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HEALTHY FOOD PRODUCTS WITH PROBIOTIC AND PREBIOTIC PROPERTIES

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Abstract: In this paper the results of the study of the structure and properties of arabinogalactan extracted from Dahurian larch as well as its influence on the formation of fermented milk products are presented. The optimum application rate of arabinogalactan in a composite mixture is determined. The choice of the starter culture for the production of a functional fermented milk product is justified.

Keywords: functional food products, probiotic, prebiotic, arabinogalactan, milk, soy-based food

INTRODUCTION

In recent years, the implementation of the healthy food policy has been one of the important trends in health maintenance, disease prevention, restoration of disturbed functions of the body, and active human longevity. Food is the most powerful factor influencing the human body state. It affects all systems and organs continuously throughout life [1].

Food is a source of energy and substances necessary for growth, development, and other human vital processes. Food products should improve metabolism and body's resistance to adverse environmental effects as well as to have physiological, revitalizing, and preventive effects on the human body [2].

An important role in maintaining health and human performance is played by the adequate and regular supply of the body with all the nutrients [3, 4]. Thus it is necessary to take into account that the total number of incoming biologically assimilable nutrients should not exceed the daily physiological need of a healthy person since it can be accompanied by undesirable side effects.

As part of the development of the optimum nutrition concept a new branch of science was formed, i.e., the functional food. It includes the development of theoretical bases of production, sale, and consumption of functional foods.

Functional foods are conventionally divided into main groups with respect to specifics of the production process: conventional foods containing a significant amount of the native physiologically functional ingredient, products with technologically reduced harmful components, and products enriched with prebiotics and probiotics, antioxidants and vitamins, micronutrients and flavonoids, minerals and other substances essential to the human body [5].

A current area of research is the development of technologies for manufacturing fermented milk products with probiotic properties [6]. It is believed that at the beginning of the XXI century dairy products with probiotic cultures will occupy half of the existing market of chemical drugs and thereby solve the problem of prevention and treatment of many human diseases [7, 8, 9].

The use of probiotics based on live microorganisms from the normal human microflora is an important element of the concept of healthy nutrition of the population and one of the most effective ways to prevent human gastrointestinal tract disorders and treat thereby developing disorders of digestive, immune, and endocrine systems [10, 11].

The possibility of application of probiotics in dairy products is determined by the availability of milk, its low cost, multicomponent composition, possibility of modification, and easy fractionation [12]. In introducing probiotic bacteria (before or after souring), it is necessary to take into account that their viability is a valid unit of measurement of the probiotic activity. Therefore, the content of bifidobacteria in probiotic products should be standardized [13].

In the fermentation of raw milk the focus should be made on the selection of strain of starter microflora. Poor development of bifidobacteria in milk is associated with oxygen dissolved in it, so the co-culture of bifidobacteria with lactic acid bacteria has a number of advantages. Lactobacilli bind oxygen dissolved in milk and thus create anaerobic conditions favorable for the growth of bifidobacteria.

Numerous studies [14, 15] found that dairy products fermented with probiotic bifidobacteria and lactobacilli stimulate the immune system and protective functions of the body and supply a number of essential amino acids and B vitamins. Their ability to reduce the level of urea and cholesterol in the blood as well as anticarcinogenic and antimutagenic activities which perform protective and detoxificating functions were found. Bifidobacteria contribute to the absorption of lactose [15].

According to researchers [16], the mechanism of stimulation of growth of normal microflora of gastrointestinal tract with probiotics consists in inhibiting the growth of pathogens, immunocompetent cell activation, stimulation of growth of the endogenous microflora resulting from the production of vitamins and other growth promoting factors, neutralizing toxins, and change of microbial metabolism that manifests itself in the increase or decrease of enzyme activity. Because of such properties of probiotic cultures as the survival rate and proliferation in the human intestine, the viability in passing through the gastrointestinal tract, the metabolic activity, and the ability to provide therapeutic effects [17], they are an essential component of functional foods.

In developing dairy products containing bifidobacteria a great role in correction and activation of the habitat of bifidobacteria and lactobacilli is played by prebiotics. Numerous studies have pointed out that prebiotics stimulate the growth of the "right" microorganisms, i.e., bifidobacteria and lactobacilli. Thus, the research on the development of domestic functional prebiotic products is promising and relevant [18, 19].

Prebiotics include nondigestible food ingredients that improve health by selective stimulation of growth and metabolic activity of bacteria living in the colon. Prebiotics are not susceptible to hydrolysis by human digestive enzymes. They are not absorbed in the upper digestive tract. They are selective substrate for the growth and metabolic activity of beneficial microorganisms [3].

Prebiotic properties are most pronounced in fructose oligosaccharides, inulin, galacto-oligosaccharides, lactulose, and lactitol. Prebiotics are found in dairy products, corn flakes, cereals, bread, onions, field chicory, garlic, beans, peas, artichokes, asparagus, bananas, and many other products. Lactulose [20, 21] and gum arabic are generally accepted prebiotics.

In recent years, the food additive Fibregum has become increasingly popular. Fibregum is the exudate of acacia which is a glycoprotein whose macromolecules consist of protein core and arabinogalactan side chains. Because of the combination of technical characteristics and nutritional properties, Fibregum is recommended for use in the manufacture of various food products. Dietary fibers of Fibregum can stimulate the growth of beneficial bacteria and promote the formation of short chain fatty acids that have a beneficial effect on the physiology of the human body [22, 23].

When producing dihydroquercetin from larch, a significant amount of biologically active substances can be obtained as a byproduct. One such biologically active substance is arabinogalactan.

From the middle of the last century domestic and foreign scientists have been studying its structure and properties. Arabinogalactan is found in immunomodulating herbs (Echinacea purpurea, Baptisia tinctoria, Angelica Acutiloba, and Curcuma longa), but the study of the arabinogalactan extracted from larch is of the greatest interest, since it constitutes a significant part of its biomass. The heartwood of some species of larch comprises up to 35% of arabinogalactan [24].

In recent years, the study of the biological activity of arabinogalactan has been intensified. The difference of arabinogalactan from many polysaccharides has been reported in terms of physico-chemical properties such as low viscosity of concentrated aqueous solutions, high solubility in water, resistance to acid environment, thermal and hydrolytic stability, and good dispersing ability [25].It was found that arabinogalactan has a significant membranotropic and antimicrobial action against certain bacteria as well as immunomodulating property [26, 27] and antimutagenic activity [27].

The prebiotic ability is of particular interest in studying the properties of arabinogalactan. The results of studies of foreign and domestic scientists show the effect of larch arabinogalactan as a nutrient medium for Lactobacilli and Bifidobacteria since it is a fermentable fiber.

Arabinogalactan serves as food to beneficial symbiotic bacteria in the colon. In turn it helps to reduce the growth of pathogenic bacteria [24, 28, 30, 29, 30].

Over the past few years because of its multifunction properties arabinogalactan has been aggressively introduced in the production of dietary supplements and foods. Thanks to the dispersing ability it is relevant to use arabinogalactan in the development of formulas of yogurt, juices, pastries, confectionery, powdered milk, and other foods rich in mineral supplements and vitamin complexes.

Arabinogalactan mixes well with all kinds of food and does not affect the organoleptic properties of the finished product. It is a source of dietary fiber and has a positive impact on the gastrointestinal tract. It can be recommended as a nutraceutical or functional food supplement in the human diet [31].

Based on the above, studies were carried out in order to assess the possibility of using arabinogalactan extracted from the larch as a functional ingredient adding probiotic and prebiotic properties, improving traditional technologies, intensifying the process of fermentation of soy milk mixture, improving physical, chemical, and organoleptic properties, and microbiological parameters of the fermented milk product.

SUBJECT AND METHODS OF RESEARCH

The whole experiment cycle consists of a number of interrelated steps.

At different stages of work there were the following objects of study: composite blend (skim milk and soy-based food) in the ratio of 70:30, arabinogalactan extracted from larch, which according to TU 9325-008-706-921-52-08 is produced and sold under the trade mark "Lavitol-arabinogalactan" at ZAO Ametis, Blagoveshensk, Amur region, Russia, the starter culture composition YF-L811 (Streptococcus thermohilus, Lactobacillus delbrueckii subsp. bulgaricus) and BB-12 (Bifidobacterium lactis) in the ratio of 1:1.

At the first stage of the experiment, the structure and properties of arabinogalactan extracted from larch were investigated.

At the second stage, the effect of the arabinogalactan portion on the dynamics of the acid formation of the clot in the fermentation of the composite mixture was determined. Syneresis properties, effective viscosity, and microbiological properties of the final clot with respect to the portion of arabinogalactan in the mixture were considered. The technological stage of introduction of arabinogalactan in the product was defined.

At the third stage, the effect of the portion of arabinogalactan on the product storability was investigated.

In carrying out the experimental part, the complex of generally accepted and standard research methods was used including chemical, physical, microbiological, biochemical, and rheological methods.

RESULTS AND DISCUSSION

Arabinogalactan is the main part of intracellular wood polysaccharides that performs protective functions and contains bioactive nutrients.

The structure of arabinogalactan has been studied by infrared spectroscopy. Infrared spectra of arabinogalactan are shown in Fig. 1.

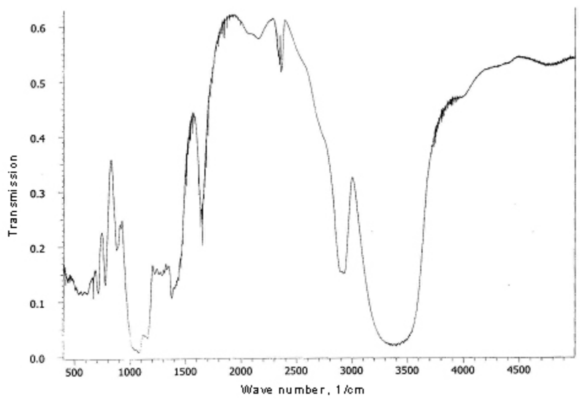

Fig. 1. The IR-spectrum of arabinogalactan.

In the IR spectrum of arabinogalactan there were intense absorption bands characteristic of deformation vibrations of cycles (716 cm^{-1}, 781 cm^{-1}, 884 cm^{-1}, 1085 cm^{-1}, 1162 cm^{-1}).

The carbonyl group has significant absorption bands at 1647 cm^{-1}. Absorption bands characteristic of stretching vibrations of C-O manifest themselves in bands 1085 cm^{-1} and 1162 cm^{-1}. Vibrations of hydroxyl groups are in the area of 2913 cm^{-1}. Broad peaks are characteristic of associated hydroxyl groups 3385 cm^{-1}.

In terms of organoleptic parameters arabinogalactan is an amorphous pale cream dry powder with a light almost impalpable pine smell and faint sweet flavor.

In the course of work the effect of the portion of arabinogalactan extracted from the larch on the formation of qualitative properties of the fermented milk product was investigated, in particular on the dynamics of titratable acidity, effective viscosity, syneresis, organoleptic, and microbiological properties of clot samples. The technological stage of introduction of arabinogalactan in the product was defined.

In the experiment, five samples of the composite mixture (skimmed milk and soy-based food in the ratio of 70:30) enriched with arabinogalactan in an amount of 0.5 to 2.5% were used. The sample of the mixture without arabinogalactan served as the control.

The mixture was fermented with the starter culture composition YF-L811 (Streptococcus thermohilus, Lactobacillus delbrueckii subsp. bulgaricus) and BB-12 (Bifidobacterium lactis) in the ratio of 1:1.

The effect of arabinogalactan on the organoleptic characteristics of the product was examined at various stages of the technological process.

The experiment showed that in the introduction of arabinogalactan in the mixture before the heat treatment, the sample of the clot had a sour-milk taste, was moderately sweet, white colored with cream tint, uniform throughout the mass, smoothly consistent, and moderately viscous and sticky. In the introduction of arabinogalactan in the mixture before fermentation, the clot was obtained which was characterized by a loose consistency with a significant separation of serum. In the introduction of arabinogalactan in the product after fermentation an extensive destruction of the clot takes place. Thus, it is recommended to introduce arabinogalactan in the mixture before heat treatment.

During the fermentation the dynamics of the acid formation of clots depending on the portion of arabinogalactan was investigated. The fermentation was carried out at a temperature of (40 ± 2) ?C for 6 hours. The titratable acidity was determined at intervals of 1 hour. The results are presented in Fig. 2.

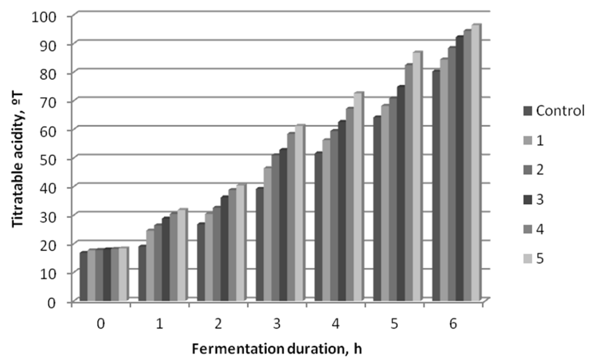

Fig. 2. The titratable acidity of the clot depending on the portion of arabinogalactan: control is without arabinogalactan, 0.5% (1), 1.0% (2), 1.5% (3), 2.0% (4), and 2.5% (5).

The analysis of the obtained data suggests that as a result of introduction of arabinogalactan in the composite mixture the fermentation time is greatly reduced. It can be associated with an increase in dry matter content in the mixture and the stimulating effect of the introduced polysaccharide on the microflora of starter cultures. It was found that if the portion of arabinogalactan is 0.5, 1.0, 1.5, 2.0, and 2.5% the titratable acidity of samples increases intensively by 4.5%, 11.3%, 17.0%, 20.1%, and 23.0%, respectively, compared to the control sample.

The effect of the portion of arabinogalactan on the number of viable microbial cells in the resulting sour-milk clot was investigated. The results are shown Table 1.

Table 1. The effect of the arabinogalactan portion on the number of viable microbial cells

Portion of arabinogalactan	Bacterial culture type		
	Streptococcus thermohilus	bacillus delbrueckii subsp. bulgaricus	Bifidobacterium lactis
0.5	$8 \cdot 10^7$	$6 \cdot 10^7$	$6 \cdot 10^7$
1.0	$4 \cdot 10^8$	$8 \cdot 10^7$	$2 \cdot 10^8$
1.5	$3 \cdot 10^9$	$4 \cdot 10^8$	$6 \cdot 10^8$
2.0	$5 \cdot 10^9$	$6 \cdot 10^8$	$8 \cdot 10^8$
2.5	$7 \cdot 10^9$	$7 \cdot 10^8$	$9 \cdot 10^8$
Control	$3 \cdot 10^7$	$5 \cdot 10^6$	$2 \cdot 10^7$

The analysis of table data shows that the introduction of a portion of arabinogalactan up to 1.5% leads to an increase in the number of viable microbial cells in the product compared to the control sample. However, it should be noted that the introduction of 2.0 and 2.5% of arabinogalactan had no significant impact on the increase in the number of viable microbial cells. This is possibly associated with a sufficient accumulation of lactic acid and other metabolic products and the high density of the bacterial population of microorganisms.

The structure of the clot is formed in souring and depends on various factors. In particular, it depends on used raw materials and a kind of starter cultures as well as on the introduced various food components. In this connection, the effect of the introduced portion of arabinogalactan on the effective viscosity of investigated clots at different shear rate was examined. The control sample is without arabinogalactan.

The nature of the change of the effective viscosity of the product depending on the amount of introduced arabinogalactan is presented in Fig. 3.

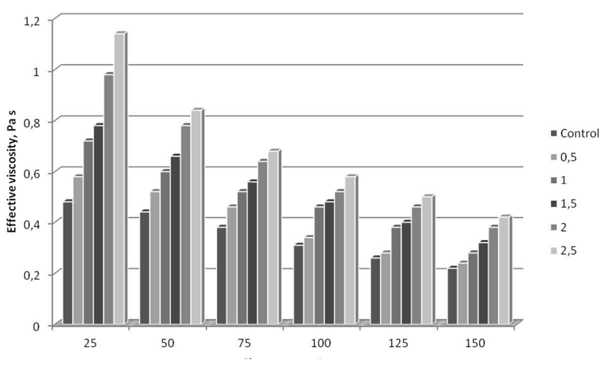

Fig. 3. The dependence of the effective viscosity on the shear rate in the fermented milk product with different portions of arabinogalactan:
0.5% (1), 1.0% (2), 1.5% (3), 2.0% (4), and 2.5% (5), control is without arabinogalactan.

It was found that with the increasing portion of arabinogalactan in the samples structural and mechanical properties are modified, the viscosity increases in proportion to the increase in the content of arabinogalactan. In the mixture which does not contain arabinogalactan (control sample) the clot was less viscous. It is noted that in the case of the mass concentration of arabinogalactan of 2.0 and 2.5% the consistency of the clot was excessively viscous. The product with a mass fraction of 1.5% arabinogalactan had the most appropriate viscosity.

In the finished samples syneresis properties of the clots have been examined. The results of these studies are presented in Fig. 4.

The results of the experiment revealed the ambiguous dependence of syneresis properties of clots on the portion of arabinogalactan introduced into a mixture. With the increasing portion of polysaccharide in the product the decrease in the syneresis ability of clots by 1.2, 1.4, 1.6, 1.7, and 1.8 times was observed respectively as compared with the control sample. It confirms water-binding properties of arabinogalactan.

The analysis of organoleptic characteristics of the analyzed samples indicates that the introduction of arabinogalactan has no effect on the taste and smell of the product. All the obtained clots had high quality organoleptic characteristics, i.e., they had taste and smell of sour milk, the soy component was almost not felt, the color was white with a cream tint, they were uniform throughout the mass.

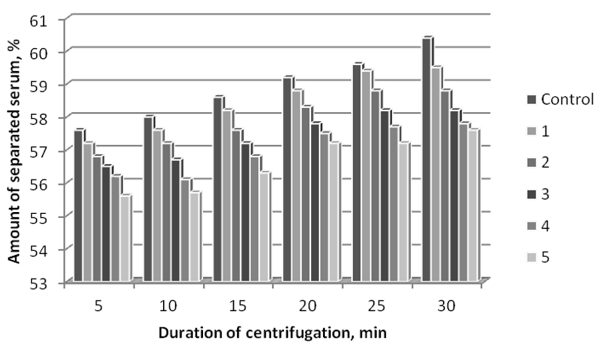

Fig. 4. The syneresis ability of clots depending on the arabinogalactan portion:
control, 0.5% (1), 1.0% (2), 1.5% (3), 2.0% (4), and 2.5% (5) of the mixture weight.

The biological value is an important indicator characterizing the quality of dairy products. It is determined by the presence of complex nutrients in the product.

In order to determine the biological value of the obtained soy-milk-based product enriched with arabinogalactan, its amino acid composition has been analyzed. It makes it possible to assess its biological value with a greater degree of correctness. The amino acid composition of the product is shown in Fig. 5.

The biological value of dairy products was also assessed by the amino-acid score. The calculation results of amino-acid score of essential amino acids are given in Table 2.

The results have indicated that the analyzed dairy product based on soy-milk mixture enriched with arabinogalactan extracted from Dahurian larch has a high biological value.

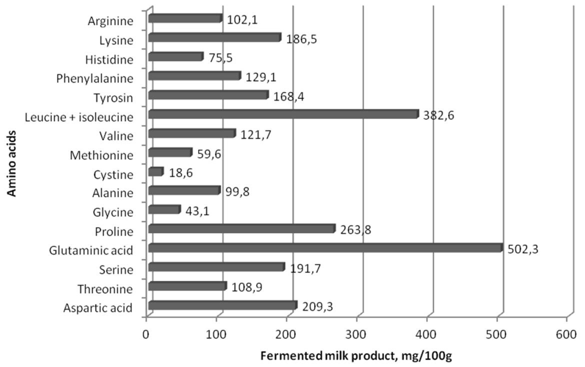

Fig. 5. The amino acid composition of the fermented milk product

According to the calculation results the amino-acid score of essential amino acids, i.e., leucine, isoleucine, threonine, and phenylalanine + tyrosine, exceeds 100%. It has been noted that the limiting amino acids are methionine + cystine (84.5%) and valine (92.1%).

Table 2. The composition of essential amino acids and amino-acid score of the fermented milk product in comparison with the recommendations of the FAO/WHO

Amino acid	FAO/WHO g/100 g of protein	Fermented milk product	
		g/100 g of protein	Score. %
Valine	5.0	4.60	92.1
Leucine	4.0	5.78	144.6
Isoleucine	7.0	10.12	145.0
Lysine	5.5	7.05	128.3
Methionine + cystine	3.5	2.96	84.5
Threonine	4.0	4.12	103.0
Phenylalanine + tyrosine	6.0	11.06	184.4

The production of functional foods with extended shelf-life is an important and promising direction in the food industry.

The storability manifests itself in the immutability of sensory, chemical, and physical properties throughout the storage life of the product [32, 33]. The quality of the finished product greatly depends on the variability of its basic constituents such as proteins, carbohydrates, and, in the first place, lipids which are oxidized when processed and stored.

The design of new functional foods should include careful planning and testing of the shelf life of the finished product. A comprehensive approach to this problem involves the analysis of the composition of the product, technological process parameters, packaging, environmental factors, chemical and biochemical reactions, and the types of microorganisms present [34].

The antioxidant activity of natural products is one of the important indicators that determine their biological value.

Arabinogalactan is a polysaccharide with a wide range of useful properties, but there is almost no data on its antioxidant activity in literature.

Based on the above, the effect of the introduction of the portion of arabinogalactan on the storability of the fermented milk product was investigated.

When storing prepackaged in plastic five samples of products containing different portions of the introduced arabinogalactan, at (40 ± 2)C the change in the number of viable cells of lactic acid bacteria and bifidobacteria were observed for 10 days.

The number of viable microbial cells was determined on second, fourth, sixth, eighth, and tenth day of storage. The sample without arabinogalactan served as a control one.

The results showed that in the samples with arabinogalactan during the storage for two, four, six, eight, and ten days the number of viable cells of bifidobacteria maintained at the desired level ($5\cdot10^8$, $3\cdot10^8$, $1\cdot10^8$, $7\cdot10^7$, $3\cdot10^7$ CFU/cm^3) and corresponded to microbiological requirements on the eighth day of storage, which was not the case for the control sample in which on the sixth, eighth, and tenth day of storage the quantitative indicator of bifidobacteria content was $1\cdot10^6$, $8\cdot10^5$, $2\cdot10^5$ CFU/cm^3.

CONCLUSIONS

The obtained results suggest that arabinogalactan extracted from Dahurian larch has a stimulating effect on bifidobacteria and lactobacilli. It is proved that its introduction makes it possible to obtain a product with a high number of viable cells of the probiotic microflora in the final product.

The use of arabinogalactan intensifies the fermentation of the composite mixture (skimmed milk and soy-based food in the ratio of 70:30) which accordingly reduces the production cycle of the product.

The application rate of 1.5% arabinogalactan in the composite mixture has been found. It is recommended to introduce arabinogalactan in the composite mixture before its pasteurization which makes it possible to obtain a product with high physical-chemical, rheological, and organoleptic characteristics.

The expediency of the use of arabinogalactan in the production of dairy products is proved.

Summing up the above, we can conclude that the study of the enrichment of food with components with prebiotic properties is relevant in the field of technology of production of functional foods.

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