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Enhancement of the Adsorptive and Antimicrobial Properties of the Yeast Cell Walls by Enzymatic Processing.

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ABSTRACT

Processed *Saccharomyces cerevisiae* is a perspective source of functional components for food and functional products. Application of the different methods of yeasts treatment allows to obtain products which are effective for toxins binding. In the current research, parameters of the cell wall enzymatic hydrolysis were studied for functional properties modification of yeast samples. The results showed that mannano-, glucano-, proteolytic enzymatic systems and their complexes used for hydrolysis of yeast biopolymers allowed to increase adsorptive and antimicrobial properties of the yeasts walls.

Keywords: yeast**,** cell wall, enzyme, biocatalysis, enterosorbent

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INTRODUCTION

Application of chemical products and pesticides in agriculture and toxigenic effects of industrial wastes are increasing significantly the content of dangerous components in plants used in food and feed industries. Taking into account the health risks for human and farm animals associated with the agricultural products quality, there is a need to find effective ways to eliminate harmful substances from raw materials on the stage of its processing and during ingestion. Many different methods based on application of mineral, carbon, and microbial adsorbents have been used for removing of toxic substances [1, 2]. According to results of researches, yeast cells capacity for toxin binding can be improved by heat-, acid- and ultrasound treatment [3]. The most important aspect of the biological products obtaining is the preservation of the adsorptive properties of cell wall polymers. Nowadays, interest to the sorption materials produced with biotechnological methods has increased significantly. Enzymatic hydrolysis is the most effective method, which can be used for biopolymers transformation and destruction without high temperature treatment, acid and alkali usage [4, 5, 6].

The cell wall represents about 25% of total cell weight and consists of the two layers [7, 8]. Main polymers of upper layer are mannoproteins, which are covalently linked with β-1,3 / 1,6-glucans of the cell walls inner layer. The β -1,3 / 1,6-glucans can bind to glucan-receptors of white blood cells and stimulate immune system. Retrieval of the glucan preparations needs mannan biopolymers destruction because of the bonds of mannoproteins glucanextractable fraction with a glucan layer. But the mannan is also a valuable polysaccharide with a health-improving characteristics. Mannoproteins allow to block intestinal colonization by pathogenic bacteria and to enhance intestinal immune system and intestinal morphology [9, 10]. Mannanooligosaccharides of the the yeasts are able to absorb the wide spectrum of toxins. After mannan biopolymers destruction, mannan molecules have coils form in the solution with antigenic side chains being inside these coils, so the mannan preparations activity decreases significantly. Biosorptive capacity of mannan can be increased with high-temperature and alkali treatment, but the biotechnological process is the most promising way of treatment.

The functional abilities of biological products from the microbial biomass depend on the biochemical, structural and fractional composition of the polymers with the sorptive properties. Applying of the original sorption properties of yeast cell walls polymers allows to use their preparations as substances for reduction of the negative effects of metals ions, free radicals, harmful bacteria. In the current study, the influence of the enzymatic processing of cell wall polymers on the functional properties of yeast biomass was investigated.

MATERIALS AND METHODS

The *Saccharomyces cerevisiae* (RCAM 01138) yeast biomass was used as the object of the research. The strain was obtained from the collection of microorganisms of the Russian Research Institute of Food Biotechnology. The strain was grown at 30°C for 6 hours on the culture medium, which contained clarified molasses; ammonium sulphate, diammonium phosphate and potassium chloride. The yeast cells were obtained by centrifugation of the media at 5,000×g for 10 minutes and double washing with water. The yeast cells were mixed with distilled water to 20% concentration of total solids in the suspension, incubated at 80°C for 15 minutes for pasteurization and native enzymes inactivation and then cooled to 50 °C.

The following enzymes and their complexes were used in the current study:

- Mannanase- preparation containing α-1,3-1,6- mannanolytic enzymes derived from controlled condition growth of *Penicillium canescens*;
- Glucanase - glucanolytic enzymes complex derived from controlled condition growth of *Myceliophthora fergusii* for β-1,3-1,6-glycosidic bonds hydrolysis in the glucan molecules;
- Amyloprotoorizine enzyme complex containing several proteases (proteinases and peptidases) derived from controlled condition growth of *Aspergillus oryzae*;
- Enzyme system- 1 (ES-1)- complex containing Mannanase and Amyloprotoorizine preparations;
- Enzyme system- 2 (ES-2)– complex containing Mannanase, Glucanase and Amyloprotoorizine preparations.

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Proteolytic activity of Amyloprotoorizine was assessed according to method developed by Sigma-Aldrich using casein as a substrate [11].

The mannanase activity of preparations was estimated according to the modified method of Somogyi-Nelson measurement. One unit of mannanase activity (ManU) was defined as the amount of enzyme required to release one µmole of mannose reducing-sugar equivalents per minute from carob galactomannan (10 mg/mL) in sodium acetate buffer (100 mM), pH 4.0 at 40°C.

The method of Somogyi-Nelson was used for glucanolityc activity determination of enzyme preparations. One unit of glucanase activity (BGU) was defined as the amount of enzyme required to release one µmole of glucose reducing-sugar equivalents per minute from barley β -glucan (10 mg/mL) in sodium acetate buffer (100 mM), pH 4.7 at 50°C.

The enzyme compositions and their dosages used in the experimental are presented in table 1.

Table 1- Experimentation enzymes dosages

Preparation	Enzyme dosage, U/100 g of yeast		
	ManU	BGU	PU
Mannanase	100	0	0
Glucanase	0	100	0
Amyloprotoorizine	0	0	20
Enzyme system- 1	100	0	20
Enzyme system- 2	50	50	20

Enzymatic processing of yeast biomass was carried out at 50°C, pH 6.2±0.1 (natural pH of yeast water suspension) for 12 hours.

Results of the polysaccharides hydrolysis was estimated by reducing sugars accumulation using Somogyi-Nelson method based on measuring the colorimetric reaction intensity of the reducing sugars compounds with the Somogyi-Nelson reagent [12]. The degree of protein hydrolysis was rated as the amino nitrogen content increase using a biuretic method.

The antimicrobial activity of the samples was determined as the ability to inhibit the test culture (E.coli) growth on the solid agar medium Endo.

The absorption capacity of the samples was assessed as the ability to adsorb heavy metal (arsenic) salts from their water solutions at 30°C for 30 minutes. The residual concentration of arsenic ions was determined according to GOST R 51766 “Food staples and products. Atomic absorption method of arsenic determination”.

Statistical analysis were performed using one-way analysis of variance conducted at a confidence level of 95%.

RESULTS AND DISCUSSION

Experimental data of researchers showed necessity of more in-depth study of the mannoproteins and glucans role in the adsorption processing of mycotoxins, metal ions, pathogenic microorganisms, etc. The influence of enzymatic hydrolysis at the cell wall polymers is unclear and needs understanding of the process chemistry. In the current study, the effectiveness of different enzyme compositions on the cell walls polymers degradation was studied.

At the first step of the investigation, amount of low molecular weight substances obtained by enzymatic hydrolysis of yeast mannoproteins and glucans was assesed. The concentration of reducing sugars and amine nitrogen in the supernatant was measured after solid fraction separation. The supernatant after high-temperature exposure of yeast biomass suspension was considered as control sample (fig.1 a,b).

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The results revealed significant differences between enzymatically treated and the control sample. Action of the individual enzymes (glucanase, mannanase, proteases) led to increase of the amine nitrogen accumulation (mannoproteins hydrolysis product) to 93-284% compared to the control sample (317, 578 and 630 mg% respectively) (Fig. 1a).

Analysis of the results of cell walls treatment showed that the mannoproteins of the upper layer are affected to mannanolytic and proteolytic action almost on the same level. Using of mannanase caused bio-transformation of polymers and allowed to release protein fraction to soluble form of supernatant. The results indicated that glucanase –extrable proteins of cell walls hydrolyzed at a lesser degree.

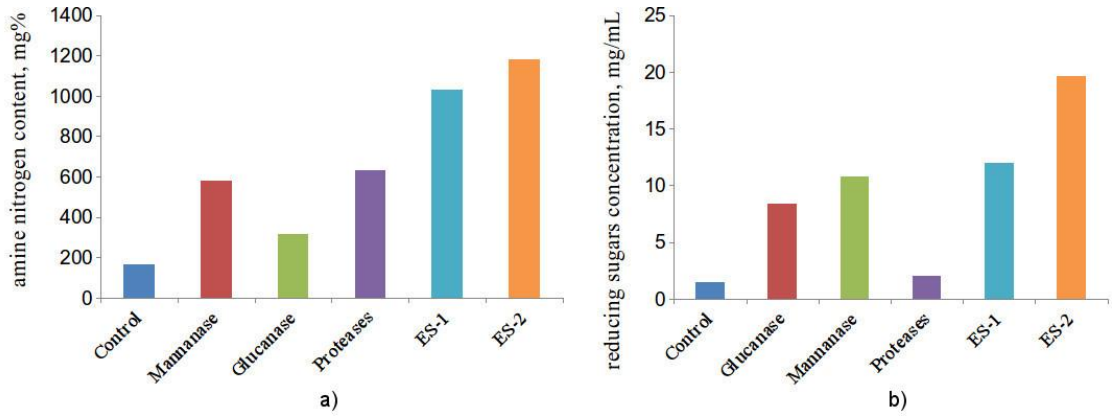

Figure 1- The influence of biocatalytic systems on the degree hydrolysis of cell wall polymers
a- amine nitrogen concentration; b- reducing sugars concentration

The highest amount of amine nitrogen was determined after treatment with ES-1 and ES-2 complex systems. The amine nitrogen concentrations in corresponding samples were 1030 and 1180 mg%, respectively.

It has been shown that enzymatic hydrolysis of mannan-containing part of cell wall polysaccharides was more effective than destruction of glucan. According to the fig.1b, the highest concentration of reducing sugars was observed in samples processed with mannanolytic enzymes used separatly and in the complexes ES-1 and ES-2. The study of the low-molecular sugars accumulation confirmed the assumption of easier hydrolysis of the cell wall mannoproteins in comparison with glucanase –extrable proteins. Fig.1b showed that most significant effect of the cell wall polymers enzymatic hydrolysis was revealed during the complex treatment with mannanolytic, proteolytic and glucanolytic biocatalysts (ES-2).

Taking into account that the functional properties of the yeast preparations depend on the treatment type of raw material (high-temperature, ultrasound, acid-treatment etc.) and polymers structure, the ability of cell wall hydrolysates to bind harmful elements was assessed [1, 13, 14].

Figure 2 shows that biotechnological processing of the yeast biomass allowed to increase the antimicrobial activity of the samples against the E.coli cells as compared with a control. The highest percentage of test culture growth inhibition was related to samples treated with mannanase and glucanase. Antimicrobial activity was 96% (fig.2a, b). The proteases additions to the systems based on mannanolytic enzymes lead to reduction of antimicrobial capacity to 87% (fig.2 d).

Results of previous studies reported that main antimicrobial properties were demonstrated by mannoproteins and glucans of yeast cells. The protease complex with high level of proteinases and peptidases (Amyloprotoorizine) was used for proteolysis of yeast biomass. The antimicrobial activity of the obtained sample with reduced protein content had the intermediate value (91%).

Complex enzymatic system used to the cell wall polymers degradation decreased antimicrobial activity of the samples to the greater extent than other samples, which were obtained after biocatalytic degradation (fig.2 e). The antimicrobial activity of the control sample was 39%.

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Due to covalent binding, the yeast cell wall surface can adsorb metal ions. The current study showed positive results concerning the absorption capacity of the hydrolysates to the arsenic from water-salt solution (fig.3).

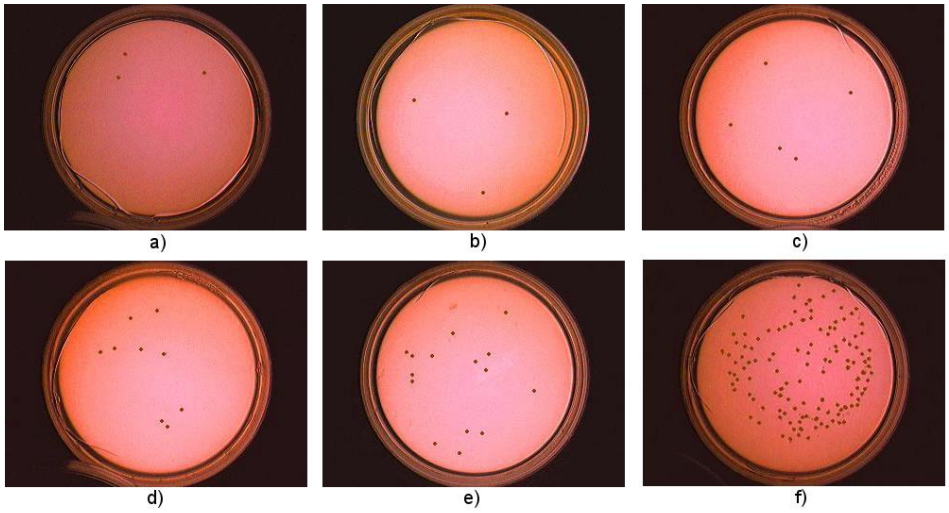

Figure 2- Antimicrobial activity of the yeast samples after various enzyme composition using: *a- mannanase; b- glucanase; c- proteases; d- mannanase+proteases; e) mannanase + glucanase +proteases; f) control*

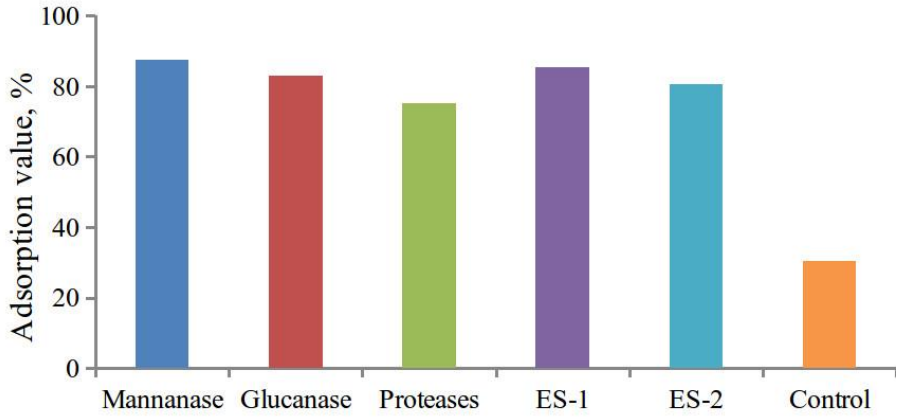

Figure 3- Metal-ion adsorbing ability of the yeast samples after hydrolysis with various enzyme compositions

The experimental data showed that the high degree of the arsenic ions binding was observed after biocatalytic destruction of the cell wall polymers using the mannanases and mannanases with proteases (ES-1) complex. The percentages of the metal ion concentration were 87.5 and 85.3%, respectively (fig.3).

The glucanolytic treatment of the yeast cell walls allowed to increase absorption capacity upper to the 52.5% as compared to a control sample. Furthermore, ES-2 complex action on the yeast biomass decreased the effectiveness of biocatalytic transformation of the cell wall polymers in case of point of metal ions binding of the samples.

CONCLUSIONS

Results of investigation indicated the possibility to enhance the functional properties of yeast preparation due to enzymatic processing. It was revealed that carbohydrate biopolymers of mannoproteins of yeast cell walls were most susceptible to the enzymatic action. Mannano-, glucano- and proteolytic enzymes

usage allows to increase the adsorptive activity preparations to the 44,8-57,0 % and antimicrobial activity to the 45-57 % in comparison with high-temperature treated sample.

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REFERENCES

[1] Nikolaev VG, Mikhailovsky SV, Nikolaeva VV, Oleschuk AM, Lisnichuk NE. Enterosorption: the state of the issue and prospects for the future. Bulletin of the problems of biology and medicine 2007; 4: 7-17. (in Russian).

[2] Debarshi R, Gaur P, Verma N, Pathireddy M, Singh KP. Bioremediation of arsenic (III) from water using baker yeast Sacchromyces cerevisiae. International Journal of Environmental Bioremediation & Biodegradation. 2013; 1: 14-19.

[3] Karazhiyan H, Mehraban Sangatash M, Karazhyan R, Mehrzad A, Haghighi E. Ability of different treatments of Saccharomyces cerevisiae to surface bind aflatoxin M1 in yoghurt. J. Agr. Sci. Tech.. 2016; 18: 1489-1498.

[4] Kurbatova EI, Rimareva LV, Borhcheva YA, Davydkina VE. Biotechnological bases of plant and microbial raw materials cell walls enzymatic degradation to obtain products with biologically valuable natural substances high content. Proceedings of international congress “Biotechnology: State of the art and perspectives". 2017; 1: 204-205.(in Russian).

[5] Serba EM., Overchenko MB., Davydkina VE, Shelekhova NV, Rimareva LV, Polyakov VA. Scientific and practical aspects of BAA obtaining based on the conversion of secondary bioresources. Storage and processing of farm products. 2015; 2: 44-50. (in Russian).

[6] Ibragimova SI, Tikhonova OV, Tolstikhina TE, Vasileva BF, Sinitsyn AP, Tsurikova NV, Zorov IN, Efremenkova OV. Hydrolysis of *Saccharomyces cerevisiae* Cell Wall by Enzyme Complexes from Basidial Mushrooms. Biotechnology in Russia. 2012; 6: 53-60.

[7] Kalebina TS., Kulaev IS. The role of proteins in the formation of the molecular structure of the yeast cell wall // Advances in Biochemistry. 2001; 41: 105-130. (in Russian).

[8] Aliverdieva DA., Malygin AG., Lagutina LS., Sholtz KF. Isolation of cell membranes from *Saccharomyces cerevisiae* for evaluation of their protein composition. APPL BIOCHEM MICRO+. 2004; 40: 296-299.

[9] Czop JK, Kay J. Isolation and characterisation of betaglucan receptors on human mononuclear phagocytes. J Exp Med. 1991; 173: 1511–1520.

[10] Savitskaya KI. Disturbances of microecology of the gastrointestinal tract and chronic intestinal diseases. Terra Medica. 1998; 2: 13–15. (in Russian).

[11] Cupp-Enyard, C. Sigma’s non-specific protease activity assay – Casein as substrate. J.Vis. Exp. 2008, 19, 899.

[12] Nelson N. A photometric adaptation of the Somogyi method for determination of glucose. J. Biol. Chem; 1944; 153:375-380.

[13] Wang J, Chen C Biosorption of heavy metals by Saccharomyces cerevisiae: A review. BIOTECHNOL ADV. 2006; 24: 427–451.

[14] Aronbaev SD, Nasimov AM, Aronbaev DM Biosorption of uranium (VI) in aqueous solutions by cellular shells of brewing yeast *Saccharomyces cerevisiae*. Water: chemistry and ecology.2011; 12: 51-55. (in Russian).