Prospects for use of nanofiltration buttermilk and whey concentrates in the technology of fermented milk products with an increased mass fraction of protein

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Abstract. In compliance to the studies data on the composition and properties of protein concentrates of buttermilk and whey obtained by the method of nanofiltration have been expanded and systematized. It has been found that buttermilk and whey concentrates with a mass fraction of solids of 20 % contain up to 7 and 2.3 % protein, respectively. The dispersion composition and the nature of the concentrates microstructure have been studied using an electron microscope. When buttermilk has been concentrated by nanofiltration, the average diameter of the dispersed particles has not increased and amounted to (130 ± 30) nm, and the spatial mesh size has decreased by 3.2 times. When whey has been concentrated, the particle size has increased by 1.7 times while the spatial mesh size has decreased by 1.3 times. The regularities of the joint development of lactic acid and bifidobacteria in a milk base have been revealed with a different ratio of nanofiltration buttermilk and whey concentrates to substantiate the rational composition of the milk base. It has been proven that fermented milk products based on buttermilk and whey concentrates have increased biological value. The developed products contain 44-46 % more essential amino acids, the balance coefficient of amino acid composition is 17-18 % higher than those of cultured buttermilk.

1. Introduction
At the moment, the government’s priorities include tasks to increase production and expand the range of food products that contribute to maintaining a healthy lifestyle [1].

Among the modern directions of the development of the science of food technologies for healthy nutrition is the creation of functional products, the modern market of which is 65 % represented by dairy products [2].

The level of consumer awareness in the organization of healthy nutrition poses the challenge of developing new combined systems based on affordable, natural, high-grade raw materials and improving technologies while maintaining high consumer properties of the final product, low energy value, and a full range of biologically active substances. The composition of buttermilk and whey, primarily as sources of complete protein, allows attributing them to products with high nutritional and biological value, what determines the expediency of their effective use [3].

The structural-mechanical properties of acid clots can be controlled by changing the mass fraction of solids of the raw materials input. In the technology of some domestic fermented milk products, the most common method of increasing the solids mass fraction is the introduction of skimmed milk...
powder or whey protein powder concentrates. Abroad, ultrafiltration is more often used for these purposes [4].

An advanced way to increase the mass fraction of protein in buttermilk and whey is nanofiltration, which proceeds without phase transformations of the processed product and contributes to a significant reduction in the loss of biologically active substances [5]. Concentration by nanofiltration is preferable compared, for example, with reverse osmosis and condensation by evaporation, since the content of mineral substances increases slowly due to permeation of monovalent and partially divalent ions [6].

To balance the amino acid composition of buttermilk concentrates, it is possible to enrich them with whey concentrates, because whey proteins are among the most valuable proteins of animal origin, as they are a source of essential amino acids. They have the highest digestion rate; digestibility of whey proteins is 98 % [7].

It is possible to ensure high therapeutic properties of fermented products based on buttermilk and whey concentrates with a high content of complete protein by selecting an association of starter cultures that provide probiotic properties, provided that they have a well-expressed sour-milk product taste and smell and a uniform, moderately viscous consistency [3].

2. Materials and methods
When conducting experimental studies, certified equipment and modern research methods were used. The amino acid composition of the products was determined by high-efficient liquid chromatography using the specialized Aracus system; electron microscopic investigations were carried out using an EM-410 transmission electron microscope (Philips, the Netherlands).

The objects of study were: buttermilk, skimmed cheese whey, buttermilk and cheese whey concentrates with a mass fraction of solids from 10 to 20 % obtained by the method of nanofiltration, two versions of the milk base with a ratio of buttermilk concentrate to whey concentrate 75:25 and 50:50, respectively, and fermented dairy products based on them.

3. The study of buttermilk and whey concentrates obtained by nanofiltration.
For the concentration of buttermilk and whey, the TIA nanofiltration unit (France) equipped with polypyterazinamide membranes with a molecular weight cut-off of 200 D was used. Based on the recommendations of the membrane manufacturer, the concentration process was carried out at a temperature of 20 °C and a pressure of 2.5 MPa.

According to the preliminary studies, the filtration rate under these conditions significantly decreases with an increase in the mass fraction of solids in concentrates above 22 %. This is apparently due to the high concentration polarization on the membrane surface owing to the viscous and hydrodynamic conditions of product expiration. In accordance with the foregoing, the concentration of buttermilk and whey was carried out to a solids mass fraction in concentrates of 20 %.

The composition and properties of buttermilk and cheese whey concentrates obtained by nanofiltration are shown in table 1.

Thus, when buttermilk was concentrated by nanofiltration by 2.5 times, concentrates with a mass fraction of protein up to (7.03 ± 0.3) % were obtained. When concentrating cheese whey by 3.3 times, the mass fraction of whey proteins increased from 0.63 to 2.28 %.

In the course of fermented milk products manufacture, the pH of the medium significantly affects the development of starter microflora. As a result of studies, it should be noted that a decrease in active acidity with an increase in the mass fraction of solids is less significant compared to an increase in titrated acidity. With an increase in acidity by 2.2-2.6 times during the concentration of buttermilk from 8.1 to 20 %, the pH changes insignificantly – by 0.27 units. In whey concentrates, with an increase in titrated acidity by a factor of 3-4, when concentrated from 6.06 to 20 %, the pH changes by 0.3 units. Apparently, this is due to the presence in the concentrates of a number of buffer systems — protein, phosphate, citrate, and lactate ones [8].
Table 1. The composition and properties of buttermilk and whey concentrates

| Product               | Mass fraction of solids, % | Mass fraction of protein, % | Asidity, °T | pH, unit. |
|-----------------------|-----------------------------|----------------------------|-------------|-----------|
| Buttermilk            | 8.10±0.40                   | 3.30±0.50                  | 14.7±2.0    | 7.14±0.06 |
| Concentrate 1         | 10.40±0.20                  | 3.60±0.70                  | 16.4±2.8    | 7.06±0.08 |
| Concentrate 2         | 12.00±0.30                  | 4.60±0.30                  | 19.2±1.5    | 7.03±0.07 |
| Concentrate 3         | 13.90±0.30                  | 5.50±0.50                  | 23.0±1.1    | 6.99±0.06 |
| Concentrate 4         | 16.30±0.40                  | 6.20±1.02                  | 26.6±2.0    | 6.97±0.06 |
| Concentrate 5         | 18.40±0.20                  | 6.60±0.70                  | 30.1±2.5    | 6.94±0.04 |
| Concentrate 6         | 20.10±0.50                  | 7.03±0.30                  | 32.0±1.0    | 6.87±0.21 |
| Whey                  | 6.06±0.20                   | 0.63±0.15                  | 9.7±1.5     | 6.66±0.05 |
| Concentrate 1         | 9.80±0.39                   | 0.90±0.10                  | 13.7±0.5    | 6.49±0.01 |
| Concentrate 2         | 12.30±0.33                  | 1.13±0.06                  | 15.7±1.1    | 6.46±0.01 |
| Concentrate 3         | 13.94±0.13                  | 1.30±0.10                  | 18.7±1.1    | 6.43±0.01 |
| Concentrate 4         | 15.99±0.30                  | 1.87±0.16                  | 23.3±0.6    | 6.39±0.01 |
| Concentrate 5         | 17.92±0.18                  | 2.09±0.08                  | 26.3±0.5    | 6.37±0.01 |
| Concentrate 6         | 19.99±0.09                  | 2.28±0.06                  | 29.3±1.2    | 6.36±0.12 |

Electron microscopic studies of the microstructure of buttermilk, whey and their concentrates with a mass fraction of solids of 20 % were carried out in order to predict the quality indicators (primarily consistency) of fermented milk products based on them (table 2).

Table 2. Characterization of the microstructure of buttermilk, whey and their concentrates obtained by nanofiltration

| Object of study         | The size of the dispersed particles, nm | The size of the spatial mesh cells, microns |
|-------------------------|----------------------------------------|-------------------------------------------|
| Buttermilk              | 130±30                                 | 4.2±0.7                                  |
| Buttermilk concentrate (20 % solids) | 130±30                                 | 1.3±0.4                                  |
| Whey                    | 34±7                                   | 2.3±0.5                                  |
| Whey concentrate (20 % solids) | 58±9                                   | 1.8±0.5                                  |

It should be noted, that dispersed particles are present in the buttermilk both in isolated form and in the form of loosely coupled components, while dispersed particles in nanofiltration concentrate of buttermilk are mainly in bound form. When buttermilk was concentrated by nanofiltration, the average diameter of the dispersed particles did not increase and amounted to (130 ± 30) nm, and the mesh size decreased by 3.2 times, which is probably due to the increased intermolecular interaction of structural elements per volume unit of the dispersion medium [9].

When whey was concentrated, the particle size increased by 1.7 times with a decrease in the mesh size by 1.3 times, which is probably due to the enlargement of dispersed particles and the formation of individual free aggregates.

Thus, in the process of nanofiltration, structuring of the buttermilk and whey protein phase was noted. Apparently, during the formation of a sour milk clot in the process of fermentation, the elements of the protein phase of concentrates will be the centers of formation of a new continuous protein phase.

4. The influence of the milk base composition on the activity of the starter microorganisms development

To obtain fermented milk products balanced in amino acid composition, enrichment of buttermilk concentrates with whey concentrates in the ratio of 75:25 (variant 1) and 50:50 (variant 2), respectively, is provided. The characteristics of the concentrates and infant formula based on them are shown in Table 3.
Table 3. The composition of the milk base

| Sample               | Mass fraction, %, solids | protein  | fat       | Lactose  |
|----------------------|--------------------------|----------|-----------|----------|
| Buttermilk Concentrate | 20.10±0.50               | 7.03±0.12| 0.78±0.08| 10.58±1.2|
| Whey Concentrate     | 19.99±0.09               | 2.28±0.26| 0.10±0.01| 14.95±1.4|
| Variant 1            | 20.07±0.35               | 5.17±0.46| 0.70±0.06| 11.91±1.8|
| Variant 2            | 20.05±0.29               | 4.44±0.33| 0.55±0.05| 12.08±1.0|

To develop a technology of milk-based fermented dairy products containing selected combinations of buttermilk and whey concentrates, starter culture associations were selected that provide probiotic properties under condition that the milk has a pronounced fermented milk taste and smell and a uniform, moderately viscous consistency. For this, acidophilus bacillus and bifidobacteria are included in the composition of the starter culture. The introduction of thermophilic streptococcus into the starter culture will ensure the formation of a clot that is sufficiently resistant to destruction.

To study the patterns of the joint development of lactic acid and bifidobacteria, fermentation of the milk base with the selected cultures was carried out simultaneously. The ratio of cultures in the starter microflora of *Lactobacillus acidophilus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium* (A: T: B) was 1: 1: 3; 1: 2: 2 and 1: 3: 1, respectively. Fermentation was carried out at a temperature of (37 ± 1) °C until a sufficiently viscous clot was obtained. The duration of ripening was (4.0 ± 0.5) h.

The number of viable cells of lactic acid bacteria in the product ranged from 6.8·10⁸ to 9.9·10⁸ CFU / cm³, depending on the type of milk base and the starter culture composition. The minimum number of viable cells of bifidobacteria was noted in samples with a ratio of cultures in starter microflora A: T: B equal to 1: 3: 1. The largest number of viable bifidobacteria cells was observed in both samples of the milk base: from 4.3·10⁷ to 4.8·10⁷ CFU / cm³ with a ratio of cultures of 1: 1: 3.

According to the data obtained, both samples of the milk base are a favorable environment for the development of the studied starter microorganisms and allow obtaining products with probiotic properties.

5. The study of the biological value of products

The calculation results for the content of essential amino acids in 1 g of product protein are presented in Figure 1.

According to the data obtained, in a milk base containing 25 % whey concentrate, the content of valine, methionine (+ cysteine), threonine and tryptophan is close to the content of these acids in the “ideal protein”; a milk base with a whey concentrate content of 50 % provides a leucine content higher by 12.9 %, isoleucine, lysine, phenylalanine (+ tyrosine) higher by 16–32.6 % compared with the FAO / WHO amino acid scale.

To characterize the biological value of the developed products, the coefficient of balance and imbalance of the amino acid composition, as well as the index of essential amino acids were calculated [8] (table 4).

Table 4. Indicators characterizing the biological value of products

| Indicators                        | Fermented buttermilk (control) | Milk-based product Variant 1 | Milk-based product Variant 2 |
|-----------------------------------|--------------------------------|------------------------------|------------------------------|
| The balance ratio of amino acid composition | 0.51                           | 0.68                         | 0.69                         |
| The amino acid imbalance coefficient | 0.50                           | 0.32                         | 0.31                         |
| Essential amino acids index       | 0.80                           | 1.26                         | 1.24                         |

The calculation results of indicators characterizing the biological value of the product (table 4) indicate a higher balance of the amino acid composition of products protein with the selected milk base compared with the control sample. The balance coefficient of new products is 17-18 % higher,
and the unbalance coefficient is 18–19% lower compared to the control sample. Index of essential amino acids increased by 44–46% compared with the control sample, which indicates the high biological value of the developed products.

![Figure 1. The content of essential amino acids, mg per 1 g of protein.](image)

Thus, the use of the milk base, that includes buttermilk and whey concentrates obtained by nanofiltration, allows obtaining biologically complete products.

On the basis of experimental data and recommended daily intake of nutrients in foodstuffs for a healthy person [10], the degree of meeting the need for essential amino acids was determined. When using a single portion of a packaged product (usually 200 g), the degree of meeting the need for individual amino acids will be 12.4–30%, this will ensure the supply of essential amino acids in an amount sufficient for protein biosynthesis in the human body.

6. Conclusion
In the course of the studies, the feasibility of increasing the mass fraction of milk protein in buttermilk and whey by concentrating them with nanofiltration was proved. The use of a milk base that includes nanofiltration concentrates of buttermilk and whey in the studied ratio, allows you getting biologically complete fermented dairy products.

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