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INFLUENCE OF *PLEUROTUS OSTREATUS* PREPARATIONS ON FERMENTATION PRODUCTS OF LACTIC ACID CULTURES

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Abstract

Pleurotus ostreatus is well-known and commercially important edible basidiomycetes. Polysaccharides, obtained from *P. ostreatus*, are suitable candidates for research and development of new functional foods and nutraceuticals. *P. ostreatus* preparations can provide to the products additional therapeutic properties, such as anti-cancer, anti-inflammatory and hypoglycemic properties. Yoghurt is the popular base for functional products. Useful additives can be simple added into yoghurt, but they can also impact on process of milk fermentation by lactic acid bacteria: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*.

The object of our study was polysaccharide preparations, obtained from submerged cultivated *P. ostreatus* biomass. It was obtained 3 preparations: preparation P1, which was collected after removing from biomass lipids and low-molecular compounds by 80% ethanol repeated; preparation P2, collected after ethanol extraction and extraction in a boiling water bath and then concentrated by evaporation and precipitated with five volumes of 96% ethanol solution; and preparation P3, taken as the solid residue remaining after the ethanol and aqueous extractions. Preparations were added to milk in different concentration before the introduction of lactic acid cultures. The titrated acidity was analyzed by acid-base titration with sodium hydroxide. The water holding capacity was defined as the ratio of the weight of the fermented bunch to the weight of the total fermented milk product after 24 hours storage from the time of preparation.

It was found, that preparation P1 mostly increase the acidity of fermented milk for both cases and the water-holding capacity of *L. bulgaricus* fermented milk. Preparation P2 enlarges both parameters, but only in case of *L. bulgaricus*, and preparation P3 enhanced the acidity of *S. thermophilus* fermented milk and the water-holding capacity of milk fermented by each bacteria.

Thus, the addition of the *P. ostreatus* preparations increase the rate of acidity growth and the moisture-holding capacity of the clot.

***Key words**: Functional food, Pleurotus ostreatus, Lactic acid fermentation, Polysaccharides, Lactobacillus bulgaricus, Streptococcus thermophilus.*

1. Introduction

Polysaccharides, particularly β-glucans, isolated from basidiomycetes, are of considerable interest due to their various useful medical properties. For a large number of genera and species of fungi the immune stimulating activity was demonstrated in paper of Vannucci *et al.,* [1]. Pronounced hypolipidemic effect was also detected related to consumption of biomass of the *Pleurotus ostreatus* mushroom in Shamtsyan *et al.,* [2] paper. It is believed by Gargano *et al.,* [3], that polysaccharides isolated from *P. ostreatus* fungus culture can be used for development of new functional products and nutraceuticals. Such preparations from *P. ostreatus* could provide additional preventive and

functional properties to widely used ordinary food products, such as: immunostimulating, hypoglycemic, antitumour, anti-inflammatory and hypoglycemic properties, as it was described in Giavasis [4].

Air pollution in metropolises, bad eating habits, sedentary lifestyle, and excessive use of preparations - these factors negatively affect the human body. Therefore, the theory of "healthy nutrition" developed in recent decades, based on the consumption of foods enriched with various functional additives and probiotics, is becoming increasingly relevant as mentioned by Shenderov [5]. It has been shown by Bigliardi [6], that functional products reduce the risk of developing food-related diseases, including improving the functioning of the intestine and heart, promoting better immunity, or weight normalization.

One of the following beneficial and widely used foods in the world are dairy products. Yogurt is a functional food product containing probiotic cultures and amino acids that accumulate in the process of splitting the milk proteins by the enzymes of these cultures, as it was mentioned in Ahmad *et al.,* [7]. Many manufacturers enrich yogurts with various vitamins, minerals and natural flavor additives.

It would be interesting to combine the useful properties of yogurt and β-glucans of basidiomycetes. The first step towards the creation of such a functional product based on yogurt is to check the effect of polysaccharide additives on individual yoghurt cultures. This article examines the effect of different concentrations of various preparations of polysaccharide from *P. ostreatus* on development and level of milk fermentation by monocultures that make up yogurt: *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Some physical-chemical parameters of obtained dairy products were also evaluated.

Submerged biomass of this fungus was used as a source to obtain preparations of polysaccharides as it was mentioned in Giavasis [4]. Besides other advantages, submerge cultivation method permits to obtain homogenic biomass, which simplifies its processing, product isolation, as well as the stability of the properties of the finished product and its relative standardization, according to Biliaderis [8].

As a result of the work, the effect of obtained polysaccharides on the physical-chemical properties of the fermented dairy product was studied. It was determined that preparations of polysaccharides of the *P. ostreatus* are promising beneficial ingredients for fortification of dairy products.

2. Materials and Methods

As a producer, *P. ostreatus* culture was used from the museum of the Department of Microbiological Synthesis Technology of the St. Petersburg State Technological Institute.

To study the effect of the preparation on lactic acid bacteria, two starter cultures obtained from State Institute of Dairy industry were used, based on strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in the form of lyophilized dried preparations. Dairy products were made from ultra-high-temperature treated milk (Valio, "Galactica" plant) with a fat content of 1.5%; for preparation of inoculum, milk of the "Valio" company with a fat content of 0% was used.

The inoculum of *P. ostreatus* was first incubated in test tubes on sloped wort agar for 7 - 10 days at the temperature of 28 - 30 ^{0}C. Then, few pieces of mycelium measuring about 50 mm^2 were transferred with the agar medium to conical flasks with glass beads and 150 mL of the nutrient medium of the following composition (g/L): Glucose - 10; $MgSO_4$ - 0.5; Peptone - 2.5; NaCl - 0.5; KH_2PO_4 - 0.6; $CaCl_2$ - 0.05; K_2HPO_4 - 0.4; Yeast Extract - 2.0.

Inoculate has been farther grown under steady-state conditions at the temperature of 28 - 30 ^{0}C for 7 - 10 days until the surface of the medium completely overgrew with mycelium. The raised surface inoculum was crushed with glass beads by shaking the flasks. The resulting suspension was used as inoculate for the fermentation step. Submerged cultivation was carried out in Erlenmeyer flasks with 100 mL of medium on a rotary shaker with a rotation frequency of 230 min^{-1} at the temperature of 28 - 30 ^{0}C. 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 ^{0}C.

To isolate the polysaccharides, the dried biomass was first ground, then the lipids were removed by double extraction with an 80% aqueous solution of ethyl alcohol, to make fungal cell walls more permeable. Water-soluble polysaccharides were separated by triplicate aqueous extraction. The scheme for isolation of preparations from the biomass of *P. ostreatus* is shown in the Figure 1.

In the process of isolation of polysaccharides, three preparations were obtained: oyster mushroom biomass treated with 80% ethanol - preparation P1; water-soluble polysaccharides isolated from aqueous extract - preparation P2; and the biomass of oyster mushrooms remaining after ethanol and aqueous extraction is P3.

To study the effects of preparations on the lactic acid bacterial cultures of *L. bulgaricus* and *S. thermophilus*, they were pre-cultured on milk in test tubes at the temperature of 4 ^{0}C until complete clotting. One of the three preparations of a certain mass was initially added into sterile glasses, and then they were filled with milk

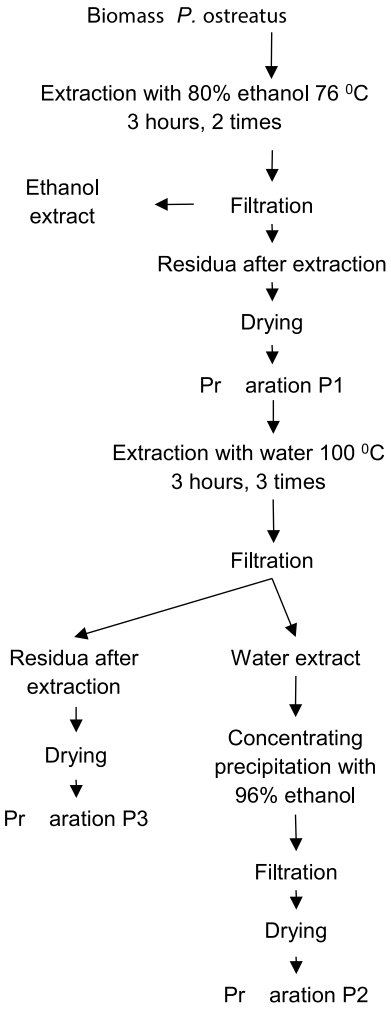

Figure 1. Obtaining preparations P1, P2 and P3 from *P. ostreatus* biomass

up to a volume of 100 mL. At the end, the inoculum was added (3% of the sample volume).

For both yoghurt cultures, the following dosages of the preparations were taken:

- Preparation P1 in concentrations of: 0.1%; 0.5%; 1.0%; 1.5%.
- Preparation P2: 0.1%; 0.25%; 0.5%.
- Preparation P3: 0.1%; 0.5%; 1.0%; 1.5%.

Analysis of titratable acidity was carried out according to the indicator method described in GOST (Russian State Standard) 3624-92 "Milk and dairy products. Titrimetric methods for determination of acidity" [9].

Water-holding capacity (WHC) and the syneresis of fermented milk were studied according to the procedure described in Sodini [10]. Samples of fermented milk (about 20 cm^3) (Y) after cooling to 4 °C and 24 hours of storage were centrifuged for 10 minutes at 4000 min^{-1} at 20 °C. The released serum (W) was removed and weighed. The WHC of fermented milk is calculated by the formula: WHC = (Y - W) / Y × 100% [10].

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

3. Results and Discussion

The effect mushroom preparations on the fermentation of milk by individual cultures of lactic acid bacteria was studied. The results of these studies for *S. thermophilus* are shown in Figures 2 - 4.

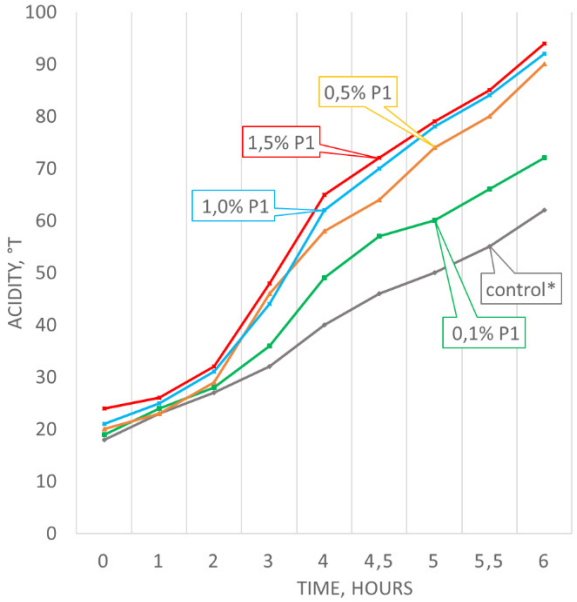

Figure 2. (* - sample without preparations) - Dynamics of titratable acidity during fermentation of milk by *S. thermophilus* with the addition of P1 (p < 0.05)

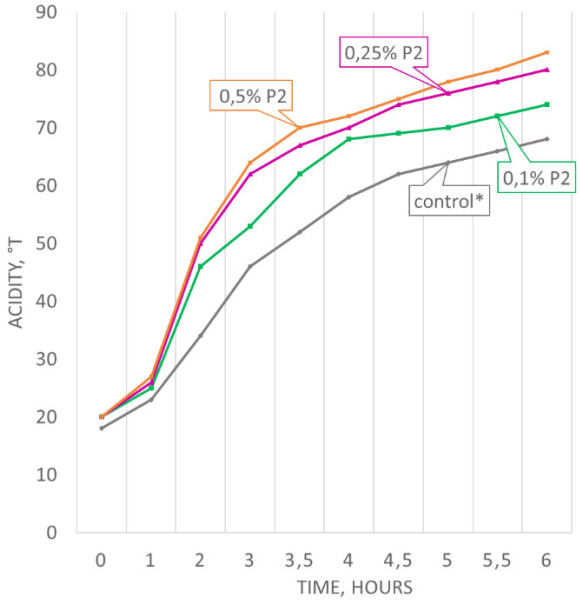

Figure 3. (* - sample without preparations) - Dynamics of titratable acidity during fermentation of milk by *S. thermophilus* with the addition of P2 (p < 0.05)

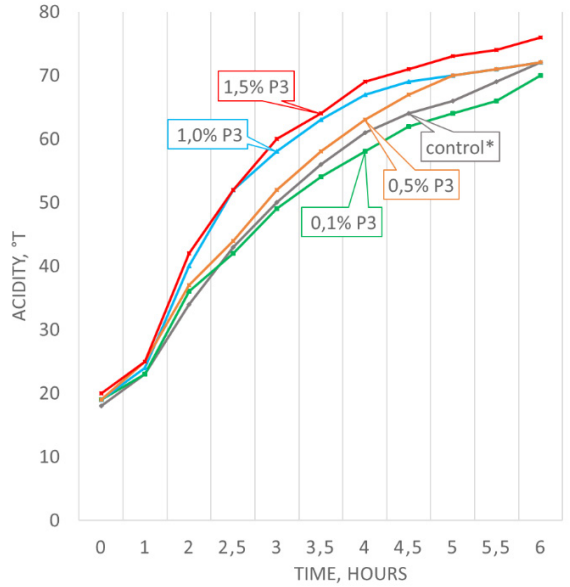

Figure 4. (* - sample without preparations) - Dynamics of titratable acidity during fermentation of milk by *S. thermophiles* with the addition of P3 (p < 0.05)

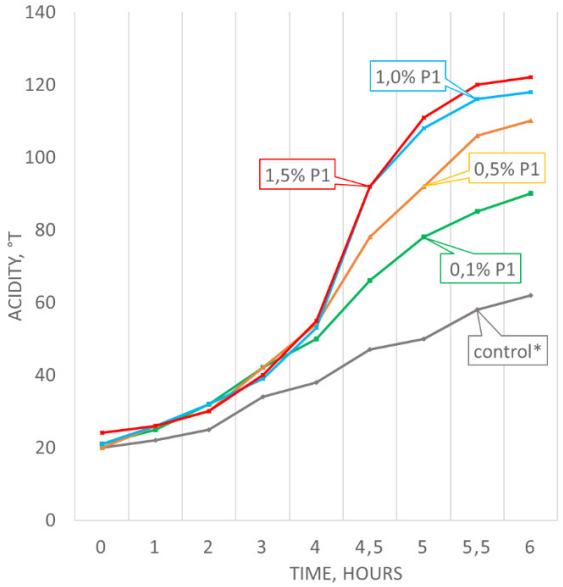

Figure 5. (* - sample without preparations) - Dynamics of titratable acidity during fermentation of milk by *L. bulgaricus* with the addition of P1 (p < 0.05)

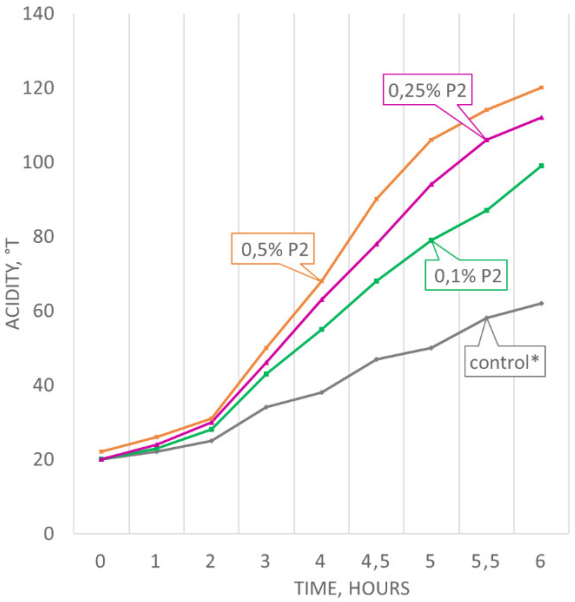

Figure 6. (* - sample without preparations) - Dynamics of titratable acidity during fermentation of milk by *L. bulgaricus* with the addition of P2 (p < 0.05)

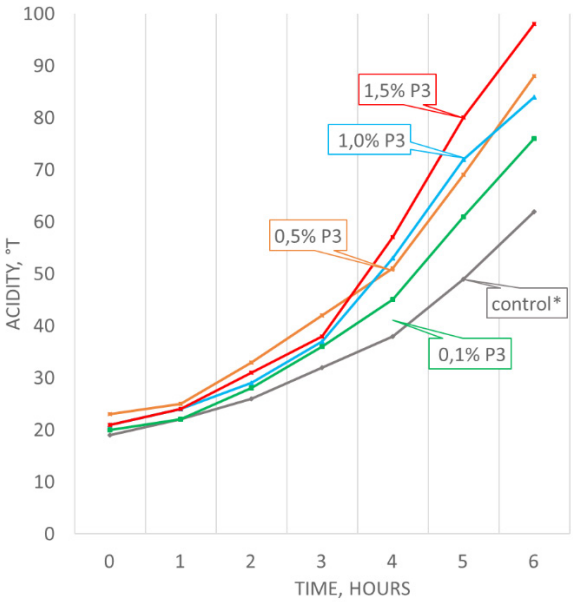

Figure 7. (* - sample without preparations) - Dynamics of titratable acidity during fermentation of milk by *L. bulgaricus* with the addition of P3 (p < 0.05)

From the presented in figures 2 - 4 it is clear, that the addition of preparations P1, P2 and P3 to milk before fermentation by *S. thermophilus* increases glycolytic activity of bacteria. The addition of preparation P1 to milk before fermentation in concentrations of: 0.1%, 0.5%, 1.0% and 1.5%, after 6 hours results in increase of acidity for: 16%, 45%, 48%, and 52% respectively, compared to the control sample. The preparation P2 added to milk at concentrations of: 0.1%, 0.25%, and 0.5% after 6 hours of fermentation causes an increase in acidity of: 12%, 21%, and 26%, respectively, compared to the control sample. The preparation P3, added to milk at concentrations of: 0.1%, 0.5%, 1.0% and 1.5%, after same period of fermentation causes an increase in acidity of: 6%, 9%, 9% and 15% respectively, compared to the control sample. Thus, for the culture of *S. thermophilus*, the best results were detected for ethanol treated fraction of polysaccharides P1 at 0.5% concentration and for water-soluble fraction P2 at 0.25%. The addition of P3 did not significantly affect the glycolytic activity of the *S. thermophilus* culture.

The effect of preparations on the fermentation of milk for *L. bulgaricus* are shown in Figures 5 - 7.

From the presented in figures 5 - 7 it could be seen that the addition of all studied preparations to milk, fermented by *L. bulgaricus* also causes a significant increase in glycolytic activity and this effect was even higher, than for streptococcus culture. The preparation P1, added to milk at concentrations of: 0.1%, 0.5%, 1.0% and 1.5%, causes an increase in acidity of: 52%, 77%, 90%, and 102%, respectively, compared to the control sample. The preparation P2 added to milk in concentrations of: 0.1%, 0.25%, and 0.5% causes an increase in acidity of: 60%, 81%, and 94%, respectively, compared to the control sample. And even preparation P3, which was not active in case of *S. thermophilus*, added to milk at concentrations of: 0.1%, 0.5%, 1.0% and 1.5%, causes an increase in acidity of: 23%, 35%, 42% and 58% compared to the control sample. Thus, for the culture of *L. bulgaricus*, the best results were shown by maximum concentrations of all preparations. The most efficient was addition of water-soluble fraction of mushroom polysaccharides P2.

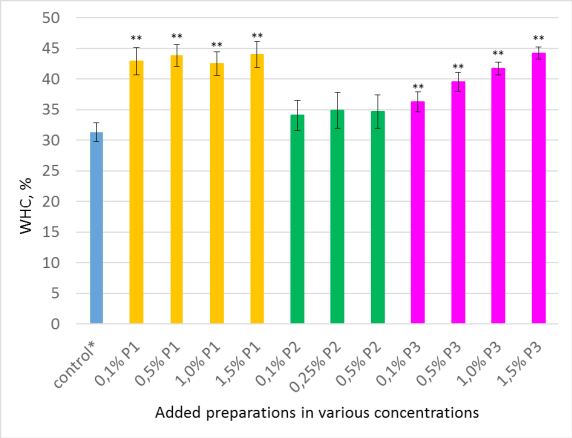

Figure 8. (* - sample without preparations) - Effect of preparations on the WHC of milk clots of products with *S. thermophilus* (- p < 0.05)**

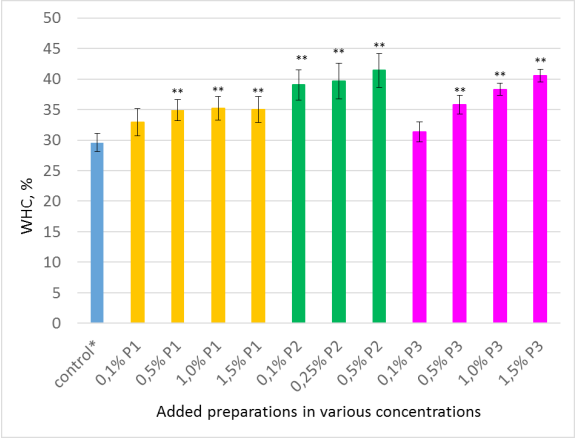

Figure 9. (* - sample without preparations) - Effect of preparations on the WHC of milk clots of products with *L. bulgaricus* (- p < 0.05)**

One of the important physical-chemical parameters, which determine the texture of a dairy product, is WHC. The results of determination of the relation of WHC from added preparations are presented in Figures 8 and 9.

From the data obtained, it can be seen that the addition of all preparations to milk fermented by *S. thermophilus* causes an increase in WHC of the milk clot. The preparation P1 added at a concentration of: 0.1%, 0.5%, 1.0%, and 1.5% causes an increase in MTC of: 37%, 40%, 36%, and 40%, respectively, compared to the control sample. The P2 preparation added at a concentration of: 0.1%, 0.25%, and 0.5% causes an increase in WHC by: 9%, 11%, and 11%, respectively, compared to the control sample. The preparation P3, added at a concentration of: 0.1%, 0.5%, 1.0%, and 1.5%, causes an increase in WHC by: 16%, 26%, 33% and 41% respectively, compared to the control sample. Thus, for the culture of *S. thermophilus*, the best results showed P1 and P3 at a concentration of 1.5%, the addition of P2 did not have a significant (statistically significant) effect on the moisture retention capacity of the *S. thermophilus* culture.

In the case of *L. bulgaricus*, similar results were obtained. The preparation P1 added at a concentration of: 0.1%, 0.5%, 1.0%, and 1.5% causes an increase in WHC by: 11%, 18%, 19%, and 18% compared to the control sample. The P2 preparation added at a concentration of: 0.1%, 0.25%, and 0.5% causes an increase in WHC by: 32%, 34%, and 40%, respectively, compared to the control sample. Formulation 3 (P3) added at a concentration of: 0.1%, 0.5%, 1.0%, and 1.5% causes a: 6%, 21%, 29%, and 37% increase in WHC, respectively, compared to the control sample. Thus, for the culture of *L. bulgaricus*, the best results were shown by preparations of P2 in a concentration of 0.5% and P3 at a concentration of 1.5%.

4. Conclusions

- The addition of *P. ostreatus* preparations to milk increased the glycolytic activity of yoghurt cultures of *S. thermophilus* and *L. bulgaricus*. The acidity of milk fermented by *S. thermophilus* as a result of the addition of P1 was the highest. In the studied ranges of preparations doses, their increase result in increase of the growth of the titrated acidity of fermented milk products. Acidity of milk, fermented by *L. bulgaricus*, increased as a result of the use of each of the preparations, higher results showed preparations P1 and P2.

- The introduction of preparations into the milk before fermentation increased the WHC of the clots. This might be because the increase of dry substances in the content strengthens the structure of the fermented milk product and the fact that microorganisms, when sorbed on a solid carrier, use components of the nutrient medium better.

- The addition of polysaccharides positively affects the process of lactic acid fermentation, carried out by the studied cultures of microorganisms. The addition of polysaccharide preparations before the lactic fermentation stage reduces the fermentation time and improves the physico-chemical properties of fermented milk products. The results obtained suggest the possibility of using fungal polysaccharides containing β-glucans to create functional foods based on fermented milk products.

5. References

[1] Vannucci L., Krizan J., Sima P., Stakheev D., Caja F., Rajsiglova L., Saieh M. (2013). *Immunostimulatory properties and antitumor activities of glucans*. International journal of oncology, 43, (2), pp. 357-364.

[2] Shamtsyan M., Antontceva E., Panchenko A., Petrishchev N. (2014). *Hypolipidemic and hypocholesterolic action of submerge cultured mushrooms*. Journal of Hygienic Engineering and Design, 7, pp. 96-99.

[3] Gargano L. M., van Griensven D. L. J. L., Isikhuemhen S. O., Lindequist U., Venturella G., Wasser P. S., Zervakis I. G. (2017). *Medicinal mushrooms: Valuable biological resources of high exploitation potential*. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, 151, (3), pp. 548-565.

[4] Giavasis I. (2001). *Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals*. Current Opinion in Biotechnology, 26, pp. 162-173.

[5] Shenderov B. A. (2001). *Medical microbial ecology and functional nutrition. Vol. III: Probiotics and functional nutrition* (in Russian). Grant, Moscow, Russia.

[6] Bigliardi B., Galati, F. (2013). *Innovation trends in the food industry: the case of functional foods*. Trends in Food Science & Technology, 31, (2), pp. 118-129.

[7] Ahmad A., Munir B., Abrar M., Bashir S., Adnan M., Tabassum T. (2012). *Perspective of β-glucan as functional ingredient for food industry*.

URL: https://www.omicsonline.org/perspective-of-glucan-as-functional-ingredient-for-food-industry-2155-9600.1000133.php?aid=5165. Accessed 17 September 2017.

[8] Biliaderis C. G. (2006). *Microbial polysaccharides*. In: Biliaderis C. G., Izydorczyk M. S. (Eds.), Functional food carbohydrates, CRC Press, Boca Raton, Florida, USA, pp. 167-213.

[9] GOST. (1992). *GOST 3624-92: Milk and milk products. Titrimetric methods of acidity determination*.

[10] Sodini I., Lucas A., Oliveira M. N., Remeuf F., Corrieu G. (2002). *Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing*. Journal of Dairy Science, 85, (10), pp. 2479-2488.