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Creating A Biologically Active Supplement With Synbiotic Properties

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Abstract

The work is aimed at studying the chemical composition of cedar oil cake, which is protein-and-carbohydrate residue, after production of cedar oil. It has been found that microwave extraction with ethyl alcohol increases ecological purity and quality of cedar oil cake. An increased protein and a reduced fat content was also noted, indicating the efficiency of the developed extraction method. Soft modes of extraction reduce the denaturation processes in the oil cake proteins, as evidenced by small reduction in the amount of available lysine during processing. The fermentation conditions of cedar oil cake with bifidobacteria starter culture have been chosen. It has been found that cedar oil cake has prebiotic properties, and stimulates the growth of bifidobacteria. High survival rate of bifidobacteria during freeze-drying and prolonged storage of fermented oil cake has been noted. Based on the research results, a biologically active supplement (BAS) with synbiotic properties has been developed, which is characterized by high content of bifidobacteria viable cells and dietary fibers.

Keywords: cedar oil cake, bifidobacteria, synbiotic properties, prebiotics, probiotics, available lysine, dietary fibers.

INTRODUCTION

In recent years, food products containing so-called bifidogenic factors, i.e. prebiotics that stimulate growth and development of bifidobacteria, have become popular in the international market.

The concept of "prebiotics", first formulated by Glen R. Gibson, is used to refer to substances or dietary supplements, most of which are not adsorbed in the human intestine, but are beneficial to the human organism by selective stimulation of growth and activation of metabolism in useful representatives of its intestinal microflora. Thus, prebiotics can be called stimulants or promoters of probiotics, bifidobacteria in particular. Among prebiotics, the most popular are poly- and oligosaccharides, galactooligosaccharides, soybean oligosaccharides extracted from natural sources or obtained by biotechnological and synthetic methods. They are produced and sold as separate products in the form of enriching supplements to various food products, as well as in combination with probiotic microorganisms, and belong to synbiotics [1, 2, 3].

Currently, the problem of compensating for the lack of coarse plant food in the human diet has become more and more relevant. Many countries are engaged in deep studying of the structure, composition, and properties of dietary fibers, the technology of separating them from the source plant material, and using then as prebiotics in creating food products. The role of fibers in the diet is diverse. It is not only partial supplying energy to the human organism, but also regulating physiological and biochemical processes in the digestive organs [4, 5, 6].

Dietary fibers play positive role in normalizing intestinal microflora, and inhibition of septic processes. It is known that normal intestinal microflora along with fiber-like oligosaccharides metabolizes polysaccharides in dietary fibers, namely, starch, hemicellulose, pectin, gums, etc. [7, 8].

It is known that dietary fibers stimulate growth of normal microflora in the gastrointestinal tract. Their consumption, especially for a long time, promotes positive changes in the functional state of the gastrointestinal tract, and has a beneficial effect on the motor and voiding functions [9, 10, 11].

Results of the foreign scientists'studies obtained on experimental animals show that when the diet contained dietary fibers, invasion of enterocytes and penetration of bacteria through the intestinal wall decreased by 90%, digestibility of fat and proteins increased, compared to the reference group [12, 13, 14].

Thus, the data obtained from various sources suggest that dietary fibers normalize functional activity of the intestinal microflora and the gastrointestinal tract in general; their properties are considered prebiotic.

In recent years, prebiotic properties of dietary fibers have been widely used in the development of new types of fermented products. Developers of new technologies seek to use available local vegetative materials and products of their processing. Wild and cultivated fruits and berries, which are rich in carbohydrates and vitamins, are traditionally used as the source of dietary fibers.

It is known that cedar forests are characteristic of the Siberian region. Traditionally, cedar nuts, due to the high content of lipids in them, are used for oil production. Processing cedar nuts leaves cedar oil cake, which is characterized by high content of proteins, carbohydrates, mineral and other biologically active substances [15, 16, 17].

In this regard, it is promising to use products of processing cedar nuts for enhancing functional properties of fermented dairy products.

The aim

The work was aimed at creating a biologically active supplement (BAS) with the use of cedar oil cake and bifidobacteria.

MATERIALS AND METHODS

The research is focused on cedar oil cake, which is protein and carbohydrates containing residue after extracting cedar oil from the seeds of Siberian pine (cedar nuts), using the method of extraction with ethanol in an ultra-high frequency electromagnetic field (UHF EMF) TS 9146-032-02069473-2002.

To detect carbons in pine oil cake, the following methods were used: starch - using the polarimetric method, sucrose - using the colorimetric method, glucose - using the glucose-oxydase method, and dextrins and cellulose – using the enzymatic method [18].

Nitrogenous substances were detected using the Kjeldahl's method. The content of available lysine was determined using the ninhydrin method based on the colorimetric reaction of separate protein fractions with ninhydrin reactant [19].

Mineral substances in cedar oil cake were detected using the atomic absorption method [20]. The method is based on atomization of the tested sample's mineralizate solution in air-and-acetylene flame. Getting into the flame, the metals present in the mineralizate solution transform into the atomic state. Absorption of light with the wavelength of proper resonance line is proportional to the value of the metal concentration in the tested sample.

Samples were prepared for analysis using the method of dry mineralization. The measurements were made on the atomic absorption spectrophotometer SOLAAR M6. Oil cake was

fermented with bacterial concentrate of bifidobacteria TS 9229-002-02069473-2005.

Viability of bifidobacteria was judged upon by the number of colony forming units (CFU) after sowing cell suspensions from appropriate dilutions onto the maleic acid hydrazide environment [21].

All experiments were repeated 3 times. The obtained data were processed using the Excel statistical software package using the Mann-Whitney test. Statistically veracious difference at $p<0.05$ is discussed.

RESULTS AND DISCUSSION

It is known that cedar nuts are not only oil raw materials, but also a source of protein, carbohydrates and minerals that go into oil cake after oil extraction. It should be noted that the quality of cedar oil cake depends on the method of oil extraction.

In further studies, the effect of microwave extraction with ethanol on the chemical composition of cedar oil cake was studied. The reference was oil cake obtained by extraction with diethyl ether.

Comparative data show that microwave extraction with ethanol improves the quality of oil cake, which is evidenced by low fat content and higher protein content. Proteins of cedar oil cake are represented by albumins, globulins, glutamins and prolamins. Cedar protein digestibility is 95%, which is comparable to digestibility of proteins in chicken eggs.

It should be noted that the biological value of cedar oil cake depends on the method and modes of extracting cedar oil, since globular proteins are able to undergo denaturation, i.e. to undergo such changes of the spatial structure that result in complete or partial loss of functional properties. The reason for denaturation may be heat action, the use of organic solvents that may compromise the system of hydrophobic contacts within the globule essential to the stability of protein.

According to Rush V. A., proteins in the kernel of pine nuts contain high amount of lysine, methionine and tryptophan. Lysine is most susceptible to changes in the technological process due to high reactivity of free E-amino groups that characterize the so-called available lysine, which is an important indicator of biological value [22].

Given the above, the effect of cedar oil extraction method on changes in available lysine has been studied. The greatest decrease in available lysine in cedar oil cake has been marked during extraction with diethyl ether. This is due to the prolonged thermal exposure for 4-7 h, which leads to denaturation of globular proteins. Short exposure to microwave heating for 6-8 min and subsequent extraction with ethanol allows reducing denaturation processes in the proteins of oil cake, which is evidenced by a slight decrease in available lysine, compared to the reference.

Carbohydrate composition of cedar oil cake is presented by polysaccharides (starch, cellulose, pentosans, dextrins) and water-soluble oligo- and monosaccharides. The literature data about the content of carbohydrates are quite contradictory. High content of starch, cellulose, dextrins, sucrose and raffinose has been noted.The amount of monosaccharides is insignificant (Table 1). Soft modes of cedar oil extraction reduce the possibility of melanoidin compounds'formation due to di- and monosaccharides' interaction with oil cake proteins, which increases its nutritional value.

Cedar oil cake contains significant amount of mineral substances that are represented by a set of macro- and micronutrients. The data in Table 2 indicate high content of potassium, magnesium, iron, zinc and phosphorus, etc.

The results show that cedar oil cake contains the entire complex of minerals, and is a unique natural source of biologically active substances, which play an important role in human vital functions.

Thus the research has shown that microwave treatment with ethyl alcohol improves the quality of products of pine nuts' processing.

In creating BAS, the use of natural raw materials has been preferred in recent years. As the obtained data show, cedar oil cake has high chemical potential, and may be considered as a source of raw material for production of a wide range of functional food products.

Given the high content of biologically active substances in pine oil cake, the hypothesis about the possibility of using cedar oil cake as a prebiotic in production of bifidobacteria-containing dairy products has been put forward.

In addition, biological treatment of oil cake will enhance absorption of proteins subjected to the action of denaturing factors and minerals.

One of the conditions for microorganisms' development is the presence of free moisture in the environment. Based on the above, the effect of the degree of cedar oil cake hydration on the dynamics of acid production and growth of bifidobacteria has been studied.

Cedar oil cake was humidified in two ways: with skim milk and with water. After reaching certain moisture content, 5% of bifidobacteria leaven were introduced into the oil cake.The results of the research are shown in Figure 2.

The data in Fig. 2 show that with increasing the degree of hydration, the acid-forming ability also increases. It is interesting that the same dynamics of acid formation is observed when oil cake is humidified with water. This is an evidence of the fact that cedar oil cake is a good breeding medium for bifidobacteria development even without the dairy substrate.

Bifidobacteria start developing in the first hours of culturing, and after 6-8 hours, the number of cells is 10^{10} CFU per 1 g, and varies between samples, depending on the humidity, only in the bifidobacteria titer (Fig. 3). Given further granulation of the fermented BAS, the most optimal is moisturizing the oil cake to moisture mass fraction of 40%.

As a result of the research, it has been found that cedar oil cake has bifidogenic properties and stimulates growth of bifidobacteria.

Currently, freeze-drying is the most advanced method of dehydration that ensures the best preservation and the best recoverability during the hydration of dried products. Besides, freeze-dried products in appropriate packaging may be stored for a long time without significant changes to their quality.

In this regard, the possibility of maintaining quality of fermented cedar meal during long-term storage was studied in further studies.

Table 1 - Chemical composition of cedar oil cake

Indicator name	Mass fraction, % of dry matter	
	Reference	experiment
Fats	2.1	0.6
Proteins	44.8	47.1
Carbohydrates, including	45.1	45.4
starch	14.3	14.5
fiber	4.9	5.1
dextrins	4.0	4.1
pentosans	2.4	2.3
sucrose	11.7	11.3
raffinose	7.4	7.6
glucose	0.2	0.2
fructose	0.2	0.3
Ash	5.6	5.2
Other substances	2.4	1.7

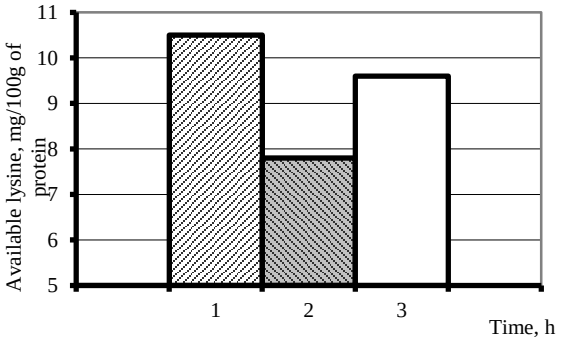

Figure 1. The effect of extraction method on changes of available lysine amount in cedar oil cake

a) 1 - reference, proteins in pinenuts'kernels,
b) 2 - cedar oil cake obtained by extraction with diethyl ether,
c) 3 - cedar oil cake obtained by microwave extraction with ethanol.

Heat drying was chosen with regard to the thermal stability of bifidobacteria. The initial temperature of drying the frozen fermented oil cake was minus 18^0C, additional drying was at 40 - 45^0C. The drying time was checked by residual moisture content (not more than 5%). Dry fermented oil cake was assessed by the following parameters: residual moisture content, solubility, and the number of viable cells of bifidobacteria.

Two variants of the fermented oil cake were subjected to drying: with the protective environment, and without it. It has been found that a higher survival rate of bifidobacteria (90%) is observed when protective environment is used. Without protective environment, high survival rate of cells is also observed, which is 86%. Therefore, a more economical method of preparing fermented oil cake without protective environment has been proposed.

As shown in the study, a high content of carbohydrates and proteins, which are natural cryoprotectants, fully protects bifidobacteria from irreversible changes during freezing and drying, and probably this can explain the high number of viable bifidobacteria cells in dry fermented oil cake.

Bifidobacteria survival rate depends on the amount of remaining intracellular water and on uniformity of dehydration. And this, in turn, directly depends on the duration of drying. It has been found that the optimum residual humidity is 5%, and the number of bifidobacteria cells equal to $(8\text{-}9)\cdot10^9$ CFU per 1 g is observed after 5-6 hours of drying.Qualitative characteristics of biologically active supplement are shown in Table 3.

As follows from the data in Table 3, wetting with milk followed by fermentation increases organoleptic properties of the finished product. The high number of viable cells of bifidobacteria gives the BAS additional properties of a probiotic that regulates composition of the microflora in human gastrointestinal tract.

Table 2 - Content of macro- and micronutrients in pine nut and its byproducts, mg/kg

Element name	Oil	Oil cake
Potassium	660.25	148.65
Sodium	195.24	31.74
Magnesium	552.36	175.06
Iron	0.78	5.78
Manganese	1.69	1.61
Copper	0.84	1.17
Zinc	2.38	1.24
Lead	0.009	0.002
Phosphorus	509.82	103.1
Calcium	3.5	2.5

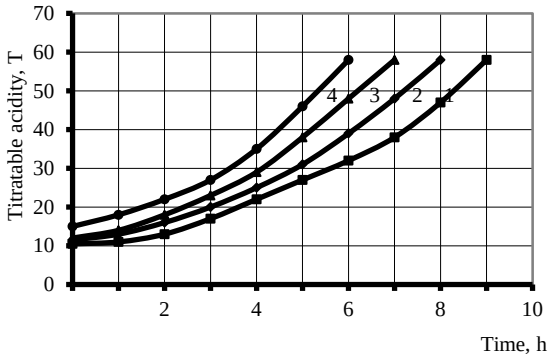

Figure 2. Influence of the degree of oil cake hydration with skim milk the dynamics of acid formation

a) 1 – hydration up to 30%; 3 – hydration up to 50%;
b) 2 – hydration up to 40%; 4 – hydration up to 60%.

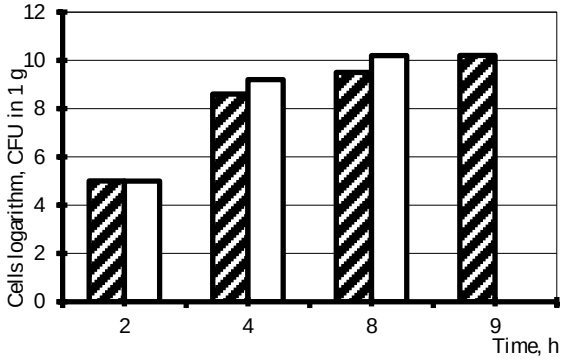

Figure 3. Bifidobacteria development during fermentation of pine oil cake

a) wetted with water
b) wetted with milk

Table 3 - Qualitative characteristics of the BAS

Indicator	BAS when the oil cake iswetted with milk	BAS when the oil cake iswetted with water
Appearance	Dry porous granules with a size of 10-20 mm	Dry porous granules with a size of 10-20 mm
Taste and odor	Clean, sour-dairy with strong flavor of cedar nuts	Clean, with the flavor of cedar nuts
Color	Cream	Cream
Mass fraction of moisture, %	4 ± 0.6	4 ± 0.5
Hydrophility, %	80 ± 1	82 ± 2
Number of cells of bifidobacteria, CFU/g, not less than	$(9\text{-}10)\cdot10^9$	$(8\text{-}9)\cdot10^9$
Bacteria of the group of coliform bacteria in 2.0 g	Missing	
E. coli in 5.0 g	Missing	
S. aureus in 2.0 g	Missing	
Mold in 10 g	Missing	
Yeast in 10 g	Missing	

Based on the research, a comprehensive BAS - a "synbiotic" has been created, which includes probiotic bifidobacteria and prebiotic substances - dietary fibers, polysaccharides and oligosaccharides, as well as micronutrients contained in cedar oil cake.

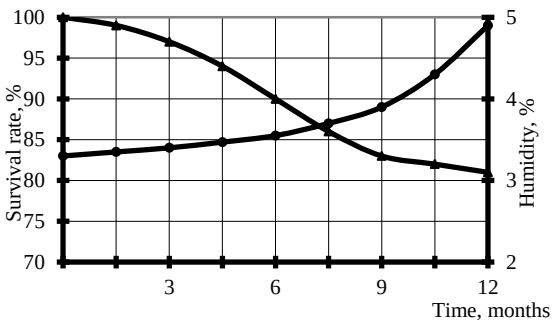

Figure 4. Bifidobacteria survival rate during BAS storage

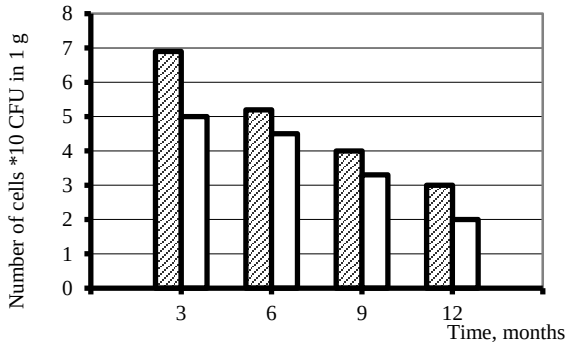

Figure 5. Changes in the number of bifidobacteria cells during storage

a) dry fermented oil cake obtained by wetting with milk

b) dry fermented oil cake obtained by wetting with water

It is known that the effect of synbiotics is based on the synergy of probiotics and prebiotics combinations, which most effectively not only implants the introduced microorganisms, probiotics, into the gastrointestinal tract of the host, but also stimulates its own microflora.

It is known that cell damage and inactivation of a part of the microbial population occurs not only during dehydration, but also during storage of dried products.

In this respect, dry fermented oil cake was assessed during storage by such indicators as the number of viable cells and moisture.

Dried fermented oil cake placed for storage for 12 months at the temperature of 4-6^{0}C was analyzed. An important indicator of cells' resistance during storage is their number or survival rate. This indicator depends on the residual moisture content and product storage conditions. Changes in the number of bifidobacteria cells during storage are shown in Figure 4.

Analysis of the obtained data showed that the survival rate correlated with the values of dry fermented oil cake mass fraction. Coefficient of correlation was 0.95. Functional dependence of the considered values has the following mathematical expression:

$$y = (-0.029x^2 - 0.115x + 7.27) * 10^{10},$$

where y is bifidobacteria cells' survival rate, %

x is product moisture, %.

The obtained data showed that after 6 months of storage, the number of cells in the concentrate decreased by 9.8%. After further storage, a decreased number of cells was observed, and after 12 months of storage, the survival rate was 80.4% (Fig. 4).

However, the absolute number of viable bifidobacteria cells remained high: $7 \cdot 10^9$ CFU in dry fermented oil cake obtained by wetting with milk, and $5 \cdot 10^9$ CFU - when wetted with water. After 12 months, these values decreased to $3 \cdot 10^9$ and $2 \cdot 10^9$, respectively (Fig. 5).

It should be noted that components of cedar oil cake – proteins and carbohydrates - act as natural cryoprotectants, and have a protective effect on the cells during long-term storage.

As it has been found before, high survival rate of bifidobacteria cells during processing and storage is probably due to the fact that, when cultured in cedar oil cake, they adhere to the surface of the dietary fiber and form a structured microbial community. It is known that adhesion on firm surfaces is a versatile reaction that adds up to microorganisms' preservation in adverse conditions, for example, in the gastrointestinal tract; they quickly adapt to these conditions, and resume active metabolism [23, 24].

Thus, polysaccharides contained in cedar oil cake have a protective effect, protect bifidobacteria from damage, and retain their vital activity during long-term storage.

CONCLUSION

As a result of the research, biologically active supplements with synbiotic properties, which contain bifidobacteria as probiotic and cedar oil cake as prebiotic,have been developed.

The study of cedar oil cake chemical composition has shown that it is a unique natural source of biologically active substances. Using microwave extraction with ethyl alcohol improves the quality of cedar oil cake.

High biochemical activity of bifidobacteria during cedar oil cake fermentation has been proven, which is an evidence of its prebiotic properties.

The optimum process parameters of obtaining the BAS that ensure high quantity of viable bifidobacteria cells in the finished product have been chosen.

The high bifidobacteria survival rate during fermented oil cake freeze drying and long-term storage has been noted.

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