Modification of the whey protein cluster for the utilization in low-calorie food technology

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Abstract. One of the urgent tasks of the dairy industry is to develop alternative modifications of the composition and properties of the whey protein cluster for the utilization in food technologies. The objective of the research is to optimize the conditions for modifying the whey protein cluster to develop competitive food products. The quality indicators of the research subjects, their composition and properties have been determined in accordance with Russian standards. Some part of the research has been carried out in in vivo and in vitro experiments. To develop the whey proteins microparticulate, a technological flowchart involving concentrating the whey protein cluster by ultrafiltration and thermomechanical treatment of the resulting concentrate has been proposed. Mathematical modelling of the process by means of neural-network approximation has been carried out to forecast the quality indicators and some directions of using whey proteins microparticulates depending on the initial technological parameters. The resulting microparticulates are characterized by a valuable composition, high water-binding, bifidogenic capacity and antioxidant activity. There have been the formulations of food products with the microparticulate content providing for the replacement of 10-40 % of fat-containing dairy raw materials, which has resulted in the energy value decrease. The technology of the developed products is characterized by the standard technological flowchart and supplemented by the stages of the microparticulate production.

1. Introduction
The development of innovative technologies of agricultural raw materials deep processing to produce new kinds of specialized, functional and fortified food products, to provide guaranteed and sustainable supply for the country's population is one of the priority areas of advancement of the dairy industry in the Russian Federation. A promising raw material source is whey, the resource potential of which exceeds 6.5 million tons, the rate of industrial processing being 40-45 % [1-3]. The main range of products manufactured on its basis is specific and limited. This does not provide the sufficient whey utilization and meet the domestic needs of Russia’s industrial sector and population. Whey proteins are of particular value in the composition of whey [4]. They have optimally balanced amino acid composition and carry out numerous functions in the human body. Membrane technologies are promising methods of producing the whey protein cluster that make it possible to fractionate protein components in a wide range and native state. New opportunities for expanding the application of whey proteins in food technologies are provided by their functional and technological properties: solubility, water-binding capacity, gel and foam formation, fat binding and emulsifying capacities [5-7]. Thus, the development of alternative modifications of the composition and properties of the whey protein...
clustering for the utilization in food technologies is an urgent task of the dairy industry. The main objective of the research is to optimize the conditions for modifying the whey protein cluster to develop competitive food products. To reach this goal the following tasks have been determined: to justify the method for the whey protein cluster modification and some directions of the rational use of modified product; to propose the technological flowchart; to determine the optimal parameters of the modification; to evaluate the quality indicators and functional and technological properties of the resulting modified product; to develop the formulations and adapt the dairy technology for the use of the modified whey protein cluster form.

2. Research Materials and Methods
The research has been carried out in research laboratories of VSUET’s departments and share-use centers, Voronezh and Moscow’s leading research centers and laboratories, as well as tested in pilot production conditions. The subjects of the research have been the curd whey obtained in the production of low-fat cottage cheese with the fat mass fraction of 9% on the basis of PJSC Voronezh Dairy plant (Voronezh, Russia), the cheese whey obtained in the production of Russian, Kalacheevsky and Tilsiter cheeses on the basis of Kalacheevsky cheese-making plant (Kalach, Voronezh Region, Russia), the whey proteins microparticulate, as well as dairy products derived from it. The native whey protein cluster has been developed in production conditions on the laboratory ultrafiltration unit equipped with polysulfonamide membranes, and on the Kieselmann ultrafiltration unit with ceramic membranes. The process of curd whey microparticulation has been carried out in PJSC "Voronezh Dairy plant" processing room with the use of the pilot unit EcoProt+ Kieselmann company manufactured.

Samples of the research subjects have been selected and prepared for analysis in accordance with the ISO 707:2008 (IDF 50: 2008) “Milk and milk products. Guidance on sampling”. The evaluation of organoleptic indicators has been carried out in accordance with ISO 22935-2:2009 Milk and milk products. Sensory analysis. Part 2: Recommended methods for sensory evaluation. Composition indicators of the research subjects and their physical and chemical properties have been determined in accordance with Russian standards [8]. The mass fraction of dry matter has been estimated by the weight loss as a percentage when drying the product sample at a constant temperature. The determination of the protein mass fraction has been done by the Kjeldahl method. The Bertrand method has been used to evaluate the lactose mass fraction in the research subjects. The determination of the fat mass fraction has been carried out by the acid method using the fat meter. The ash mass fraction has been estimated after drying the suspension in the muffle furnace. Mineral substances have been determined by means of the atomic absorption spectrometry. The active acidity has been evaluated using the potentiometric method. The titratable acidity has been determined by the titrimetric method. The dynamic viscosity has been estimated using the tuning fork vibration method on the SV-10 vibration viscometer.

The bifidogenic activity of the research subjects has been evaluated in the in vitro experiments. For this purpose, the modified Blauocco medium has been used, the Bifidobacterium bifidum microorganisms being cultured on it. The modification of the nutrient medium has involved adding the whey proteins microparticulate to it. The pre-dissolved and activated Bifidobacterium preparation has been introduced into the modified and control media at the rate of 5 doses per 1 liter of the medium. The cultivation has been carried out in anaerobic conditions. To evaluate the growth of the culture, the number of cells has been counted on fixed smears [9].

The in vivo evaluation of the antioxidant activity when inducing the free radical pathology has been carried out in the conditions of reproducing the pilot model of CCl4-induced liver damage. To study the biochemical parameters of the rat blood serum four groups of white noninbred individuals with the body weight of 200-220 g, 10 individuals in each group, have been formed by the principle of analogy, the duration of the experiment being 21 days. The antioxidant activity has been evaluated based on the blood serum indicators: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, bilirubin, cholesterol, and histologic sections of animal liver [10].
The microparticulation conditions optimization has been carried out using the developed software product made in the Python 3.5 programming language. It is designed for statistical processing of experimental data obtained during the microparticulation. The data array has included the microparticulation conditions, as well as the values of quality indicators of the resulting microparticulates. The optimization process includes analyzing the data array, building and training a neural network based on it.

The experiment has been interpreted using mathematical statistics based on the data from 5 to 10 experiments in a three-fold sequence. The graphical dependencies in the figures are presented after processing the experimental data using the least squares method.

3. Results
In the global dairy industry there is a method for modifying the whey protein cluster, which makes it possible for the protein globule to reach as close as possible the size and shape of the fat globule. It results in developing the food composition with unique properties simulating the milk fat flavor – the whey proteins microparticulate [11-12].

To develop the whey proteins microparticulate, the technological flowchart has been proposed that involves concentrating the whey protein cluster by ultrafiltration and thermomechanical processing of the resulting concentrates (Figure 1).

![Figure 1. The Flowchart of the whey protein microparticulate production: a – one-step, b – two-step.](image-url)

By changing the processing modes, you can adjust the particle size of the microparticulate, since it has a significant impact on the product viscosity, its consistency and the possibility to use it in the technology of different product line groups.

Mathematical process modelling by using the neural network approximation was carried out to predict the quality indicators and some directions of the whey proteins microparticulates application depending on the initial technological parameters. As a result of sequential neural networks training and testing, the architecture containing 1 hidden layer with 5 neurons has been recognized as the most successful option (Figure 2). The resulting architecture of the neural network and the values of weight coefficients (table 1) have formed the mathematical model, which resulted in the development of a
A software product to optimize the conditions for achieving and forecasting the whey proteins microparticulates quality.

![Figure 2. Network architecture for classification](image)

**Table 1. Weight coefficients values of synaptic links**

|     | 1       | 2       | 3       | 4       | 5       |
|-----|---------|---------|---------|---------|---------|
| 1   | 5.57085 | -3.99911| -22.7835| -18.8996| -6.3663 |
| 2   | -22.5788| -1.37797| 8.111961| 8.492995| -2.19921|
| 3   | 0.867612| -20.0283| 0.432452| 12.90843| -30.7419|

|     | 1       | 2       | 3       | 4       | 5       |
|-----|---------|---------|---------|---------|---------|
| 1   | 6.147442| 17.47173 | -8.08921| -7.91071| -5.85678 |
| 2   | 7.203244| 2.445926| -0.51973| -6.54331| -3.24957 |
| 3   | -6.73053 | 10.13949 | -15.873 | -8.93333| 21.70725 |
| 4   | -7.74047 | 4.843334| 3.660944| -3.03107| 2.42314  |

Taking into account the optimization performed, the whey proteins microparticulates of different chemical composition have been developed (Table 2), their organoleptic, physicochemical, and bifidogenic properties have been studied (Figure 3) and their antioxidant activity (Table 3).

![Figure 3. Effect of the microparticulate on the bifidobacterium growth](image)
Table 2. Composition and physicochemical properties of whey proteins microparticulates

| Indicator Name                                | Curd whey | Cheese whey |
|-----------------------------------------------|-----------|-------------|
| Factor of concentration (by protein)          | 4         | 18          |
| Mass fraction of solids, %                    | 8.5       | 18.1        | 8.9      | 18.5    |
| Mass fraction of total protein, %             | 3.4       | 10.5        | 3.5      | 11.3    |
| Mass fraction of true protein, %              | 3.0       | 9.4         | 3.2      | 9.7     |
| Mass fraction of lactose, %                   | 3.8       | 5.3         | 4.5      | 5.6     |
| Mass fraction of lactic acid, %               | 0.4       | 0.37        | 0.15     | 0.13    |
| Mass fraction of fat, %                       | 0.05      | 0.2         | 0.05     | 0.3     |
| Ash, mass, %                                  | 0.75      | 0.86        | 0.8      | 0.93    |
| Macronutrients, mg %, including sodium        |           |             |          |         |
| potassium                                     | 40.2      | 53.1        | 39.4     | 50.9    |
| calcium                                       | 120.1     | 137.2       | 113.1    | 123.0   |
| magnesium                                     | 84.3      | 82.1        | 84.0     | 81.6    |
| phosphorus                                    | 72.3      | 69.8        | 71.6     | 68.1    |
| Vitamins, mg %, including B₁                   | 0.036     | 0.045       | 0.037    | 0.041   |
| B₂                                            | 0.120     | 0.145       | 0.093    | 0.098   |
| PP                                            | 0.380     | 0.290       | 0.495    | 0.483   |
| Titrated acidity, °T                          | 60        | 75          | 19       | 25      |
| Active acidity, pH                            | 4.5       | 4.4         | 6.3      | 6.2     |
| Viscosity, MPa sec.                           | 9.7       | 25.3        | 10.1     | 27.0    |

Table 3. Effect of the cheese whey microparticulate on the biochemical parameters of the rat serum

| Indicator Name                               | Indicator Value for the group of animals |
|----------------------------------------------|-----------------------------------------|
| Indicator Name                               | 1<sup>a</sup>                           | 2<sup>b</sup>                           | 3<sup>c</sup>                           | 4<sup>d</sup>                           |
| Alanine aminotransferase (E / l)             | 70±1                                    | 81.9±3.10                               | 69±0.7                                  | 70±0.8                                 |
| Aspartate aminotransferase (E / l)           | 58±3                                    | 66.1±2.55                               | 57±2.4                                 | 58±1.7                                 |
| Alkaline phosphatase (E / l)                 | 332±10                                  | 401.7±18.95                             | 329±8                                  | 331±7                                  |
| Total protein (g / l)                        | 69±0.6                                  | 52.4±1.37                               | 69±0.6                                 | 69±0.6                                 |
| Albumin (mg / DL)                            | 29±0.4                                  | 22.3±1.47                               | 29±0.4                                 | 28.7±0.56                              |
| Total bilirubin (mg/DL)                      | 1.7±0.10                                | 2.3±0.57                                | 1.6±0.10                               | 1.6±0.07                               |
| Total cholesterol (mmol / l)                 | 1.8±0.03                                | 3.5±0.03                                | 1.8±0.03                               | 1.8±0.11                               |

<sup>a</sup> Test group - the animals received a normal feeding diet.
<sup>b</sup> Animals additionally received CCl₄.
<sup>c</sup> Animals additionally received the whey proteins microparticulate.
<sup>d</sup> Animals additionally received the microparticulate and CCl₄.

The resulting microparticulates have been characterized by the high water-binding and emulsifying capacity.

The valuable composition and properties of microparticulates have been proposed to be implemented in the technology of a wide range of food products: milk-intensive products (cottage cheese, cheese), fermented milk products (yogurt, kefir, sour cream) and products traditionally characterized by the high energy value (sauces, ice cream) [13-14]. The technology of the products developed is characterized by the standard technological flowchart and supplemented by stages of the microparticulate production.
4. Discussion
The initial stage of producing the microparticulate is the concentration of the protein cluster of pre-purified whey. This step should be carried out by means of ultrafiltration. During subsequent UV concentrates heating the whey proteins denaturation occurs. In this case, disulfide bonds involved in the formation of the structure of whey protein molecules are destroyed, and sulfhydryl groups (–SH) present in the tertiary structure are released. When protein molecules are disordered, there is an increase in the activity of the sulfhydryl groups of cysteine, the phenolic groups of tyrosine, the guanidine groups of arginine, and the ε-amino groups of lysine. Lactose, being the UV concentrate constituent, reacts with the ε-amino groups of lysine to form an enamine (Schiff base), catalyzing the first, inducing stage of the Maillard reaction [15].

The value and time of the temperature exposure have a decisive effect on the intensity of the melanoidin formation reaction. Selecting the optimal heating parameters makes it possible to directly adjust the flavor of the resulting microparticulate, minimizing the adverse organoleptic characteristics of the whey. Unfolding the protein molecules structure during heating is accompanied by the increase in their ability to aggregate and by the formation of new bonds with each other. The resulting clusters of whey protein molecules exceed the edge sizes of the colloid state, the proteins gathering in compact aggregates. To increase the efficiency of the transformation of whey protein molecules into particles, it is necessary to ensure a high denaturation rate (90-95 %). The denaturation of all whey proteins is known to occur in the temperature range of 62-78 °C. However, lactose, being the UV concentrate constituent, prevents proteins from the solubility loss during the heat treatment, stabilizing their structure against the thermal unfolding. The high lactose content in the concentrate slows down the denaturation reaction. Therefore, in order to ensure the microparticulation, it is necessary to use stronger modes of UV concentrate heat treatment – from 85 to 110 °C [16].

To form spherical microparticulate particles from clusters of denaturated whey proteins, mechanical processing is required. By changing its intensity, it is possible to adjust the particle size of the microparticulate. To prevent protein particles from the spontaneous aggregation under the conditions of low active acidity and from the precipitate formation, the thermal and mechanical impacts have to be done simultaneously during the microparticulation of the curd whey UF-concentrate. The microparticulation of the cheese whey can be carried out in both 1 and 2 stages.

The resulting whey protein microparticulates represent homogeneous, moderately transparent, white viscous liquid with a pure milk flavor and aroma. The color of the microparticulate results from the light scattering by agglomerated protein particles, which are characterized by a high degree of dispersion and are similar in size and shape to fat globules. As a result of highly intensive mechanical processing during the microparticulation, UV concentrates protein clusters are crushed to form particles of the correct spherical shape. It enables to simulate the flavor of milk fat, making the microparticulate sensory properties almost similar to those of cream. The microparticulation changes the flavor of UV concentrates. Changes in proteins and lactose during the heat treatment contribute to the formation of osmophoric components that form a pleasant flavor, and therefore, to leveling the specific organoleptic whey characteristics [17].

The chemical composition of microparticulates allows us to consider them as food products with functional properties. A significant proportion of proteins, peptides, and amino acids determine their bifidogenic properties. The fractional protein composition changes in comparison with the native whey, and the proportion of biologically more valuable whey protein fractions increases. Milk sugar in the microparticulate also acts as a prebiotic component, since it is not absorbed in the upper gastrointestinal tract and has a stimulating effect on the body's natural microflora. The bifidogenic properties of the microparticulate have been confirmed in invitro experiments. The microparticulate being added, bifidobacteria have demonstrated significantly higher growth on the modified medium. This opens up wide opportunities for the application of microparticulate in the technology of fermented milk products, giving them synbiotic properties and functionality.

The whey proteins denaturation during the microparticulation results in the activation of sulfhydryl groups. The –SH groups in native proteins are so structurally arranged that they can become reactive only after the α-helix is unfolded [18]. Their activation starts when heated at the temperature of 75 °C.
and increases with its rise. When the sulfhydryl groups are released, the oxidation-reduction potential is reduced the anti-oxidant properties of the proteins being increased. SH-containing compounds play the leading role in protecting cells from the hydroxyl radical being formed in the Fenton reaction or as a result of water molecules decomposition due to the ionizing radiation [19-20]. In addition, some products of the Maillard reaction are also characterized by the antioxidant activity. Enhancing the microparticulate antioxidative properties has been confirmed by the study of blood of laboratory animals, as well as by histological examination of liver tissues. Tetrachloromethane has induced a severe toxicological effect in the test animals, which has been indicated by a significant increase in the blood serum indicators such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, billirubin, and cholesterol. The liver of animals in this group has been characterized by degenerative obesity, necrosis and apoptosis. The liver of animals receiving the whey proteins microparticulate (Group 3) has demonstrated a substantial improvement in the cell architecture of various zones around the central veins. Most of the liver cells of animals of Test group 4 have had a neutral structure, which proves the increase in the antioxidant effect achieved during the microparticulation.

The microparticulation process has also changed the functional and technological properties of whey proteins. The high water-binding capacity is explained by the presence of hydrophilic groups of amino acids adsorbing water. The hydrate shell is formed around the protein particles of the microparticulate. The hydration of native whey proteins increases as the degree of denaturation grows. Due to their good hydration properties, microparticulates are a link between the water phase and other components of the dairy matrix. The water-binding capacity of the microparticulate increases the yield of milk-intensive products, including cheese and cottage cheese.

The modified composition and properties of the whey protein cluster have been applied in the technology of a wide range of dairy products. The developed formulations provide for the replacement of 10-40 % of fat-containing dairy raw materials with the microparticulate, which reduces the energy value while maintaining acceptable organoleptic properties. The implementation of the proposed technologies improves the efficiency and environmental friendliness of milk processing and reduces the demand for additional raw materials in the dairy industry.

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