Revisiting the question of vitamin D enrichment of milk

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Abstract. The concept of food safety in Russia includes the task of achieving self-sufficiency in dairy products of high nutritional and biological value in accordance with scientific medical standards. The article is devoted to the scientific development of the formulation of vitamin D-enriched drinking milk. The research data are relevant due to the vitamin D deficiency in the diet of the population living in the North-Western region of Russia. The main reason why drinking milk was chosen as an object for vitamin D enrichment is the fact that dairy products occupy a significant place in the human diet, with 30-40% of the total energy value of food accounting for this particular group of products.

1. Introduction
Milk is one of the most valuable human food products. Nature has created a unique nutritional value of this drink: milk can replace any product, but no product can replace milk.

Milk contains all the substances necessary for human nutrition – fats, proteins, carbohydrates, which are provided in a balanced ratio and are easily absorbed by the body. In addition, it contains many enzymes, vitamins, minerals and other important nutrients necessary to ensure normal metabolism [2].

Milk is a source of many vitamins. For instance, the daily demand for relatively scarce vitamin B2 is satisfied by 42-50% due to milk and dairy products consumption [1].

Vitamin supplementation of staple foodstuffs has proven to be useful in a number of countries over the years. This method is recognized as effective for correcting vitamin deficiencies.

Vitamin D (calciferol) – belongs to a group of biologically active fat-soluble organic substances - is one of the undoubtedly essential vitamins for humans, with vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) to be of primary importance for the human body. While vitamin D3 is synthesized in the skin from its precursor 7–dehydrocholesterol, vitamin D2 enters our body in small quantities and only from a limited number of foods (bread, milk). The content of vitamin D in milk is about 0.05 µg/100 g (in the summer due to ultraviolet rays, the vitamin content in cow milk is slightly higher).

Vitamin D – ‘‘solar vitamin’: it is synthesized in the human body under the influence of ultraviolet rays, but due to the small number of sunny days in the year, 80% of the inhabitants of the North-Western region of Russia feel its deficit. This is the conclusion made by the researchers of the Russian Federal Center for Nutrition and Biotechnology on the basis of a two-year study, which was completed in 2018. Depending on the level of insolation, this figure varies from 23 to 97% from region to region. In case of insufficient number of sunny days in the regions, food sources should provide a compensating effect.
D vitamins are an essential part of the human diet. The main function of vitamin D in the human body is to ensure the absorption of calcium and phosphorus from food in the small intestine.

A number of clinical studies have shown the following additional functions of vitamin D: participation in the regulation of cell reproduction, metabolic processes, stimulation of the synthesis of a number of hormones [3].

In recent years, there have been studies that noted the role of vitamin D deficiency in the development and persistence of infectious agents, as well as its impact on various parts of the immune response [4].

In their work, Ruggiero B. and co-authors (2015) proved the crucial importance of vitamin D in the differentiation of osteoblasts from stromal bone marrow cells in children aged 8 to 12 years by detecting the expression of VDR genes, vitamin-D-binding protein (Megalin), D-hydroxylase (CYP27B1, CYP27A1, CYP2R1 and CYP24A1) and estrogen receptor (ER). In addition, the authors revealed gender differences: as it turned out, boys’ expression of CYP27B1 and CYP24A1 was significantly higher than that of girls [6].

Additionally, vitamin D has a cytokine-modulating effect, reducing the proliferation of Th1 and Th2 cells, decreasing the production of IFN-\(\gamma\), IL-2, and IL-5, as well as inhibiting the proliferation of mitogen-activated lymphocytes [5]. Thus, this vitamin is involved in the formation of the immune response, in intercellular interactions.

Milk is the optimal object for the vital vitamin D enrichment and consequently obtaining a functional food product, the manufacture of which will make it possible to fill the deficit of calciferol in the diet of the population in those regions where it is necessary.

While choosing a food product to be enriched with this vitamin, the authors of the study were guided by the national characteristics of the consumption of this food product (milk in Russia is traditionally a commonly used product), as well as the versatility of this product, the possibility of its industrial production, the constancy of the average daily consumption, the possibility of uniform distribution of vitamin throughout the product. It is important that milk contains such a useful micronutrient as calcium, which is known to be very quickly removed from the body and poorly absorbed without the participation of vitamin D.

Thus, in order to provide the human organism with the maximum benefit from milk in regions with low insolation, it must be enriched with vitamin D, thereby replenishing the necessary amount of such a vitally important vitamin in the body.

The result of these specific studies was the development of the formulation and production technology of the functional food product – drinking milk enriched with vitamin D, which due to the physiological effects on the body of consumers allows reducing the risk of diseases associated with this vitamin deficiency in the diet, helps to preserve and improve human health.

2. Methods and Equipment
To conduct the study, a test sample of drinking milk with the addition of vitamin D was chosen. A sample without vitamin D was used as a control sample. The test sample formulation is presented in table 1.

| Name of raw material                                | Quantity |
|-----------------------------------------------------|----------|
| Drinking milk, normalized by mass fraction of fat (3,2%) | 100 g    |
| Vitamin D ND 42-2779-08-2008                        | 1.5 µg   |

According to the requirements of Methodological Recommendations (MR) 2.3.1.2432-08 “Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation” (date of introduction 18.12.2008), the specified physiological need for vitamin D for
adults and children under the age of 14 years old makes 10 µg/day, for persons over 60 years – 15 µg/day.

In order to avoid exceeding the daily values, the formulation of the test sample was determined at the rate of 15 % of the daily intake of adults and children under 14 years of age in 100 g of the product.

Figure 1 presents the Trial Production Technological Scheme of Drinking Milk Enriched with Vitamin D.

Figure 1. Trial Production Technological Scheme of Drinking Milk Enriched with Vitamin D

Drinking milk enriched with vitamin D must meet the requirements of GOST 31450-2013 Interstate Standard. Drinking milk. Technical Specifications. The product is manufactured in accordance with the requirements of the Technical Regulations of the Russia-Kazakhstan-Belarus Customs Union (CU) on Food Safety (TR TS 021/2011), according to technological instructions in compliance with hygienic requirements for the dairy industry operating on the territory of the Russian Federation.

The determination of the organoleptic indicators of the quality of the product was carried out according to GOST 28283-2015 Interstate Standard. Cow’s milk. Method of the organoleptic determination of odour and taste.

The study of physical and chemical parameters of drinking milk was carried out on the basis of the following regulatory documents:
- mass fraction of fat: according to GOST 5867-90 Interstate Standard. Milk and dairy products. Methods of determination of fat;
- determination of acidity: according to GOST 3624-92 Interstate Standard. Milk and milk products. Titrimetric methods of acidity determination;
- density determination: according to GOST R 54758-2011 National Standard of the Russian Federation. Milk and milk products. Methods for determination of density;
- mass fraction of protein: according to GOST 25179-2014 Interstate Standard. Milk and milk products. Method for determination of protein.
- mass fraction of vitamin D: according to GOST 32916-2014 Interstate Standard. Milk and milk products. Determination of vitamin D mass fraction by high performance liquid chromatography method.
3. Results
In accordance with the research scheme, the formulation of drinking milk enriched with vitamin D was developed in the following sequence:
- at the first stage, organoleptic parameters of drinking milk samples with vitamin D and without vitamin D were determined. The purpose of the organoleptic evaluation was to determine the absence of the negative impact of this vitamin on the taste, color, smell and consistency of milk;
- at the second stage of the study, physical and chemical investigations of milk samples were carried out;
- at the third stage, a tasting analysis was conducted to confirm the results of the organoleptic analysis due to a larger number of experts participating in the test;
- further work included drawing up a technological scheme for the production of a functional product;
- the next stage was the development of the production formula of milk enriched with calciferol;
- the final stage was the determination of vitamin D content by high performance liquid chromatography method at different stages of the product storage.

Two samples of the product were prepared for the research:
- milk with a mass fraction of fat of 3.2 % without vitamin D (control sample);
- milk with a mass fraction of fat of 3.2 % with the introduction of vitamin D (1.5 µg per 100 g of milk).

The results of the studies aimed at organoleptic evaluation of the experimental samples are presented in table 2.

| Sample | Appearance of the product | Consistency | Color | Taste and smell |
|--------|---------------------------|-------------|-------|-----------------|
| Milk with a mass fraction of fat of 3.2 % without vitamin D | Opaque liquid, no sediment | Liquid, homogeneous, not sticky, no flakes of protein and lumps of fat | White, uniform throughout the mass | Pure, characteristic of milk, with a taste of boiling, free from foreign flavors and odors |
| Milk with a mass fraction of fat of 3.2 % with the introduction of vitamin D | Opaque liquid, no sediment | Liquid, homogeneous, not sticky, no flakes of protein and lumps of fat | White, uniform throughout the mass | Pure, characteristic of milk, with a taste of boiling, free from foreign flavors and odors |

Further, in order to determine the impact of vitamin D on the quality of milk, a complex of physical and chemical investigations of the control and test samples was carried out (in the studied samples the mass fraction of fat (%), acidity (°T), density (kg/m³), mass fraction of protein (%) were determined). The results of the studies of the experimental samples are presented in table 3.

| Sample | Mass fraction of fat, % | Acidity, °T | Density, kg/m³ | Mass fraction of protein, % |
|--------|------------------------|-------------|----------------|---------------------------|
| Milk with a mass fraction of fat of 3.2 % without vitamin D | 3.2±0.1 | 16±0.2 | 1029±0.1 | 3.0±0.1 |
| Milk with a mass fraction of fat of 3.2 % with the introduction of vitamin D | 3.2±0.1 | 16±0.1 | 1029±0.2 | 3.0±0.2 |

To conduct the tasting evaluation, two samples of drinking milk were presented to the members of the Commission consisting of 18 experts: without vitamin D and with vitamin D. The evaluation was carried out in accordance with GOST 28283-2015.
During the tasting, the following indicators were evaluated: color, smell, consistency, taste. A five-point scale was used to make evaluation. The results of the tasting analysis of the samples are shown in figure 2.

![Figure 2. Results of tasting analysis of drinking milk samples.](image)

Analyzing the data of tables 2 and 3, it is possible to conclude that the introduction of vitamin D in milk did not affect the organoleptic, physical and chemical indicators of drinking milk quality – the indicators of milk samples with the introduction of vitamin D and the control sample are almost identical.

The tasting analysis (Fig. 2) of drinking milk samples showed that the introduction of vitamin D had no effect on the taste, smell, color and consistency of drinking milk – the average final evaluation of milk without vitamin D made by milk experts was 4.92 points, with the introduction of vitamin D – 4.94 points.

According to the MR 2.3.1.2432-08 "Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation" (adopted by Federal Service for Surveillance on Consumer Rights Protection and Human Well-being (Rospotrebnadzor) on 18.12.2008) the daily intake of vitamin D is 10 µg/day.

According to GOST R 54059-2010 Functional Food Products. Functional Food Ingredients. Classification and General Requirements, in order to obtain a functional product – fortified milk, it is necessary to add 1.5 µg of vitamin D to 100 ml of milk, which accounts for 15% of its daily intake. Thus, the formulation of drinking milk enriched with vitamin D (per 1000 kg), is presented in table 4.

| Name of raw material                                      | Quantity |
|-----------------------------------------------------------|----------|
| Drinking milk, normalized by mass fraction of fat (3.2%)   | 1000     |
| Vitamin D ND 42-2779-08-2008                              | 15       |

Then, the technological process of vitamin D-enriched drinking milk production in industrial conditions was worked out.

Production begins with the quality assessment and evaluation of raw materials. Milk should not be below grade I. The milk received by the enterprise was purified, cooled to a temperature of 4-6 °C, sorted and reserved to ensure the continuity of the technological process. Further, the raw material was sent for separation.

If it is necessary to store milk for more than four hours before the ultra-pasteurization in order to maintain thermal stability, it must be pasteurized at a temperature of T = 76±2 °C with an exposure of
20°C and cooled to T = 4±2 °C. The maximum shelf life of pasteurized milk before ultra-pasteurization is 24 hours.

Vitamin D (Regulatory Document (RD) 42-2779-08-2008) was controlled according to the established requirements of Normative and Technical Documentation (NTD). The preparation of vitamin D is a drop of colorless, transparent or slightly opalescent liquid. The preparation is stored in its original packaging in a dry place at a temperature not exceeding 25 °C and a relative humidity of 75%.

The selected best quality milk was separated in the J5-OSCP-1S Cream Separator of Semi-closed Type with Automatic Periodic Centrifugal Discharge of the sediment. With its help, the purification of whole milk was carried out simultaneously.

The normalization of the mixture was conducted in a milk container with a stirrer, by adding cream to skim milk.

Vitamin D emulsions in water or milk are known to be clinically more effective. In this regard, the following method of introducing vitamin D into the product was chosen – the necessary amount of an aqueous solution of vitamin D was mixed in a small amount of skimmed milk, after which it was introduced into the normalized mixture. The temperature of the milk was 65 °C, the mixing time – 10-15 minutes.

That was followed by a process of homogenization – fragmentation of the fat globules, by means of exposure of milk to mechanical effects caused by pressure drop with the use of the P 11-M rotary homogenizer. This operation is performed to prevent the settling of fat, to improve the consistency and taste of milk. Milk enters the homogenizer at the temperature of T = 63 °C, homogenization pressure is 15±2 MPa.

During the determination of pasteurization regimes, we took into account the fact that vitamin D is a heat-resistant vitamin, its amount does not change until it reaches 100 °C, at a temperature above this indicator, the vitamin is subject to destruction in the presence of air, which is excluded in the pasteurization unit – ultra-pasteurization of dairy products in this equipment is produced in a continuous closed flow with subsequent packaging under aseptic conditions.

Further, the complete mixture was sent to the TG-UHT-1000 ultra-pasteurization unit in the pasteurization section, where it was heated to 95 °C, kept at this temperature for 30 seconds, after heat treatment, the product was sent to the second recuperation section, then to the ultra-pasteurization section, in which it was heated to 140 °C with an exposure of 4 seconds.

After the ultra-pasteurization section, the product must be cooled quickly: first by the flow of the input cold product in the recuperation section up to +30 °C, and then in the cooling section – first by cool water, and then by ice water or other refrigerant to the required temperature (up to T = +15 °C) for further packaging of the product under aseptic conditions.

Further, the cooled mixture was sent to the filling machine for aseptic filling of milk in packages of the "Pure-Pack" - combined packaging material, which eliminates further interaction of the product with air and reduction of the introduced amount of calciferol.

Further, the product was stored at a temperature not higher than +20 °C and a relative humidity not higher than 85%. The shelf life of the product in the "Pure-Pack" package is not more than 6 months from the end of the technological process.

Immediately after the completion of the packaging process and then – at intervals of 1 month, we carried out a check of vitamin D content in the produced batch of milk according to GOST 32916-2014 Milk and dairy products. The determination of the mass fraction of vitamin D was conducted by high-performance liquid chromatography method. The "Stayer" liquid chromatograph with spectrophotometric detector was used for the research.

The results of the measurements are shown in figure 3. According to the figure, the thermal treatment and shelf life of milk samples with the introduction of vitamin D did not have a significant effect on calciferol content.

1 hour after filling milk in "Pure-Pack" packages, vitamin D content was 1.50±0.02 µg/100 g, that is, the loss of vitamin D was 3.3% (calculations were made taking into account vitamin D, contained
in milk raw materials in the amount of 0.05 µg/100 g). The error margins and the components of the method for determining the mass fraction of vitamin D at P=0.95 are considered in the calculations. The loss of vitamins at this stage is probably due to the use of high temperature during ultra-pasteurization (140 °C with the exposure of 4 seconds), but the loss of vitamin D is so small that it can be neglected, so as not to avoid ultra-pasteurization.

Further storage of samples was conducted at a temperature of 20±2 °C and a relative humidity of 80–82 %. During the storage period, changes in vitamin D content did not occur for the first 2 months, then, starting from the third month, it decreased by an average of 0.01 µg/100 g and by the end of the storage period it was 1.46 ± 0.005 µg/100 g. Thus, the use of aseptic opaque packaging allowed preserving vitamin D in the product by 97.3 % (i.e., losses amounted to 2.7 % of the initial amount of vitamin determined 1 hour after packaging).

4. Conclusion
The Order of the Government of the Russian Federation of October 25, 2010 N 1873-p “On bases of the State Policy in the Field of Healthy Nutrition of the Population of the Russian Federation for the period up to 2020” determines the development of food production enriched with essential components, specialized baby food products, functional products, dietary food products with health-promoting properties and biologically active food additives, including institutional feeding in organized groups (labor, educational, etc.) as one of the main tasks of the state policy in the field of healthy nutrition.

In some European countries, fortification of milk with the introduction of various vitamins (A, C, D, etc.) is already a common practice. In Russia, this method of increasing the nutritional value of food and the creation of functional products is an urgent task at the present stage of the Russian economy development due to the extreme scarcity of the range of domestic functional food products.

As a result of this research, we have worked out the formulation and technology of vitamin D-enriched drinking milk, recommended for reducing the deficiency of this vitamin in the North-Western region of the Russian Federation – a region with very low insolation, which is the reason why it is impossible to meet the body’s need for vitamin D by means of self-produced calciferol in the skin.

The authors found that the introduction of vitamin D in drinking milk had no effect on its basic quality indicators. The experimental sample of drinking milk with vitamin D meets the requirements of GOST 31450-2013 Interstate Standard. Drinking milk. Specifications.
During the introduction in industrial conditions, the formulation of drinking milk enriched with vitamin D was developed, the technological regimes were justified.

Determining the vitamin D loss after milk processing and at the end of its storage, it was found that the loss of vitamin immediately after bottling was 3.3 % (considering vitamin D, contained in milk raw materials in the amount of 0.05 µg/100 g), 6 months after bottling, losses amounted to another 2.7 %. Thus, for the entire period from the beginning of the production process to the end of the shelf life of the product, the loss of vitamin D in milk samples was 6 %, and the amount of vitamin D at the end of the shelf life was 1.46 %, or 14.6 % of the daily intake of the vitamin, that allows the product being considered as functional one.

Thus, the formulation and technology of drinking milk enriched with vitamin D developed during the research, can be recommended for food processing enterprises of the North-Western region of Russia to be introduced into industrial production in order to prevent vitamin D deficiency and to solve the main task of the Government of the Russian Federation – to preserve public health.

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