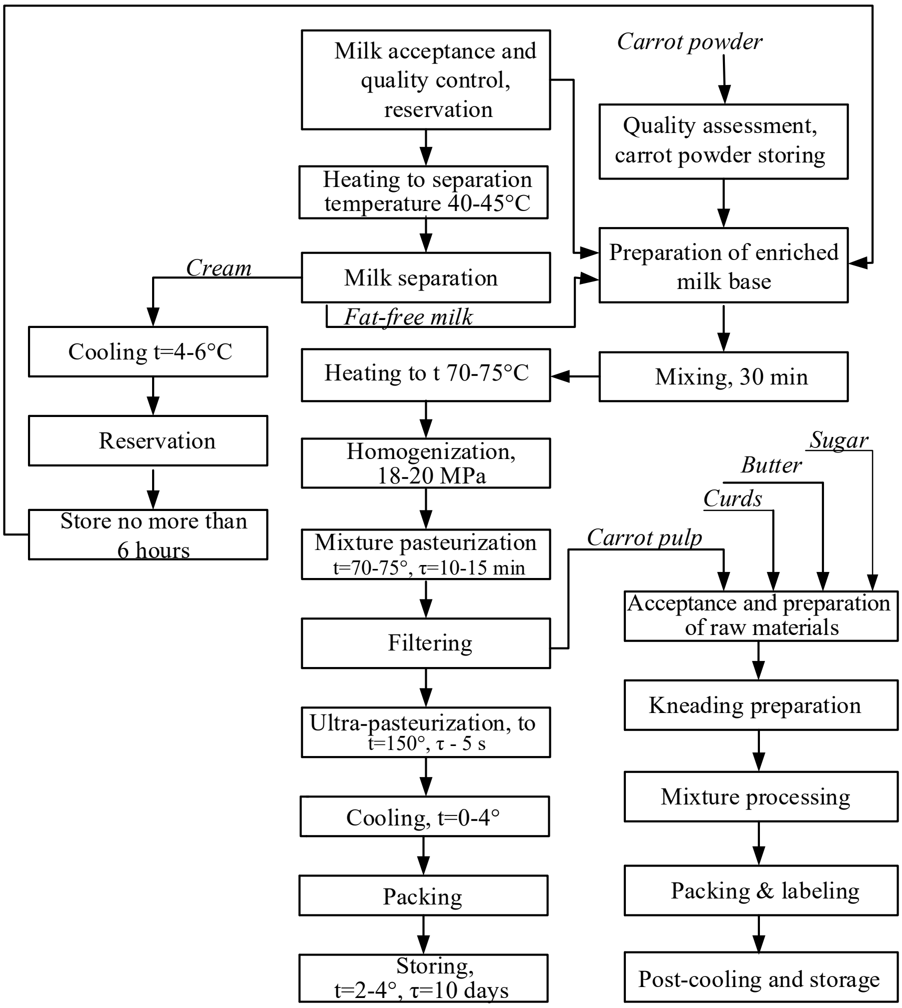

Fig. 2. Technological scheme of production of enriched A2 milk enriched with powdered carrot powder from unpeeled carrots (with peel)

The results of our research show that under the influence of a rational dose, 10 % of powders made from carrots and its peels in all prototypes of A2 milk, the content of the total amount of amino acids increases. The mass concentration of amino acids in A2 milk enriched with carrot powder made from carrot peels was 4.87 g/100 g. Similar results are characteristic of goat's milk [26].

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 1). 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 [27]. It is known that the following amino acids are indispensable for cows: Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val [28]. We have identified 7 essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine.

The hypothesis of the study was also based on our information about the important role of carotenoids that are converted by the body into vitamin A, whose action is aimed at maintaining vital body functions [29].

The research results confirm data [18] on the possibility of using carrots as a source of carotenoids since when using the proposed technique, their concentration increases to 0.1068 mg/100 ml (Fig. 1). The recommended rate of carotenoid consumption, with an established physiological effect on the body, 15 mg per day, was used to evaluate the results of research [30]. It was found that the consumption of 200 g of milk enriched with carrot powder meets the body's need for carotenoids by 1.4 %. Thus, it is possible to solve the problem of providing the body with natural vitamins [21] since the effect of synthetic vitamins added to products requires additional study [20] whereas the benefits of carotenoid in carrots have been proven [16, 17, 19].

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).

6. Discussion of results of the study of A2 milk enriched with carrot powders

It is known from literary sources that milk enriched with micronutrients and vitamins can be an effective means of reducing anemia in children under three years of age in developing countries [12, 13]. However, the disadvantages of the technique of enrichment of dairy products with macro- and microelements, vitamins include a difficult preparation for use. For example, the dissolution of fat-soluble vitamins in fat systems. In addition, their overdose can harm human health.

It is also known that under industrial conditions synthetically created vitamin-mineral complexes are used to enrich dairy products. However, the disadvantage of using synthetically created vitamins and vitamin complexes is that natural vitamins are absorbed much better than synthetic ones. In addition, due to the higher cost of vitamins of natural origin, there is practically no assortment of milk using natural food additives [18, 21].

Researchers argue that the biological value of milk is also determined by its amino acid composition since amino acids are involved in cell biosynthesis, which is very important for the vital activity of the human body [22].

A feature of the developed technique (Fig. 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 [15].

The limitation on the introduction of the developed technique industrially may be the need for additional filter equipment and double pasteurization. However, filtering equipment exists, and it is affordable. In addition, the production of enriched milk and curd masses with carrot pulp should be carried out taking into account the time of all technological operations, avoiding long-term storage of pulp, to prevent microbiological seeding. Re-pasteurization will provide an increase in the product's ability to store. The disadvantage of this technique is that manual labor is used to prepare an enriched dairy base since carrot powders are packaged in bags of different capacities.

Further research will be aimed at studying the shelf life of enriched A2 milk.

7. Conclusion

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.

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

Manuscript has no associated data.

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