Improving the environmental safety of dairy products

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Abstract. The authors studied the influence of prebiotic components on the biological value of enriched yogurt. Yogurt was produced by fermenting pasteurized normalized milk with yogurt starter containing freeze-dehydrated strains of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis and lactose with the addition of a prebiotic complex containing an inactivated yeast culture of Saccharomyces cerevisiae, dietary fibers, vitamins, amino acids, macro- and microelements. The prebiotic complex was introduced at the stage of fermentation of the mixture, at a concentration of 1%, previously dissolved in milk at 38...42 °C. The amino acid content was determined; the aminoacid score, the utility of the aminoacid composition and the utility coefficient were calculated. It was established that the biological value of proteins of yogurt is characterized by their high content, balance of four essential aminoacids and presence of three limiting aminoacids. Enrichment of yogurt with a prebiotic complex slightly increased its biological value and the ability to absorb essential aminoacids. Therefore, it is impractical to use this component to increase the biological value of the protein of traditional products. The main function of the prebiotic component of the studied yogurt is to stabilize the intestinal microflora, which determines its functional significance.

1. Introduction
Commensal microorganisms (microbiomes) play a key role in maintaining human health. Violation of the homeostasis of the microbiota - "dysbacteriosis" - is a key link in the development of a number of pathological conditions including obesity, diabetes, cardiovascular diseases and even cancer. Active use of antibiotics since the middle of the last century has led to a 22-times reduction in the overall mortality rate from infectious diseases (USA), but at the end of the 20th century, the level of septicemia almost doubled, and the mortality rate from infectious diseases increased by 50%, [1]. It was established that intestinal dysbiosis is accompanied by an increase in the production of inflammatory cytokines, dysfunction of the intestinal barrier and activation of cellular apoptosis, which may lead to the development of multiple organ failure. Therefore, the demand for the use of probiotics to restore and maintain normal intestinal microflora has increased significantly [1, 2, 3]. The results of numerous studies have shown such positive effects of probiotics as induction of
antimicrobial peptides, production of antimicrobial factors, stimulation of mucus and IgA production, prevention of cytokine-induced apoptosis of intestinal epithelial cells, regulation of the development and functioning of immune and epithelial cells, antioxidant and antimutagenic activity, etc. [4, 5, 6].

It should be noted that the use of fibers or prebiotics is extremely necessary for dietary modulation of the human intestinal microbiota. Dietary fiber consumption contributes to extensive metabolic interactions between bacterial species present in the gastrointestinal microbial community. In the bacterial environment, cross-feeding occurs, while the products obtained as a result of fermentation of polysaccharides by one bacterial species provide substrates for the growth of other bacteria present in the community [7, 8]. Thus, prebiotics stimulate the growth of beneficial bacteria in the colon selectively. They are one of the promising groups of functional food ingredients that should have the following properties: they should not be degraded and absorbed in the upper gastrointestinal tract, they should be degraded by enzymes of microorganisms in the colon and selectively stimulate the growth of bifidobacteria and/or lactobacteria, they should have functional influence on the body and should be technologically stable in the production of food [9].

Nowadays, medicinal forms of probiotics, prebiotics and fecal transplants have taken a significant place in the pharmaceutical industry and occupy a fairly high price segment. At the same time, there is an available alternative natural way to restore and preserve normal microflora – this is the regular use of fermented-milk functional products [10]. It was found that the use of fermented-milk functional products enriched with probiotics and prebiotics in the diet not only contributes to the restoration of normal microflora after the use of broad-spectrum antibiotics, but also increases the nutritional and biological value of the base product [11, 12]. As functional ingredients, components of plant and animal origin are also widely used. They are the sources of such essential components of food as vitamins, vitamers, catechins, leucoanthocyanidins, flavanones, anthocyanins and anthocyandins, etc. [13, 14, 15].

One of the promising complex prebiotics is a preparation containing inactivated yeast culture Saccharomyces cerevisiae (vini), dietary fibers (wheat bran), vitamins, aminoacids, macro - and microelements. This preparation was used to enrich yogurt [12], and stevia extract was used as a plant component. Stevia leaves contain glycosides that provide their sweet taste - stevioside and rebaudioside. These compounds are used by the food industry as sweeteners [16]. Stevioside and rebaudioside A make up respectively 5-10% and 2-4% of a/m (dry base) of the leaves. Other glycosides are represented in minor amounts (rebaudioside B, rebaudioside C, rebaudioside D, rebaudiosides E, rebaudiosides F, dulcoside F, rubusoside, and steviolbioside) [17, 18]. Rebaudioside (the sweetest compound of stevia) is a sugar substitute and is widely used in the food industry, stevioside is used in the complex treatment of diabetes, obesity, hypertension and caries prevention [19]. In addition, stevia leaves contain more than 100 phytochemical compounds with antioxidant and medicinal properties, including diterpenes, triterpenes, sterols, tannins, alkaloids, phytosteroids, saponins, flavonoids, ascorbic acid, beta-carotene, luteolin, quercetin, etc. [18]. Recently, the work on the creation of fermented milk products with high biological value has been actively carried out [20]. In this study, we set the task to assess the influence of the prebiotic complex and stevia on the biological value of yogurt.

2. Materials and methods
The biological value of the elaborated yogurt enriched with a prebiotic complex was estimated by the content of aminoacids, primarily essential ones, at the end of the intended expire date (after 10 days).

The aminoacid composition was determined by capillary electrophoresis using the capillary electrophoresis system "Kapel-105". Free aminoacids were converted to phenylthiocarbamyl derivatives (FTC-derivatives) using phenylisothiocyanate, and their ionic forms were separated in a quartz capillary under the action of an electric field. The FTC-derivatives were registered at a wave length of 254 nm in the corresponding buffer solution.

To determine the aminoacid score, the aminoacid content in the product was consistently compared with the standard FAO/WHO scale.
The aminoacid score was calculated according to the formula:

\[ AC = \frac{AC_{\text{product}}}{AC_{\text{FAO/WHO}}}, \]  

(1)

where \( AC \) is the aminoacid score of the \( i \)-essential aminoacid, in unit fractions or in percents; \( AC_{\text{product}} \) – the content of the \( i \)-essential aminoacid in the product, g/100g of protein; \( AC_{\text{FAO/WHO}} \) - the content of the \( i \)-essential amino acid in the FAO/WHO protein, g/100g of protein.

In order to assess the balance of the aminoacid composition of the protein, the utility of the aminoacid composition, which means the possibility of absorbing each aminoacid, was calculated:

\[ \alpha_i = \frac{C_{\text{min}}}{C_i}, \]  

(2)

where \( \alpha_i \) is the utility of amino acids, in standard units; \( C_{\text{min}} \) is the minimum aminoacid score, in unit fractions or in percents; \( C_i \) is the amino acid score of the \( i \)-essential aminoacid, in unit fractions or in percents.

The next stage of the calculations was the determination of the utility coefficient of the amino acid composition of proteins:

\[ \mu = \frac{\sum_{k=0}^{n} (A_k \cdot \alpha_i)}{\sum_{k=0}^{n} A_k}, \]  

(3)

where \( n \) is the number of amino acids; \( A_k \) – the amount of each aminoacid, in g/100g.

Data collection, processing and output was carried out using a personal computer with the operating system Windows ® 2000/XP. To assess the differences between the mean values in the comparison groups, the Student’s t-test was used with an error probability of \( P \leq 0.05 \).

### 3. Results and discussion

Yogurt was produced by fermenting pasteurized normalized milk with yogurt starter containing freeze-dehydrated strains of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis and lactose (produced by "VIVO Industria" LLC) with the addition of the prebiotic complex "Eubikor" (produced by "SIC "NIR" LLC, St. Petersburg) at the stage of fermentation of the mixture, in a concentration of 1%, previously dissolved in milk at 38...42 °C. The technology is implemented in the following way. The preparation of raw materials is carried out (skimmed milk is pasteurized at a temperature of 82-86 °C with an exposure of 2-3 seconds; skimmed milk powder is added; the mixture is getting normalized). Then the mixture is homogenized at a temperature of 55-65 °C and a pressure of 15.0 ± 2.5 MPa; the milk mixture is pasteurized at a temperature of 90-94 °C with an exposure of 2-3 seconds and cooled to a temperature of 38-42 °C. Next, a starter consisting of strains of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis and lactose in an amount of 5% of the mixture weight is added to the milk mixture; prebiotic complex "Eubicor" in the amount of 1% of the mixture weight and dry stevia extract in the amount of 0.3% of the mixture weight are added afterwards. Obtained mixture is poured into consumer package (for example, a glass jar or a plastic container) and packed. It is fermented at a temperature of 38-42 °C to a clot acidity of 75 °T, the finished product is cooled and stored at a temperature of 2-6 °C. The obtained product samples were subjected to a quality assessment. During the experimental part of the work, generally accepted methods and techniques for determining the organoleptic, physicochemical and microbiological parameters of the finished product were used.

The results of the study of the aminoacid composition of yogurt enriched with a prebiotic complex are shown in Table 1.
Based on many years of FAO/WHO biomedical research, a criterion for determining the quality of protein was proposed – a standard that is balanced in essential amino acids and best meets the needs of the body. The amino acid limiting biological value is the one with the lowest score.

Biological value is an indicator of the quality of food protein reflecting the degree of correspondence of its amino acid composition to the body need for amino acids for protein synthesis. The biological value of food proteins is determined by various methods. One of the available methods is the calculation of the amino acid score.

### Table 1. Aminoacid composition of yogurt enriched with prebiotic complex.

| Amino acid                  | The actual value of indicators based on the results of research (g/100g) | Yogurt without prebiotic complex (control) | Yogurt enriched with prebiotic complex (experiment) |
|-----------------------------|------------------------------------------------------------------------|------------------------------------------|---------------------------------------------------|
| Arginine                    | 0.59 ± 0.023                                                           | 0.62 ± 0.021                             |
| Lysine                      | 0.18 ± 0.006                                                           | 0.19 ± 0.007                             |
| Tyrosine                    | 0.19 ± 0.005                                                           | 0.20 ± 0.006                             |
| Phenylalanine               | 0.24 ± 0.007                                                           | 0.26 ± 0.008                             |
| Histidine                   | 0.17 ± 0.005                                                           | 0.18 ± 0.006                             |
| Leucine and Isoleucine      | 0.23 ± 0.006                                                           | 0.24 ± 0.007                             |
| Methionine                  | 0.10 ± 0.005                                                           | 0.11 ± 0.005                             |
| Valine                      | 0.23 ± 0.009                                                           | 0.24 ± 0.009                             |
| Proline                     | 0.31 ± 0.009                                                           | 0.33 ± 0.009                             |
| Threonine                   | 0.20 ± 0.005                                                           | 0.21 ± 0.005                             |
| Serin                       | 0.19 ± 0.005                                                           | 0.21 ± 0.005                             |
| Alanine                     | 0.34 ± 0.008                                                           | 0.36 ± 0.009                             |
| Glycine                     | 0.20 ± 0.007                                                           | 0.21 ± 0.007                             |
| Aspartic acid               | 0.18 ± 0.004                                                           | 0.19 ± 0.004                             |
| Glutamic Acid               | 0.16 ± 0.004                                                           | 0.17 ± 0.005                             |
| Cysteine                    | 0.038 ± 0.0004                                                         | 0.04 ± 0.0004                            |
| The sum of all amino acids  | 3.548                                                                  | 3.76                                     |
| The sum of essential amino acids | 1.18                                                                | 1.25                                     |
| The sum of relatively essential amino acids | 0.76                                                                | 0.8                                      |
| The sum of non-essential amino acids | 1.608                                                                | 1.71                                     |

A reference (ideal) protein is a hypothetical product whose composition perfectly satisfies the body’s physiological need for essential amino acids. The protein of a chicken egg and female breast milk are the closest to the ideal chemical composition.

The aminoacid composition of the reference protein proposed by the FAO/WHO Committee shows the content of each of the essential amino acids in 100 g of protein (Table 2).

In a reference (standard) protein, the amino acid score of each essential acid is taken to be 100%. The amino acid limiting biological value is the one with the lowest score. The biological value of food proteins is determined by the first limiting amino acid.
Table 2. Amino acid scale.

| Amino acid                | Reference protein, g/100g of protein | Daily nutrition for adults, mg/kg of body weight |
|---------------------------|-------------------------------------|-----------------------------------------------|
| Isoleucine                | 4                                   | 10                                            |
| Leucine                   | 7                                   | 14                                            |
| Lysine                    | 5.5                                 | 14                                            |
| Methionine + Cysteine     | 3.5                                 | 13                                            |
| Phenylalanine + Tyrosine  | 6                                   | 14                                            |
| Threonine                 | 4                                   | 7                                             |
| Valine                    | 5                                   | 10                                            |

The results of the calculation of the biological value of the proteins of the elaborated yogurt with a prebiotic complex and yogurt without a prebiotic complex are presented in Table 3 and Table 4.

Table 3. Results of calculation of the aminoacid score of yogurt enriched with prebiotic complex.

| Amino acid                | Reference protein, g/100g of protein | The essential aminoacid content in the product, g/100g | The essential amino acid content, g/100g of protein | Amino acid score, % |
|---------------------------|-------------------------------------|------------------------------------------------------|-----------------------------------------------------|---------------------|
| Isoleucine                | 4                                   | 0.24                                                 | 5.5                                                 | 50                  |
| Leucine                   | 7                                   | 0.19                                                 | 4.3                                                 | 78.2                |
| Lysine                    | 5.5                                 | 0.11                                                 | 2.5                                                 | 71.4                |
| Methionine + Cysteine     | 3.5                                 | 0.46                                                 | 10.5                                                | 175                 |
| Phenylalanine + Tyrosine  | 6                                   | 0.21                                                 | 4.77                                                | 117.5               |
| Threonine                 | 4                                   | 0.24                                                 | 5.5                                                 | 110                 |

Table 4. Results of calculation of the aminoacid score of yogurt without prebiotic complex.

| Amino acid                | Reference protein, g/100g of protein | The essential aminoacid content in the product, g/100g | The essential amino acid content, g/100g of protein | Amino acid score, % |
|---------------------------|-------------------------------------|------------------------------------------------------|-----------------------------------------------------|---------------------|
| Isoleucine                | 4                                   | 0.23                                                 | 5.4                                                 | 49                  |
| Leucine                   | 7                                   |                                                      |                                                     |                     |
| Lysine                    | 5.5                                 | 0.18                                                 | 4.28                                                | 77.8                |
| Methionine + Cysteine     | 3.5                                 | 0.10                                                 | 2.3                                                 | 65.7                |
| Phenylalanine + Tyrosine  | 6                                   | 0.43                                                 | 10.2                                                | 170                 |
| Threonine                 | 4                                   | 0.20                                                 | 4.78                                                | 119.5               |
| Valine                    | 5                                   | 0.23                                                 | 5.4                                                 | 108                 |

The limiting essential aminoacid is considered to be the aminoacid with the lowest Amino acid scope; in this case, the first limiting amino acid is isoleucine and leucine; the second limiting aminoacid is methionine and cysteine; the third one is lysine. In this case, we observe a slight increase in the biological value of the enriched product. Utility ($\alpha$) should be understood as the ability to absorb each aminoacid. This indicator is the ratio of the minimum score ($C_{min}$) to the score of each aminoacid (S) for the studied products. The calculation results are presented in Table 5.
Table 5. Comparative analysis of the utility of amino acids.

| Amino acid                             | Yogurt enriched with prebiotic complex | Yogurt without prebiotic complex |
|----------------------------------------|---------------------------------------|----------------------------------|
| Isoleucine + Leucine                   | 1                                     | 1                                |
| Lysine                                 | 0.64                                  | 0.62                             |
| Methionine + Cysteine                  | 0.7                                   | 0.75                             |
| Phenylalanine + Tyrosine               | 0.29                                  | 0.29                             |
| Threonine                              | 0.43                                  | 0.41                             |
| Valine                                 | 0.46                                  | 0.45                             |

Based on the table data, it can be concluded that the balance of the essential amino acids of the studied products in relation to the protein reference is higher in the enriched product.

The utility coefficient of the amino acid composition is of practical importance, since the possibility of utilization of amino acids by the body is predetermined by the minimum score of one of them (Table 6).

Table 6. Utility coefficient of the amino acid composition of protein.

| Amino acid                             | \( A_i \cdot \alpha_i \) Yogurt enriched with prebiotic complex | \( A_i \cdot \alpha_i \) Yogurt without prebiotic complex |
|----------------------------------------|---------------------------------------------------------------|----------------------------------------------------------|
| Isoleucine + Leucine                   | 0.24                                                          | 0.23                                                      |
| Lysine                                 | 0.12                                                          | 0.11                                                      |
| Methionine + Cysteine                  | 0.08                                                          | 0.08                                                      |
| Phenylalanine + Tyrosine               | 0.14                                                          | 0.12                                                      |
| Threonine                              | 0.09                                                          | 0.08                                                      |
| Valine                                 | 0.11                                                          | 0.1                                                       |
| The sum \( A_i \cdot \alpha_i \)      | 0.78                                                          | 0.72                                                      |
| \( \mu \)                              | 0.54                                                          | 0.52                                                      |

4. Conclusion

As can be seen from the above, the biological value of the proteins of both traditional and enriched yogurt is characterized by their high content, balance of the three essential amino acids and presence of three limiting amino acids. Enriching yogurt with a prebiotic complex slightly increased its biological value and the ability to absorb essential amino acids. Therefore, it can be concluded that it is impractical to use this prebiotic component to increase the biological value of the protein of traditional products. The main function of the prebiotic component of the studied yogurt is to stabilize the intestinal microflora, which determines its functional significance.

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