Vegetable ingredients in functional fermented milk products

V V Kryuchkova¹, I F Gorlov¹,², S N Belik³ and A S Kamlatsky⁴

¹Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Volgograd, Russia
²Volgograd State Technical University, Volgograd, Russia
³Rostov State Technical University, Rostov-on-Don, Russia
⁴Don State Agrarian University, Persianovsky, Russia

E-mail: niimmp@mail.ru

Abstract. The analysis of literature found prebiotic properties of inulin and white currant’s health-promoting properties and rich chemical compositions that allow these products to be considered functional food ingredients with bifidogenic, immunomodulating, antioxidant, prebiotic, and hepatoprotective properties, which became a reason for inulin and white currant to be used for enrichment of a dairy product. The experimental studies have established the type of starter microflora—lactic acid and bifidobacteria. The optimal method of heat treatment and technological stage and doses of inulin (3.0%) and unclarified white currant juice (3.0%) were determined, i.e., holding juice and inulin dissolved in milk at t=65±2°C for 25-30 minutes, cooling to t=14±2 °C, and adding to fermented milk product cooled to t=14±2 °C. Qualitative indicators of the fermented milk product enriched with inulin and white currant were higher than those of a traditional product. The new product met the requirements of technical regulations and can be recommended as a functional product for systematic consumption by different age groups of population without restrictions.

1. Introduction
Functional food products are known to be the most promising area in the dairy industry in recent decades. The products of that kind take into account balanced diets of different population groups and are capable of satisfying their physiological needs for energy and essential substances [1, 2].

The production of functional fermented milk products uses probiotics (starter microorganisms), prebiotics (lactulose, inulin, oligofructose, dietary fiber, etc.), and natural plant materials (berries, vegetables, fruits, seeds, and spices) that are of great value, especially due to their specific combinations of biologically active components [3, 4].

Inulin (soluble fiber) is a β-fructose polysaccharide, sweetish and soluble in water. Inulin is a typical prebiotic, it is not broken down by intestinal glucosidase enzymes; therefore it is not digested and reaches the colon where it is utilized by microorganisms and contributes to the growth of beneficial microflora (lacto- and bifidobacteria). The microflora inhibits pathogenic microorganisms and controls the growth of opportunistic bacteria, thereby cleansing the intestines and the whole body from the waste products of dangerous microorganisms. Lacto- and bifidobacteria reduce ammonia (it can provoke the growth of tumors in the body) in the intestine; adsorb carcinogens and thereby decrease the risk of developing malignant cells; reduce the risk of cardiovascular diseases (atherosclerosis, hypertension, thrombosis, etc.) by lowering blood cholesterol and regulating lipid metabolism; and promote the
synthesis of vitamins B and some important enzymes. Inulin has an immunomodulating and hepaprotective effects and normalizes blood sugar, significantly reducing the risk of diabetes. In the intestine, inulin forms a gel that contributes to normal acidity and slower digestion of food and thereby prolongs saturation time and delays hunger. Thus, the prebiotic inulin is a necessary nutrition component that ensures the health of not only the intestinal microflora, but also all organs and systems of the human body [5, 6, 7].

White currant is a berry crop that is valued for its healthy properties. Its flowers are small, yellowish-green. The fruit are small white, without a characteristic strong aroma. The main nutritional value of white currant is a high content of pectin substances that have a beneficial effect on the blood, i.e., they remove toxins from the body, salts of heavy metals—lead, mercury, and cobalt—and affect the excessive content of harmful cholesterol. Another indisputable advantage is that white berries do not contain dyes that can cause harm or food allergies. This is especially important for children, nursing mothers, and elderly people. White currant is easily digestible and can be consumed while breastfeeding to reduce the risk of hypovitaminosis. It belongs to complementary foods for babies (from 5-6 months), strengthens the immune system, and maintains health for many years [9].

Thus, the analysis of literature on experimental, clinical, and epidemiological studies devoted to the compositions and properties of inulin and white currant allowed us to consider them functional food ingredients with the effect of normalizing intestinal microflora and immunomodelling activity and use them in the development of an enriched fermented milk product.

The purpose of the study was to determine the dose, method, and technological stage of adding inulin and white currant berries to a dairy product produced and study their influence on the quality of the innovative product.

2. Materials and methods
The targets of the research were inulin powder (IP), white currant berries (WC), and fermented milk samples produced with various IP and WC doses added and various methods of adding to the milk base applied. The main research methods were determination of fat content, GOST R ISO 2446-2011; determination of protein by the Kjeldahl method according to GOST 34454-2018; sensory evaluation in accordance with GOST R ISO 22935-2-2011; microbiological analysis, GOST 32901-2014; contents of vitamins and minerals were determined by capillary electrophoresis using a Drops-105M system. The data were processed, using the Statistica 6.0 software package.

3. Results and discussion
To determine the quality of IP and WC as plant components that enrich a dairy product, of great importance is the analysis of their chemical compositions that determine the potential abilities of IP and WC to satisfy physiological needs of the human body in carbohydrates, minerals, and dietary fiber. The nutritional values of the plant ingredients established by the analysis are presented in Table 1.

| Indicator                  | Weight fraction | Daily norm, g |
|----------------------------|-----------------|---------------|
| Calories, kcal             | 49.4            | 360.0         | 1684          |
| Proteins, g /100           | 0.5±0.02        | -             | 76.0          |
| Fats, g /100               | 0.2±0.01        | -             | 56.0          |
| Carbohydrates, g/100r, incl.| 12.4±0.04      | 90±0.13       | 219.0         |
| - dietary fiber, g/100 g   | 3.4±0.01        | 85±0.12       | 20.0          |
| - pectin, g/100g           | 1.0±0.02        | -             | -             |
| Ash, g/100                 | 0.5±0.02        | -             | -             |
| Organic acids, g /100 g    | 2.0±0.02        | -             | -             |
| Water, g /100 g            | 86.4±0.12       | 10±0.11       | 2273.0        |
Table 1 shows that the main component of inulin is carbohydrates, 94.4% of them are dietary fiber. In white currant, carbohydrates also prevail (12.4%), dietary fiber made 27.4% of the total amount of carbohydrates, pectins 8.0%, and organic acids 2.0%. When dietary fiber enters the gastrointestinal tract, it improves intestinal motility, stimulates digestion, and provides nutrition and growth of beneficial lactobacilli and bifidobacteria. Pectins remove “bad” cholesterol from the body, cleanse blood vessels, and prevent diseases of the cardiovascular system. Pectins also adsorb various intoxications in the intestine by turning them into insoluble complexes and remove toxins and salts of heavy metals from the body. Moreover, organic acids of white currant inhibit intestinal infections—bacteria and some viruses. Therefore, the biologically active substances of inulin and white currant have a beneficial effect on the gastrointestinal tract and liver, cardiovascular and immune systems, and hematopoiesis; they tone up the body, so can give functional properties to fermented milk products [5, 8].

A ferment that includes different strains of microorganisms is important in the production of fortified dairy products. We investigated three types of fermentation starters, i.e., Starter I was kefir starter (kefiric fungi overspill); Starter II contained *Lactococcus lactis* subsp. *Diacetylactis*, *Streptococcus salivarissubsp. Thermophilus*, and *Lactobacillus acidophilus*; and Starter III contained *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactobacillus helveticus*, *Propionibacterium freudenreichii subsp. Shermanii*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*. Starter III was found to be the most effective in terms of sensory characteristics and an increase in acid production during fermentation (figure 1).

### Figure 1. Efficiency of acid production of enriched fermented milk products with different types of starter cultures, °T.

The obtained results indicated that the acid production to a dense clot and titratable acidity of 65-70 °T lasted for 6.5-7 hours in the fermented milk product with Starter III. This result was 1 hour faster than in the product with Starter II and 3 hours faster than in the Starter I product, which was a resource-saving factor for the enterprise. The sensory evaluation showed that the fermented milk product with Starter III had higher points due to milk lactococcus, thermophilic streptococcus, and acidophilus bacillus that enabled homogeneous texture, a smooth dense homogeneous clot, without whey separated. The product had pleasant sour-milk taste and flavour, mutually complement with the taste and flavour of inulin and white currant juice introduced.

To develop a technology, there were established doses of plant ingredients introduced, i.e., 3.0% of inulin and 3.0% of unclarified white currant juice that were applied after preliminary heat treatment at
a temperature of 65±20°C, holding for 25-30 min, and cooling to a temperature of 14±20°C after fermentation. The milk product was also cooled to a temperature of 14±20°C. Evaluation of the quality of the developed product is presented in table 2.

**Table 2.** Comparative assessment of quality of dairy products.

| Name                        | Control                   | Prototype                  |
|-----------------------------|---------------------------|----------------------------|
| Sensory characteristics     |                           |                            |
| Appearance and texture      | homogeneous texture, stirred | homogeneous, stirred, with single plant ingredients interspersed |
| Taste and flavour           | clean sour-milk taste and flavour | clean sour-milk, slightly sweet with a pleasant currant aftertaste and flavour |
| Colour                      | milky white, uniform throughout the mass | milky white with slight sediment of dark-colored particles caused by plant components introduced |
| Nutritional and energy value|                           |                            |
| Weight fraction of fat, g   | 3.2±0.10                  | 3.2±0.13                   |
| Weight fraction of protein, g | 3.0±0.15                 | 3.0±0.16                   |
| Weight fraction of carbohydrates, g | 4.2±0.14             | 4.5±0.10                   |
| Weight fraction of ash, g, incl. | 0.7±0.04                | 0.72±0.04                  |
| - pectin substances, g      | –                         | 0.03±0.01                  |
| - dietary fiber, g          | –                         | 2.7±0.04                   |
| Energy value, kcal          | 57.6                      | 58.0                       |
| Microbiological points      |                           |                            |
| Lactic acid bacteria, CFU/cm³, not less than | 2*10⁷                     | 6*10⁷                      |
| Coliform bacteria           |                           |                            |
| S. aureus                   | Not detected              | Not detected               |
| Pathogenic (incl. salmonella) |                        |                            |
| Yeast, CFU/cm³ (g), not more than | 18                       | 24                         |
| Mold, CFU/cm³ (g), not more than | 20                       | 28                         |

Table 2 shows that the prototype of the enriched fermented milk product had better sensory characteristics in comparison with Control sample. The enrichment of the fermented milk product with plant ingredients increased its nutritional value, i.e., the content of carbohydrates increased by 3.0%, including developed pectin substances (0.03%) and dietary fiber (2.7%) that are prebiotics and made the product functional. A high carbohydrate content of 4.5 g/100 g, including plant polysaccharides, contributed to the development of lactic acid bacteria in the functional product for a longer period and prolonged its shelf life. The amount of lactic acid bacteria in the enriched product significantly exceeded both the normalized indices and Control values, which proved its probiotic properties and indicated a high physiological value. The amount of yeast and mold did not exceed the values regulated by TR TS 021/2011 regarding this group of fermented milk products. No pathogens, such as Coliform bacteria, Staphylococcus aureus, or Salmonella bacteria, were found in the fermented milk samples. The content of vitamins and minerals in the fermented milk products was an indicator of increased biological value (table 3 and figure 2).
Figure 2. Macronutrient / microelement composition of dairy products.

Table 3. Vitamin compositions of dairy products.

| Indicators            | Whitecurrant | Control     | Prototype   | Deviation         |
|-----------------------|--------------|-------------|-------------|-------------------|
| Vitamin B1, mg        | 0.01±0.02    | 0.041±0.02  | 0.054±0.02  | 0.003±0.02        |
| Vitamin B2 (riboflavin), mg | 0.03±0.02    | 0.151±0.02  | 0.152±0.02  | 0.001±0.02        |
| Vitamin B4 (choline), mg | 5.00±0.03    | 0.100±0.02  | 0.330±0.02  | 0.220±0.03        |
| Vitamin B5, mg        | 0.04±0.02    | 0.190±0.02  | 0.192±0.02  | 0.002±0.03        |
| Vitamin B9 (folic), mcg | 5.00±0.03    | 0.121±0.02  | 0.273±0.02  | 0.152±0.03        |
| Vitamin PP, mg        | 0.20±0.01    | 0.641±0.02  | 0.650±0.02  | 0.009±0.01        |
| Vitamin C, mg         | 40.0±0.03    | 0.610±0.01  | 1.813±0.02  | 1.203±0.03        |
| Vitamin A, mcg        | 0.04±0.02    | 0.042±0.02  | 0.043±0.02  | 0.001±0.01        |
| Vitamin E, mg         | 0.01±0.02    | 0.420±0.01  | 0.439±0.02  | 0.019±0.01        |

Table 3 shows that white currant contains a considerable amount of vitamins C, B4, B9, B5, E, and PP, so due to enrichment, the prototype had a significantly increased content of vitamins, i.e., Vitamin B4 3.3 times, Vitamin C 3 times, Vitamin B9 (choline) 2.25 times, Vitamin B5 by 21.1%, Vitamin E by 2.4%, and Vitamin PP by 1.6%. The vitamins take part in almost all metabolic processes, namely, vitamins PP, B1, and B2 in energy metabolism, B6 and B12 in protein metabolism, B9 in the exchange of nucleic acids, and B5 in fat metabolism and formation of coenzymes and prosthetic groups. Vitamin B5 is part of coenzyme A and determines the acetylation and synthesis of nucleic acids and proteins, normalizes the Krebs cycle, formation of acetylcholine, ATF, phospholipid, and other biologically active substances. Vitamin PP normalizes the cholesterol metabolism, decreases the rate of the atherosclerotic process, causes the regression of soft cholesterol plaques, and reduces the excessive content of cholesterol in cell membranes. Vitamin E is one of the main antioxidants of the human body that protects cell membranes from the destructive effect of oxygen-containing metabolites and is an essential component involved in the immune response. Thus, the enrichment of an innovative fermented milk product with plant components enabled increasing the amount and concentration of fat-and water-soluble vitamins, providing all the vital functions of the body.

The contents of macro- and microelements in the human body is quite constant, however, rather serious deviations from the norm can occur, which can lead to the development of pathologies of a different nature. The data in figure 2 show that the prototype had an increased dose of mineral substances, especially a high dose of alkaline substances—potassium, phosphorus, and calcium—that exceeded Control sample by 10.0%, 6.21% and 4.78%. According to figure 2, iron and zinc had the greatest increase. Iron is one of the essential trace elements involved in basic functions of life support, i.e., the production of iron-containing molecules and normal functioning of iron-dependent reactions. It
is part of more than 70 enzymes, is contained in hemoglobin, myoglobin, and respiratory enzymes that catalyze the processes of respiration in cells and tissues.

4. Conclusion
The study established that inulin and white currant are highly effective functional ingredients. The type of starter microflora used in production of the fermented milk product and optimal method of the temperature treatment of plant ingredients were determined. The technological stage of adding and doses of inulin (3.0%) and unclarified white currant juice (3.0%) were established, namely, holding juice and inulin dissolved in milk at t=65±2°C for 25-30 minutes, cooling to t=14±2 °C, and adding to fermented milk product cooled to t=14±2 °C. The qualitative indicators of the functional product were quite high and met the requirements of TR TS 033/2013. The fermented milk product enriched with inulin and white currant juice can be recommended as a functional product for systematic consumption to different age groups of the population without restrictions.

References
[1] Gorlov I F, Nelepov Yu N, Slozhenkina M I, Korovina E Yu and Simon M V 2014 Development of new functional products based on the use of sprouted chickpeas Everything about meat 1 28-31
[2] Slozhenkina M I, Gorlov I F, Kryuchkova V V, Serkova A D and Belik S N 2019 Vegetable ingredient in cheese product Potravinarstvo Slovak Journal of Food Sciences 1 (13) 1018-25
[3] Wong C, Harris PJ and Ferguson LR 2016 Potential Benefits of Dietary Fiber Intervention in Inflammatory Bowel Disease Int J Mol Sci 17(6) 919
[4] Cerdó T, Ruiz A, Suárez A and Campoy C 2017 Probiotic, Prebiotic, and Brain Development Nutrients 9 (11) 1247-9
[5] Hijová E, Bertková, and Štofilová J 2019 Dietary fiber as prebiotics in nutrition Cent Eur J Public Health 27(3) 251-5
[6] Dróżdż P, Šėžienė V and Pyrzynska K 2017 Phytochemical Properties and Antioxidant Activities of Extracts from Wild Blueberries and Lingonberries Plant Foods Hum Nutr 72(4) 360-4
[7] Klimenko A I, Kryuchkova V V, Skripin P V, Druker O V, Kontareva V Yu, Gorlov I F, Mosolova N I, Belik S N and Mishchenko A A 2017 Pat. of the Russian Federation No 2681291 publ. 12.15.2017
[8] Kryuchkova V V, Kontareva V Yu, Skripin P V and Savitskaya T S 2020 Pat. of the Russian Federation No 2681987 publ. 03.14.2020
[9] Barclay T 2010 Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses J. Excipients and Food Chem 1 27–50