

ORIGINAL RESEARCH PAPER

INFLUENCE OF FORTIFICATION OF DAIRY PRODUCTS BY *PLEUROTUS OSTREATUS* BETA-GLUCANS ON PRODUCT CHARACTERISTICS

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Received: July, 06, 2018
Accepted: August, 23, 2019

Abstract: Dairy products are often used as a base for functional food products. Preparations obtained from *Pleurotus ostreatus* possess antitumor, immunomodulating, anti-inflammatory, antioxidant, antibiotic, radioprotective and hypoglycemic actions. A special attention should be paid to beta-glucans, which are demonstrating significant biological activities. The aim of our research was to study the influence of fortification of dairy products with *Pleurotus ostreatus* beta-glucans on product characteristics. Preparations of beta-glucans obtained from submerged cultured *P. ostreatus* biomass were the object of our study. Three different preparations were obtained using different extraction protocols. Preparations were added to the milk in different concentrations just before its inoculation with different starter cultures to obtain various dairy products fortified with beta-glucans. An influence of the addition of different doses of mushroom preparations on the characteristics of produced dairy products was studied. It was found, that fortification with beta-glucan containing preparations, which is adding new functional properties to final dairy products, has no negative effect on the organoleptically properties of the products. It can also shorten the fermentation period and increase the product viscosity. Obtained results are demonstrating the possibility and motivation of the fortification of dairy products with mushroom beta-glucans to develop of new prospective functional products.

Keywords: *acidophilus* milk, functional food, mushrooms,
polysaccharides, ryazhenka, yogurt

INTRODUCTION

Medicinal mushrooms have been used as decoctions and essences and as an alternative medicine in Korea, China, Japan and eastern Russia for hundreds of years. The wide variety of compounds that occur naturally has proven to be protective against the development of tumors and inflammatory processes. The most studied compounds are polysaccharides that are present in the entire structural composition of fungi. Among the impacts there is an antitumor effect and an activation of the immune response. It was found that most of the taxonomic groups of higher basidiomycetes possess biologically active polysaccharides. Whether collected in the natural environment or artificially grown fruit bodies, cultured and purified mycelia or cultural liquids – all of them contain polysaccharides that have important properties for medicine [1, 2].

Several polysaccharides have been isolated from the basidiomycetes and are represented by homo- and heteropolymers, especially β -configuration glucans, which are widely found in the bacterial and fungal cell walls [3]. Fungal polysaccharides are mainly represented as homoglucons with different kinds of glucosidic bonds, such as (1 \rightarrow 3), (1 \rightarrow 6)- β -glucoside and (1 \rightarrow 3)- α -glucoside bonds. β -glucans are polysaccharides contained in the cell walls of various crops (oats, barley), algae, microorganisms and fungi [4]. β -glucans consist of the main chain, where D-glucose is connected by a β -1,3 bond, and the lateral branches formed by β -1,6 bond at different intervals. The lateral branches of the molecule impart a form and stiffness to the cell wall [5].

In recent years, β -glucans have attracted close attention due to their possible use as medicines, namely: as immunomodulators, as substances with onco- and radioprotective properties, as a remedy directed against diabetes, etc [1, 2]. Among the other investigated fungal polysaccharides, various glycoproteins and heteroglucons are most often studied. It was found that high molecular weight glucans are more effective as antitumor and immunostimulant preparations than glucans with low molecular weight [6]. It could be noticed, that mushroom mycelia biomass, including biomass of *P. ostreatus*, has a hypolipidemic and hypocholesterolemic action [7]. In connection with the numerous reports on the effectiveness of fungal polysaccharides in reducing hyperglycemia as well as in the other fields of medicine, β -glucans are believed to have a huge and yet undiscovered potential for the treatment of diabetes and related cardiovascular diseases [1, 8]. In some articles it is noted that as a result of the use of glucans (as syrup and as supplement to milk), the incidence of episodes and the duration of acute respiratory infections in children are decreased, as well as the number of accompanying symptoms [9, 10].

An attractive idea is to use basidiomycetes preparations, including extracts, not only as an independent drug or its component, but as a biologically active additive and as part of functional food products. In the case of the latter, additional biological effects are given to products that may have their own positive properties [2].

Prebiotics are partially or completely indigestible food components that selectively stimulate growth and/or metabolism of one or several groups of microorganisms living in the large intestine, providing a normal composition of the intestinal microbiocenosis [11, 12]. In essence, prebiotics provide nutrition to microorganisms of the gastrointestinal tract. From the chemical point of view, all prebiotics are carbohydrate compounds of different structure: disaccharides (lactose and lactulose), oligosaccharides, polysaccharides and psi-fiber fibers. The mechanism of action of all

prebiotics is the same: without splitting the enzyme systems of the human small intestine, they reach the large intestine, where they are utilized, mainly by bifido- and lactic acid bacteria [13, 14]. The main biological effects of prebiotics are:

- promote the growth of bifido- and lactic acid bacteria,
- stimulate intestinal peristalsis,
- increase calcium assimilation,
- exert hypocholesterolemic effect,
- reduce the risk of developing tumors.

Combination of pro and prebiotics to prevent intestinal microbiocenosis (and in some cases also for its correction) seems quite justified, since prebiotic components provide energy not only to the intestinal microflora, but also to microorganisms introduced from the outside. In this regard, prebiotics can be introduced into both yoghurts and other fermented milk products [15].

Addition of the new beneficial properties to dairy products is a common option. Fermented products normalize metabolism, strengthen immunity, form a healthy mucosa of the intestine, promote the elimination of toxic substances and promote the improvement of digestion [16].

Yoghurt is a fermented product made from milk using bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* [17]. A search and study of ingredients such as probiotics, as well as functional additives, which individually or collectively improve the properties of the product or add additional benefits, is conducted. As a result, bio-yogurt acquires additional properties, still possessing all the advantages of conventional types of yoghurts [18]. To improve the nutritional value and functional properties of yogurt, various fillers and additives are introduced into their composition, especially those that increase their therapeutic and prophylactic effect. The use of food additives and fillers rich in dietary fibers, such as pectins, microcrystalline cellulose, vegetable gums, vegetable and fruit additives, makes it possible to add additional functional properties to yogurt [19, 20].

Ryazhenka, also known as fermented baked milk, is a traditional dairy milk drink obtained from the baked cow milk as a result of the lactic acid and alcohol fermentation. The prolonged heating increases the concentration of useful components in milk, since some liquid is evaporated from it. Long-term heat treatment allows eliminating all pathogenic bacteria from the product. Ryazhenka has a taste of baked milk and a cream color with a brownish hue.

Fermented acidophilus milk is fermented by acidophilus bacteria. The consistency of the product is viscous, and the taste is very specific. Since the expressed dairy milk taste is not popular with everyone, acidophilus milk is often mixed with sugar, cinnamon, honey and other. This product, which has a huge list of useful properties, as many other dairy products is recommended to drink in cases of gastrointestinal disorders.

MATERIALS AND METHODS

Culturing

P. ostreatus culture was taken from the museum of the Department of Technology of Microbiological Synthesis, Saint Petersburg State Institute of Technology (Technical University).

Initial culture was cultivated under submerged conditions in flasks at temperature of 28 – 30 °C for 7 days on the rotary shaker IR-1LT (Labtech, Moscow, Russia) using semi-synthetic media. Semi-synthetic medium contained (g·L⁻¹): glucose – 15; peptone – 2.5; yeast extract 2.0; KH₂PO₄ – 0.6; K₂HPO₄ – 0.4; NaCl – 0.5; CaCl₂ – 0.05; pH of the media before sterilization – 5.8 – 6.0.

From the resulting culture of *P. ostreatus*, the mycelium was separated from the culture liquid by filtration through a paper filter under vacuum. Wet biomass was dried in an oven at the temperature of 50°C.

Obtaining of preparations

Dried submerged biomass was treated with 80 % of ethanol at 78 °C to make fungal cell walls more permeable and to remove lipids and low-molecular compounds from the biomass. A residue of the biomass after an ethanol treatment was marked as '*Preparation P1*'.

Preparation P1 was treated with boiling water for 3 hours to extract water-soluble fractions of compounds. The extraction was repeated 3 times. After separation on vacuum-filter all aqueous fractions containing water-soluble compounds were collected and combined. Obtained aqueous extract was concentrated and high-molecular compounds were precipitated from the concentrate by 5 volumes of ethanol. After the filtration the residue was used as '*Preparation P2*'.

After the extraction of water-soluble compounds for Preparation P2, the remaining water-insoluble residue was marked as '*Preparation P3*' and also studied.

All preparations were dried to the constant weight in drying oven in air stream at the temperature of 50 °C and milled. The mass of the preparations was determined by the gravimetric method.

Determination of the concentration of β -glucans in mushroom preparations

The concentration of β -glucans in preparations P1 and P2 was determined using the enzymatic ' β -Glucan Assay Kit (Yeast & Mushroom)' (Megazyme, USA) as it was shown by Zhu et al. [21]. The content of α -glucans and total glucans were measured. The content of β -glucans was calculated.

Preparation of dairy products

Dairy products were made from ultra-high-temperature (UHT) treated milk ('Valio', Galactica plant, Gatchina, Russia) with a fat content of 0.01 %. To prepare fermented baked milk (ryazhenka) in traditional way, the UHT milk has been additionally exposed to the temperature of 92 – 95 °C for 3 hours.

For the fermentation of milk the following lyophilized starter cultures were used:

- starter for a fermented acidophilus milk – probiotic preparation ‘Vitaflor’ containing the *Lactobacillus acidophilus* culture (State Research Institute of Highly Pure Biopreparations, St. Petersburg, Russia)
- starter for a fermented baked milk (ryazhenka), containing culture of the *S. thermophilus* (Barnaul biofactory, Russia)
- starter for yoghurt containing cultures of *S. thermophilus* and *L. bulgaricus* (‘Panteley Toshev’ EOOD, Sofia, Bulgaria).

Starters were pre-cultured on the UHT milk in flasks at the temperature of 40 °C until complete clotting. To study the influence of added preparations on the fermentation process a definite amount of one of three preparations P1, P2 and P3 was initially transferred into experimental glasses, and then they were filled up with the warm milk. Finally, the inoculum was added (5 % of the sample volume). The samples have been maintained in thermostat at 40 °C, the temperature recommended by starter producers, for 6 – 7 hours. Samples were periodically mixed and the levels of titratable acidity were determined.

For all starters, the following dosages of the preparations were taken:

- preparation P1 in concentrations of 0.1 %; 0.5 %;
- preparation P2 – 0.1 %; 0.25 %;
- preparation P3 – 0.1 %; 0.5 %.

Study of characteristics of dairy products

Study of qualitative indexes of the finished products was performed in comparison with the control. Titratable and active acidity, dynamic viscosity and some organoleptically characteristics were determined in control and experimental samples.

Titratable acidity during fermentation and for final products was controlled by acid-base titration with phenolphthalein indicator according to Russian State Standard GOST 3624–92 [22].

Viscosity measurement was carried out at 4 ± 1 °C by rotational viscometer Brookfield type MT-202 (Metrotex, Russia) equipped with №2 spindle at 6 rpm.

Samples were evaluated for some organoleptic properties such as taste, smell, color, consistency by the blind taste test with participation of 6 male and 12 female volunteers of 21 - 50 years age. Participation was free for anyone who wished to take part and had mainly students and staff of the Department of Technology of Microbiological Synthesis.

Statistical evaluation of the data

All experiments were carried out in three replicas, with a statistical processing of the data, the level of confidence was taken as 0.95, and the Microsoft Office Excel program was used.

RESULTS AND DISCUSSION

Three different preparations from *P. ostreatus* biomass were obtained:

- preparation P1 – submerged mycelia biomass of oyster mushroom treated with 80% ethanol for removing of most part of lipids and low molecular weight compounds, brown powder, partially water-soluble, contains 33.5 % of β -glucans,
- preparation P2 – water-soluble compounds (water-soluble polysaccharides and water-soluble high molecular weight compounds), light-brown powder, contains 23.8 % of β -glucans,
- preparation P3 – a residue remaining after the ethanol and aqueous extractions, contains water-insoluble polysaccharides, like insoluble β -glucans, chitin or chitin-glucan complex and other water-insoluble high molecular compounds, dark-brown powder, contains 41.2 % of β -glucans.

Further purification of β -glucans will lead to significant increase of the preparations costs and for use as functional supplements in food industry, achieved purity and quantity of β -glucans will be acceptable. In addition, further purification could lead to lose of synergetic effect of β -glucans and other active compounds, i.e. proteins, protein-glucan complexes, etc, definitely present in obtained extracts.

The influence of the preparation from *P. ostreatus* on the milk fermentation process with different starter cultures: *L. acidophilus*, *S. thermophilus*, combination of *S. thermophilus* and *L. bulgaricus* was studied. Dairy products: fermented acidophilous milk, fermented baked milk (ryazhenka) and yogurt were obtained.

During the fermentation process titratable acidity of all prepared dairy products was determined. An addition of the preparations P1, P2 and P3 into milk before the fermentation in case of fermented acidophilus milk and fermented baked milk preparation did not lead to a significant increase of the glycolytic activity of the cultures. The addition of the preparations into milk considerably increased the glycolytic activity of yogurt starter cultures (Table 1).

Level of titratable acidity after 4 hours from the beginning of fermentation for all samples (which contain mushroom preparations) was the same as the level of titratable acidity for control samples after 6 hours of fermentation. It may indicate the prebiotic effect of used functional additives.

Table 1. Dynamics of titratable acidity during fermentation of milk by yogurt starters with the addition of preparations P1, P2 and P3 in various concentrations ($p < 0.05$)

Period, hours	Titratable acidity of samples [°T]						
	control	concentration P1 [%]		concentration P2 [%]		concentration P3 [%]	
		0.1	0.5	0.1	0.25	0.1	0.5
0	20±1	20±1	21±1	20±1	22±1	20±1	22±2
2	29±1	35±2	40±2	41±1	43±1	35±2	35±2
3	40±2	55±2	60±3	58±2	58±2	56±2	55±3
4	59±2	76±1	87±2	86±1	88±2	81±2	79±2
5	73±3	85±1	93±1	92±2	95±1	89±2	88±2
6	83±2	92±2	98±3	96±2	98±2	95±2	92±1

At the end of the fermentation, the obtained samples of dairy products with and without β -glucans had the following levels of active acidity (pH values), indicated in Table 2.

Table 2. Active acidity of finished samples of dairy products ($p < 0.05$)

Dairy product	Active acidity of finished samples - pH						
	control	concentration P1 [%]		concentration P2 [%]		concentration P3 [%]	
		0.1	0.5	0.1	0.25	0.1	0.5
Acidophilus milk	4.31±0.01	4.31±0.01	4.25±0.01	4.25±0.01	4.26±0.02	4.30±0.01	4.26±0.02
Fermented baked milk	4.92±0.02	4.89±0.01	4.85±0.01	4.90±0.02	4.86±0.01	4.93±0.01	4.91±0.01
Yogurt	4.76±0.01	4.74±0.02	4.73±0.02	4.72±0.01	4.70±0.01	4.68±0.02	4.68±0.01

One of the important structural-mechanical parameters which determine the texture of a dairy product is viscosity. Therefore, for all obtained product samples, the dynamic viscosity was measured. The results of these studies for viscosity are shown in Figures 1 – 3.

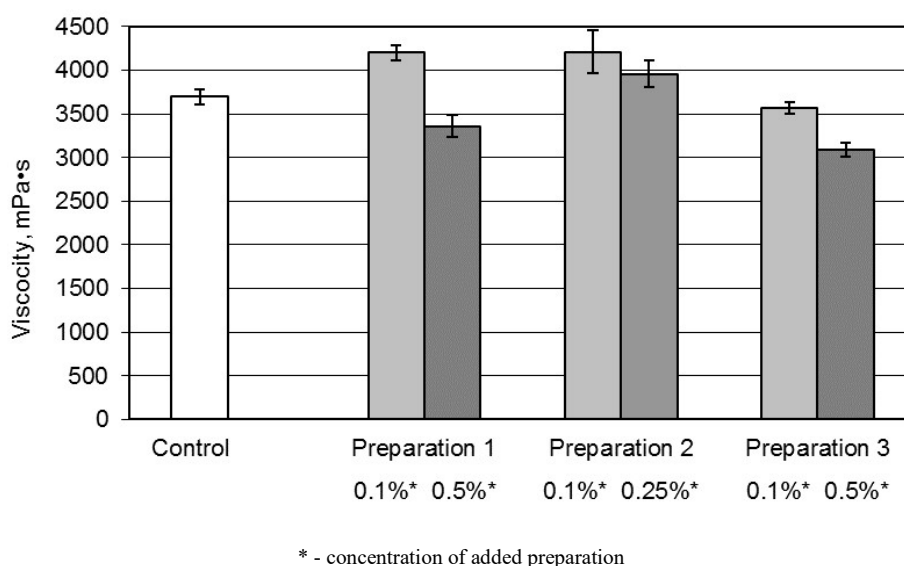


Figure 1. The influence of addition of the preparations P1, P2 and P3 on viscosity of fermented acidophilus milk samples ($p < 0.05$)

It appears, that viscosity of samples with added preparations P1, in concentration 0.1 %, and P2 to milk before fermentation increased compare to control samples (Figure 1). This is probably due to the presence of water-soluble β -glucans in both preparations, which can improve the structure of dairy products. It is important to note that increasing the concentration of water-insoluble preparations P3 leads to a decrease in the viscosity of the obtained products.

In the fermentation process, the casein micelles form floccules by attaching the particle to the particle. Observed vague microstructure corresponds to a low viscosity and intensity of gel. During fermentation, micelles ‘fuse’, forming a compact, dense structure, which improves the gel texture, increases its viscosity and water holding capacity.

Clots of dairy products usually have mixed character with a predominance of irreversibly depleting or thixotropic-reversible bonds. The ratio of these bonds depends on several factors, the correct use of which produces clots with desired properties.

Apparently, the addition of high concentrations of water-insoluble preparations leads to disruption of the formed clot structures, or prevents their formation in the dairy product, and thus prevents the holding of liquid in the structure. For the preparation of acidophilus milk enriched with polysaccharides of *P. ostreatus* preparations P1 and P2 in a minimum concentration of 0.1 % may be recommended.

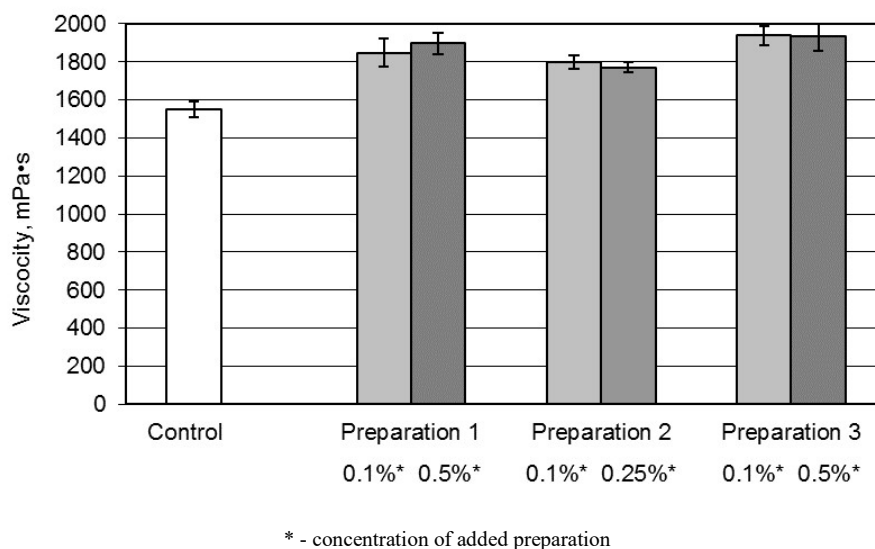


Figure 2. The influence of addition of the preparations P1, P2 and P3 on viscosity of fermented baked milk samples ($p < 0.05$)

From the Figure 2 data it could be concluded that the addition of all the preparations to baked milk before fermentation by *S. thermophilus* increased the viscosity of samples as compared with control samples.

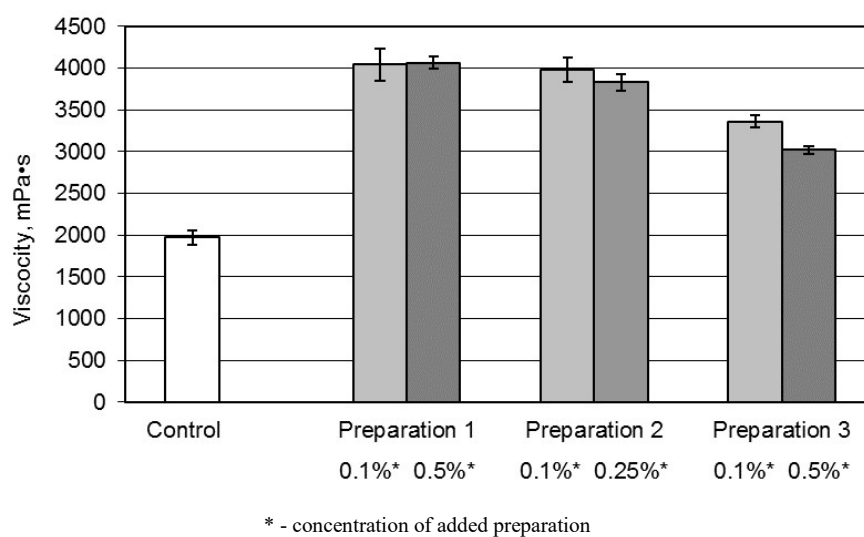


Figure 3. The influence of addition of the preparations P1, P2 and P3 on viscosity of yogurt samples ($p < 0.05$)

From the data presented in the Figure 3 the addition of preparations significantly increases the viscosity of yogurt samples. Apparently, an increase in the viscosity is explained by the strengthening of the structure of yogurt with water-soluble polysaccharides contained in preparations P1 and P2. In case of preparations P1 and P2, the viscosity increased approximately twice compared to the control. The preparation P3 is completely water-insoluble and the viscosity of the obtained samples decreases with increasing concentration of the β -glucans, but remains substantially higher than the control.

In the Figure 4 the picture of control and experimental yogurt samples after 24 hours of storage at the temperature of +4 °C is presented. There is a significant syneresis in the control samples, whereas it was not observed in the experimental samples.

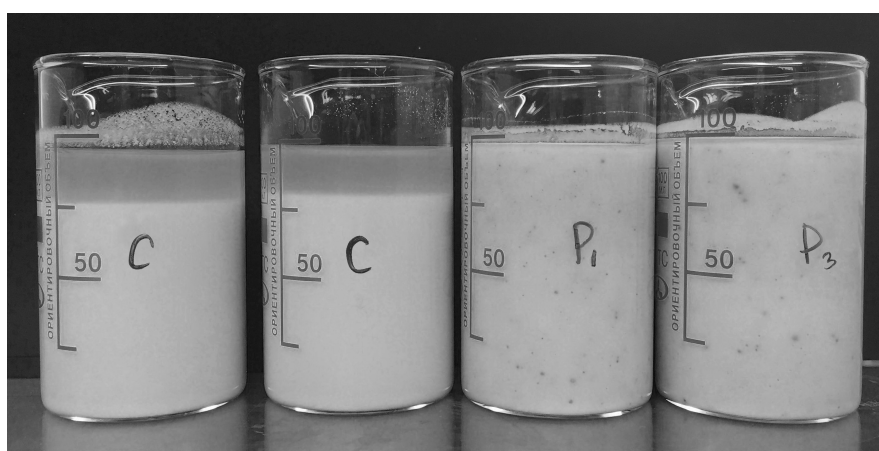


Figure 4. Yogurt samples (C – control samples, P1 and P3 – experimental samples with addition of preparation P1 and P3 in concentration of 0.5 %)

The organoleptic characteristics, such as taste, smell, color and consistency were analyzed. Characteristics determined for some dairy products with preparations are shown in Table 3.

A small coloring of the finished product was observed for all dairy products where preparation 1 was used as an additive. Color intensity increased with the increase of concentration. Besides that, small brown colored particles of preparation remained in the products. Preparation P2 is water-soluble; therefore, it was completely dissolved in samples. This preparation colored samples into creamy-white. Preparation P3 behaves like Preparation P1, but it had a lower coloring power (Figure 4).

Our results correspond to the results of the influence of addition of bacterial exopolysaccharides on yogurt viscosity [23]. In our previous studies the resembling effect was demonstrated: addition of preparation P1 in concentration of 0.5 and 1.0 % improved share stress rate and apparent viscosity of yogurt [24]. Also, when the influence of preparations obtained from *P. ostreatus* on acid-milk cultures of *S. thermophilus* and *L. bulgaricus* was studied, all the preparations reduce the fermentation period and improve the physical-chemical properties of fermented milk products [25].

Table 3. Effect of preparations P1, P2 and P3 on the sample's organoleptically quality

Dairy product	Concentration of preparation	Taste and smell	Color	Consistency
Fermented acidophilus milk	control – 0 %	pure fermented milk	white	homogeneous, viscous
	P1 – 0.1 %	pure fermented milk	white	homogeneous, viscous, there are small particles of brown color
Fermented baked milk	control – 0 %	pure fermented milk with nutty flavor	light-brown	homogeneous, viscous
	P1 – 0.1 %	pure fermented milk with nutty flavor	light-brown	homogeneous, viscous, there are small particles of brown color
	P3 – 0.1 %	pure fermented milk with nutty flavor	light-brown	homogeneous, viscous, there are small particles of brown color
Yogurt	control – 0 %	pure fermented milk	white	heterogeneous, serum separation, weak
	P1 – 0.1 %	pure fermented milk	white	homogeneous, viscous, there are small particles of brown color
	P2 – 0.1 %	pure fermented milk	creamy-white	homogeneous, viscous

CONCLUSIONS

Due to the sequential ethanol and aqueous extractions various preparations from mycelia biomass of *P. ostreatus* were obtained.

The influence of various preparations from *P. ostreatus* biomass on the glycolytic activity of starter cultures used for the preparation of fermented dairy products, such as acidophilus milk, fermented baked milk and yogurt was studied. It has been determined that preparations P1, P2, and P3 have no significant effect on the acid-producing activity of the cultures for acidophilus milk and fermented milk, but stimulate the growth of cultures for yogurt, causing a reduction in the coagulation time of milk by 2 hours compared to the control. Perhaps, this indicates the prebiotic action of the functional supplement.

It is shown that the addition of preparations into milk before fermentation improves the structural-mechanical parameters of finished dairy products in most cases. Due to addition of 0.1 % of P1 and P2 to milk, the viscosity of fermented acidophilus milk increased for 13 - 14 % compared to the control in both cases. After the addition of preparation P1 to the milk in concentration of 0.5 %, and P2 and P3, in all concentrations, the viscosity of fermented baked milk increased respectively for 25 %, 14 - 16 % and 24 - 25 % compared to the control. Viscosity of yogurt, fortified with

preparation P1 and P2 increased approximately twice compared to the control, in the case of the preparation P3 0.1 % the increase was 70 – 71 %, and for P3 0.5 % the increase was 53 %.

Based on the results obtained for the studied products and starters the following combinations of fermented milk products used preparations and their concentrations are recommended:

- for fermented acidophilus milk – preparation P1 in concentration 0.1 %;
- for fermented baked milk – preparation P1 in concentration 0.1 % and preparation P3 in concentration 0.1 %;
- for yogurt – preparations P1 in concentrations of 0.1 % and preparation P2 in a concentration of 0.1 %.

β -glucans preparations added in tested dairy products do not degrade the organoleptic characteristics of the obtained dairy products, do not introduce foreign flavors and improve consistency and viscosity of the finished dairy products.

The obtained results suggest the possibility of utilization of *P. ostreatus* polysaccharides preparations containing β -glucans to create functional foods based on dairy products.

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