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The Cedar Cake Influence on the Consumer Properties of the Bioproduct

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Abstract

The influence of the cedar cake on biochemical activity of bifidobacteria in milk fermentation was investigated. It has been observed that the fermentation process intensifies and the concentration of viable cells of bifidobacteria increases with the increase in the dose of the cedar cake, which indicates its prebiotic properties. High-molecular polysaccharides of the cedar cake contribute to the formation of thixotropic-reversible bonds in the fermentation of the protein system and form a stable viscous consistency.

The correlation between the content of dietary fibers and morphological changes of bifidobacteria has been demonstrated. The presence of morphologically various intercellular contacts, i.e. the cohesion, providing high adaptation of bifidobacteria to the new environment has been shown. The increase in the cedar cake dose in the nutrient medium leads to the increase in the adhesive properties of bifidobacteria, which are able to colonize the food fibers' surface and attach to them with the formation of mucous biofilms. This testifies to the good survival of bifidobacteria in the gastrointestinal tract of a human.

Keywords: bifidobacteria, cedar cake, cohesion, adhesion.

INTRODUCTION

In recent years, the development of a new direction in the food industry, the so-called functional nutrition, which implies the use of such products of natural origin that in systematic use have a regulating effect on the body as a whole or on its certain systems and organs, has been widely recognized throughout the world. On a global scale there is an ongoing work on the creation of new functional nutrition products possessing both wide spectra of application and spotted orientation towards the specific organ, system, and disease [1; 2; 3; 4].

Over the past decades, the problem of filling the shortage of coarse vegetable food in the diet of a man has gained momentum due to the wide implementation of the functional nutrition theory. Due to this, the deep researches of the structure and content of fibers' properties, the technologies of their extraction from initial vegetative resources, and their use at the creation of foodstuffs are underway in many countries [5; 6; 7].

The role of food fibers in nutrition is diversified. It consists not only in the partial supply of the human body with energy but also in the regulation of physiological, biochemical processes in digestive organs. The fibers-rich food has a positive effect on digestive processes.

Thus, the data obtained from various sources indicate that the dietary fibers normalize the functional activity of intestinal microflora and the gastrointestinal tract as a whole, and exhibit prebiotic properties [8; 9].

Siberian cedar nut is a useful natural source of medicinal biologically active substances. The cake, which is currently practically unused, is one of the products of its processing. The special feature of the chemical composition of cakes is represented by the significant content of dietary fibers, high-molecular polysaccharides, essential amino acids in the composition of proteins, polyunsaturated fatty acids in the composition of lipid fraction, vitamins B, tocopherols, protein fractions, trace elements, which indicates the prospect of their use as a high-value additive for food.

The objective of the work is **t**o study the cedar cake influence on the consumer properties of a milk bioproduct.

MATERIALS AND METHODS

The experimental studies were conducted in the laboratory of the Department of Technology of Milk and Dairy Products, Commodity and Expertise of Goods and in the laboratory of the Bifivit Small Innovation Enterprise (SIE), the FSBEI HE East Siberia State University of Technology and Management (ESSUTM).

Bifidobacterium longum DK-100 obtained from the All-Russian collection of industrial microorganisms FGUP GosNII Genetika, activated by the biotechnical method developed in ESSUTM has served as the object of the research. The vegetable additive - cedar nut kernel cake TU 9146-001-5313736-06 - was used in the study.

The basic physicochemical and microbiological parameters of raw materials, starters and fermented milk products were determined by standard and generally accepted methods in research practice. Active acidity was determined as per GOST R 53359-2009, quantitative accounting of bifidobacteria - as per MUK 4.2.999-00.

Adhesive properties were studied on formalinized erythrocytes as per the expanded method of V.I. Brilis. A mixture of formalized human erythrocytes O(I) of the Rh+ blood group and a suspension of the microorganism ($1{\cdot}10^9$ cells/ml) was incubated at t=37 ºC for 30 minutes, shaking the mixture on a regular basis. Thereafter, the preparation was prepared, dried, fixed and stained as per the Romanovsky-Gimza method. The adhesion was studied under the light microscope, and the count was led, taking into account a total of not less than 50 erythrocytes. The following indicators were used when assessing the adhesive properties: average adhesion index (AAI) - average number of microorganisms attached to one erythrocyte; the adhesion coefficient (C) - the percentage of erythrocytes, having adhered microorganisms on their surface; and the adhesion index of microorganisms (AIM) which is the average number of microbial cells on the erythrocyte (only the red blood cells participating in the adhesive process were taken into account). Microorganisms were considered as nonadhesive in the case of the definition of AIM in the range from 1.76 to 2.5, medium-adhesive - from 2.51 to 4.0, and high-adhesive - for AIM $\leq$ 4.1.

The morphology of the bacteria was studied by the sample preparation as per Gram, followed by the microscopic examination in an immersion system with a 90 lens. Photographs of microcapsules of bacterial cells were made using the "BIOR" USB digital microscope. The process of structure formation of the samples was studied on a Brookfield RVDV-II + PRO rotational viscometer in the ESSUTM Shared Knowledge Center [10].

Statistical data processing was carried out using the Statistica 6 program package. The nonparametric Mann-Whitney test was used (to compare independent samples). The differences were considered as significant if the error probability was $p \geq 0.05$.

RESULTS AND DISCUSSION

At the first stage of the research, the cedar cake influence on biochemical activity of bifidobacteria was studied. Biochemical activity was judged by the acid-forming ability and the number of viable cells of bifidobacteria. The results are shown in Figure 1.

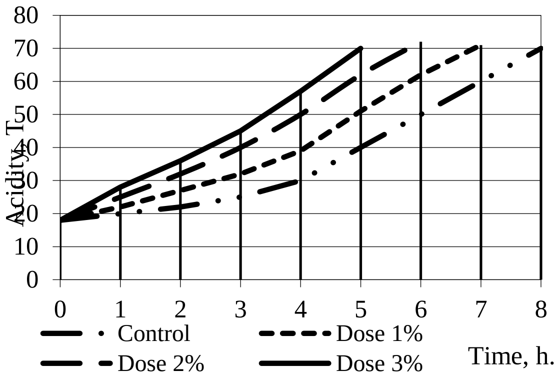


Figure 1. Cedar cake influence on the acid-forming ability of bifidobacteria

It follows from the data of Figure 1 that with an increase in the cedar cake dose the acid-forming ability of bifidobacteria increases, and the fermentation process intensifies. In the experimental sample, the fermentation process ends in 6 hours, whereas in the control sample the clot is formed after 8 hours. At the same time, the viscosity of the protein clot changes, and at the 3% dose of cake the consistency of the product becomes excessively viscous.

It was found in the quantitative accounting of bifidobacteria that the introduction of the cedar cake activated the growth of bifidobacteria (Figure 2).

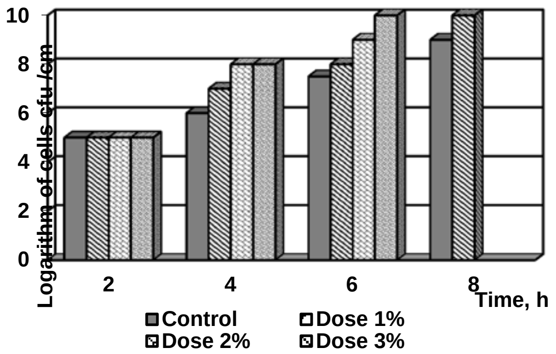


Figure 2. Influence of different doses of the cedar cake on the growth dynamics of bifidobacteria

As follows from Figure 2, the number of viable cells of bifidobacteria after 6 hours of cultivation in prototypes with the cedar cake is 10^{10} cfu per 1 cm^3, and in the control sample - 10^8 cfu per 1 cm^3. This indicates that the cedar cake has prebiotic properties, stimulates the growth of bifidobacteria, increases the number of viable cells in the finished product and shortens the fermentation time.

Consistency is one of the most important indicators in the management of the biotechnical products' quality, representing a collection of rheological properties of viscous liquid. Along with the appearance, color, smell and aroma, the consistency of fermented milk products determines the consumer properties of the finished product. Starter culture plays an important role in the formation of structural and mechanical properties of the bioproduct. Bifidobacteria form easy flowing clots with gentle consistency.

It should be noted that in practice the sensory assessment of consistency is widely used, the results of which are obtained empirically by assessing the behavior of the product during deformation. However, the results of the sensory assessment are subjective. Therefore, it is necessary to use instrumental methods to ensure the control and management of the manufactured products' quality. In this regard, the rheological characteristics of the protein clot on the rotational viscometer were investigated during further studies.

The change in the dynamic viscosity of an object from the speed of rotation of the spindle under the mechanical action was studied (Fig. 3). The viscosity-speed relationship reflects how much the system is destroyed and whether it can restore its properties after the mechanical action of the spindle.

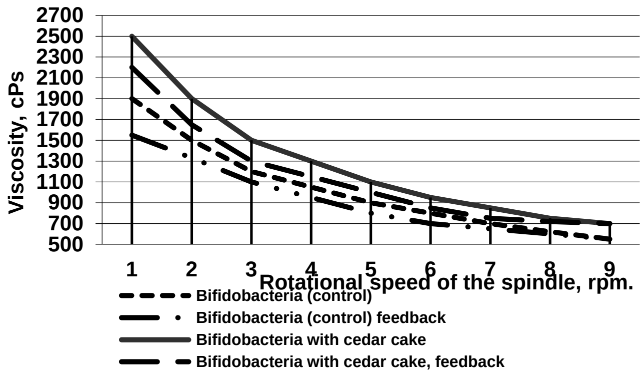


Figure 3. Dynamic viscosity dependence on spindle rotation speed

The results of the research in Fig. 3 show that the dynamic viscosity of both the control sample and the prototype specimens decreases with the increase in the spindle speed and is 550 and 670 cPs, respectively. However, as evidenced by the increase in the viscosity of both the control sample and the prototype, the fermented milk systems studied are able to restore structural and mechanical properties after the mechanical impact.

The obtained curves are not superimposed on each other, which demonstrates the thyxotropic behavior of the system in both the control sample and the prototype, which is consistent with the data obtained earlier in the symbiotic starter creation (Fig. 3) [11]. It should be noted that with the increase in the rate of destruction of the protein system, the ability to restore the structure decreases. The sample viscosity after the mechanical impact increases in all cases, as evidenced by the values of dynamic viscosity: bifidobacteria (control-feedback) - 1,550 cPs, and in the experimental sample of bifidobacteria with the cedar cake it reaches 2,200 cPs. The protein system with the cedar cake is the most recoverable one after mechanical impact. The values of dynamic viscosity for a protein clot with the cedar cake for the undamaged structure and the structure subjected to the mechanical impact differ to a lesser degree than in the control sample.

High-molecular polysaccharides of the cedar cake regulate the structure formation process, which makes it possible to obtain a clot with stable structural and mechanical properties.

In further studies, the "thixotropic index", or the effective viscosity index at loading and unloading, was studied (Table 1).

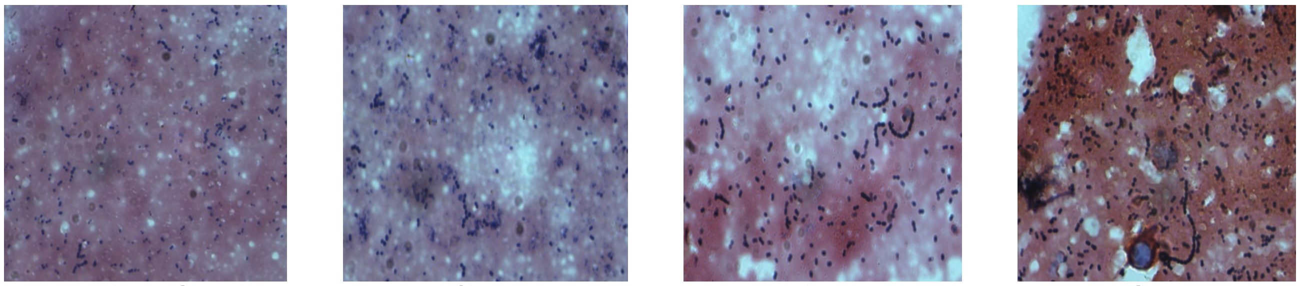
It follows from the table data analysis that thixotropic index is greater than one for all samples and they are characterized by thixotropic-reversible bonds. It has been found that the degree of restoration of the structure in the protein system with the cedar cake is of higher importance.

Thus, the analysis of the structural and mechanical properties of the samples has shown that the introduction of the cedar cake during fermentation promotes the formation of thixotropic-reversible bonds, which ensures the greatest capacity of the destroyed clot to restore the structure and forms a viscous and stable consistence.

It should be noted that the growth of cells of bifidobacteria in a nutrient medium with the cedar meal is accompanied by a change in morphology (Figure 4).

Table 1. Thixotropic index

Samples	Effective viscosity, mPa*s		Thixotropic index at		Structure recovery rate
	Maximum strain rate	Minimum strain rate	loading	unloading	
Bifidobacteria (Control)	20.28	16.32	3.55	2.99	1.18
Bifidobacteria with the cedar cake	24.70	16.57	3.69	2.47	1.49



a) control b) 1% c) 2% d) 3%

Figure 4. Microprint of B. longum DK-100 bifidobacteria (Scale 1x1,000)

It can be seen from the data provided that at the introduction of the cedar cake in a nutrient medium the topography of interlocation of coccoid, rhabdoid and multiseptated units changes and demonstrates structurally-functional adaptations of bifidobacteria to the changed cultivation medium (Fig. 4).

The morphological transformation correlates with the physiological heterogeneity of cells in the population. The introduction of the cedar cake promotes the cohesion of cells, the intensification of intercellular contacts and the formation of multicellular systems and provides adaptive resistance of cells in the nutrient medium.

It is known from the literary sources that the mechanisms guaranteeing the stability of the microbial consortium include intercellular interactions-cohesion and strong attachment of cells to the substrate (adhesion) [12, 13].

Data on the study of adhesive properties of bifidobacteria are presented in Table 2.

Table 2. Adhesive properties of *B. longum DK-100* bifidobacteria

Parameter name	AAI	CEP, %	AIM	Adhesiveness
B. longum DK-100 strain	4.2	84	4.76	highly adhesive
Bioproduct with 2% cedar cake	4.4	86	5.11	

It follows from Table 2 that the cedar cake increases the adhesive properties of bifidobacteria. This is probably due to the ability of bifidobacteria to colonize the surface of dietary fibers and attach to them with the formation of mucous biofilms. The results of our observations coincide with the opinions of other authors about the adhesiveness of probiotic microorganisms [14, 15].

The increase in the adhesive properties of bifidobacteria confirms the expediency to use the cedar cake at the creation of bioproducts. This testifies to the good survival and high adaptation of bifidobacteria in the gastrointestinal tract of a human.

Based on the studies conducted, a technology for the production of bioproducts with the cedar cake has been developed. The cedar cake is dissolved in a small amount of milk, pasteurized at a temperature of 70-72 ^{o}C and held for 30 minutes, cooled and then introduced into pasteurized milk in the amount of 2%. As a starter, bacterial concentrate of *B. longum DK-100* bifidobacteria is used in the amount of 2 E of activity per 200 l of normalized milk. Then everything is thoroughly mixed and left for fermentation for 4-5 hours to the acidity of 60-65^{o}T. The product is stored at a temperature not higher than 6 °C.

The qualitative characteristics of the product are presented in Table 3.

Table 3. Qualitative characteristics of bioproducts with the cedar cake

Indicators	Bioproduct with the cedar nut kernel cake
Organoleptic indicators	
Appearance and consistency	Homogeneous, tender, viscous, with small grains of the cedar cake
Taste and smell	Clean, with a pleasant fermented-milk taste, with a pronounced flavor of cedar nuts
Color	Milky white
Physical and chemical indicators:	
Acidity, ^{0}T	60-80
Mass fraction of fat, %	2.5
Mass fraction of protein, %	3.5
Dietary fibers, mg/100 g, above including, soluble insoluble	0.6 0.45 0.15
Microbiological indicators	
Number of bacteria, bifidobacteria, CFU/g, above	10^{9}
Coliforms, product weight (g, cm^{3}), in which it is not allowed	0.1
S.aureus, product weight (g, cm^{3}), in which it is not allowed	1
Pathogenic bacteria, incl. salmonella, product weight (g, cm^{3}), in which it is not allowed	25
Storage conditions, ^{0}C	6$\pm$2

The data presented in Table 3 indicate that the bioproduct has good organoleptic properties, contains a high number of viable bifidobacterial cells and prebiotic substances in the form of soluble and insoluble dietary fibers.

Thus, the bioproduct with the cedar cake has high consumer properties.

CONCLUSION

1. As a result of the conducted researches, it has been established that the cedar cake use in the production of a fermented milk product increases its consumer properties.
2. It has been established that the cedar cake has prebiotic properties, intensifies the fermentation process and increases the number of viable cells of bifidobacteria, which positively affects the usefulness of the product.
3. Dietary fibers and high-molecular cedar cake polysaccharides regulate the process of structure formation and rheological properties of the product.
4. The morphological picture of bifidobacterial cells is changing, which manifests itself in the aggregation and adhesion of cells on the dietary fibers of the cedar cake.

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