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ORIGINAL SCIENTIFIC WORKS

NEW PROCESSES FOR OBTAINING MANNOPROTEINS FROM BEER YEAST SEDIMENTS AND THEIR BIOCHEMICAL PROPERTIES

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Abstract: The paper provides information on a new process of extracting mannoproteins from yeast sediments from waste from the beer industry. The process is characterized by the use of wastes that pollutes the environment, the reduction of the time, the autolysis temperature and the amount of ethyl alcohol used. The content of mannoproteins is 41.34 - 44.8 % dry biomass, being 7 - 16 % higher compared to the control variant. The mannoproteins obtained according to the process have a varied biochemical composition and high catalase and superoxide dismutase activities. The obtained results present an opportunity for application in zootechnics, food, pharmaceuticals, medicine and agriculture.

Keywords: *Brewer's yeast sediments, carbohydrate content, enzyme activity, mannoproteins, protein content*

INTRODUCTION

In recent decades, microorganisms have been widely used by the scientific community to study biodiversity and as valuable biotechnological objects, with versatile applications in various fields. Yeasts, which can synthesize a rich complex of bioactive substances that play an important role in the vital activity of living organisms, were studied as sources of biological substances with high biosynthetic potential. The advantage of the utilization of yeasts in biotechnology consists primarily in their fast adaptability because they grow easily, the culture media and cultivation conditions for optimizing biosynthesis can be directed, they are resistant to foreign microflora, do not pollute the air with spores and can use nutrients that have a low cost of production [1]. Another area of major interest is the use of industrial yeast waste after the completion of the fermentation process of beer or wine as a raw material to produce biologically active substances. The use of yeast waste can reduce the negative impact on the environment, processing and production costs [2].

A component of interest in the structure of the yeast cell wall are mannoproteins. They present most of the glycoproteins in the cell liner and are made of two important classes of compounds. The first class plays a structural role in the cell wall consisting of about 40 % protein and 60 % carbohydrates. While the second-class mannoproteins are located in the periplasmic space between the cell wall and the plasma membrane, having enzymatic function [3 – 5]. Therefore, due to its specific biochemical and enzymatic composition, mannoproteins play an important role in maintaining cellular integrity, catalyze various biochemical reactions and participate in the immune and autoimmune response of living organisms, which gives them a high potential for application in animal husbandry, medicine, food, cosmetology and agriculture.

But the production of mannoproteins is often limited by the rigid wall of yeast cells, requiring efficient methods of extraction. At present, extraction processes are based on the use of enzymes, nanoparticles, ultrasound and mechanical destruction, each used separately or in combination, but which are ultimately quite expensive and require special equipment [6]. For these reasons, studies with the development of simpler and more energy-efficient procedures for the extraction of mannoproteins are becoming necessary.

Based on the above, the aim of this paper was to develop new procedures for obtaining mannoproteins from brewer's yeast sediments and to determine the biochemical properties.

MATERIALS AND METHODS

Objects of research

As research material was used the yeast biomass *Saccharomyces cerevisiae* of lower fermentation from the waste of the beer industry, offered by the Kellers brewery (Budesti).

The solutions for autolysis used in this study are glacial acetic acid in the calculation of 3 mL of acid per 1 L of suspension and sodium phosphate buffer with *p*H - 7.8.

Methods of achieving research

The determination of the dry biomass was performed gravimetrically according to the usual method by drying the biomass in the oven at +105 °C to constant mass and recalculating the dry matter [7]. The total carbohydrate content was determined at PG T60 VIS Spectrophotometer at 620 nm wavelength with the use of antron reagent and D-glucose as standard [8]. The protein was determined by spectrophotometric method using standard bovine serum albumin as a standard sample [9]. Catalase (CAT) was determined by the spectrophotometric method, which is based on the ability of hydrogen peroxide to interact with molybdenum salts, forming a stable colored complex [10]. Superoxide dismutase (SOD) activity was established spectrophotometrically, the method is based on inhibiting the reduction of the tetrazolium-nitroblue salt in the presence of TEMED and riboflavin [11].

The statistical analysis of results was done using statistical software kit Statistics 9.0. The obtained data results of 3 - 5 repetitions were expressed by calculating the mean, standard deviation and confidence interval for an average. All differences were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

A new approach in the reuse of industrial yeast waste after beer production is their use for the development of modern biotechnologies. Yeast biomass can be used as a raw material for the extraction of cellular components, including mannoproteins [12].

Autolysis is used to obtain mannoproteins from the cell wall of brewer's yeast, which is a procedure for destroying the cell wall by hydrolysis of intracellular biopolymers. Cell concentration, *p*H, temperature and duration are the parameters that influence the autolysis ratio of yeast cells [13]. The duration of the process is usually long and lasts from 24 hours to several days [14]. Thus, the autolysis process causes the desintegration of the cell wall and consequently contributes to the release of mannoproteins [15].

Thus, it became important to elucidate the influence of extraction methods on the mannoprotein content in order to select the most efficient process. The closest solution (control) was the process of extracting mannoproteins from the biomass of the yeast strain *Saccharomyces cerevisiae* CNMN-Y-18 cultured using TiO_2 nanoparticles at a concentration of 10 mg/L as a growth stimulator [16].

Initially, the aim was to research various simpler and more energy-efficient methods of autolysis and destruction of the yeast cell wall to make it easier to obtain mannoproteins and increase the nutritional and biological value. To optimize the yeast autolysis conditions in each variant, 30 g of thawed brewer's yeast biomass was used as working material, which was subjected to different autolysis conditions in ratio 1 : 1. Table 1 shows the autolysis induction factors that were used.

According to the obtained results, it was established that the autolysis of yeast biomass with the use of sodium phosphate buffer allows to obtain maximum values of mannoprotein content (Figure 1).

Table 1. *Autolysis induction factors of the beer yeast sediments for obtaining mannoproteins*

Induction factors	Temperature	During autolysis
3 % acetic acid [17]	+55 °C	8 hours
3 % acetic acid (distilled water)	+37 °C	8 hours
3 % acetic acid (distilled water)	+45 °C	8 hours
3 % acetic acid (tap water)	+37 °C	8 hours
phosphate buffer	+37 °C	8 hours
phosphate buffer	+45 °C	8 hours

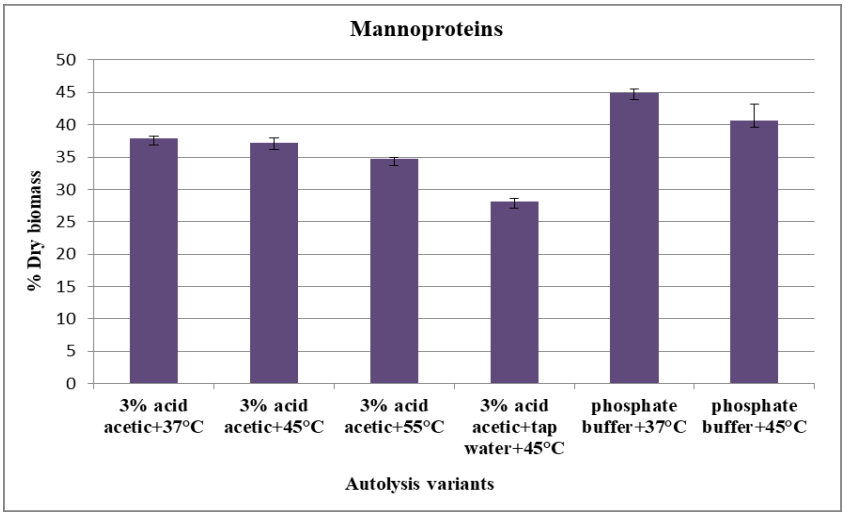

Figure 1. *The amount of mannoproteins obtained from the yeast biomass after autolysis by different methods*

In this context, the obtained results were used to develop procedures for obtaining mannoproteins from industrial yeast waste from beer production. The general scheme of achievement is presented in the Figure 2.

Thus, the procedure for obtaining mannoproteins is performed as follows:

Stage I. Initially, the biomass that was brought from the brewery, was centrifuged to remove the remaining liquid and frozen at -18 °C for storage.

Stage II. Subsequently, the biomass of thawed brewer's yeast is mixed with sodium phosphate buffer, *p*H - 7.8 (1 : 1 ratio). The obtained suspension is subjected to autolysis at +37 °C or +45 °C for 8 hours, with periodic stirring. At the end of the autolysis process, the suspensions were centrifuged at 3500 rpm. for 15 minutes.

Stage III. The deposit (cell walls) after centrifugation were treated with 1N NaOH solution (1 : 5 ratio) and hydrolyzed at 80 ± 5 °C for 2 hours. After hydrolysis the suspensions were centrifuged at 3500 rpm. for 15 minutes.

Stage IV. The obtained alkaline supernatants are sedimented with 96 % ethyl alcohol in a volume of 1 : 2. Upon sedimentation with alcohol, white-beige flakes are formed with a viscous consistency which represents the mannoprotein fraction. The liquid and sediment are separated.

Stage V. Subsequently the stabilization of the mannoproteins after the dry matter takes place.

Stage VI. At the final stage, the conditioning, labeling and packaging of the obtained mannoproteins are performed.

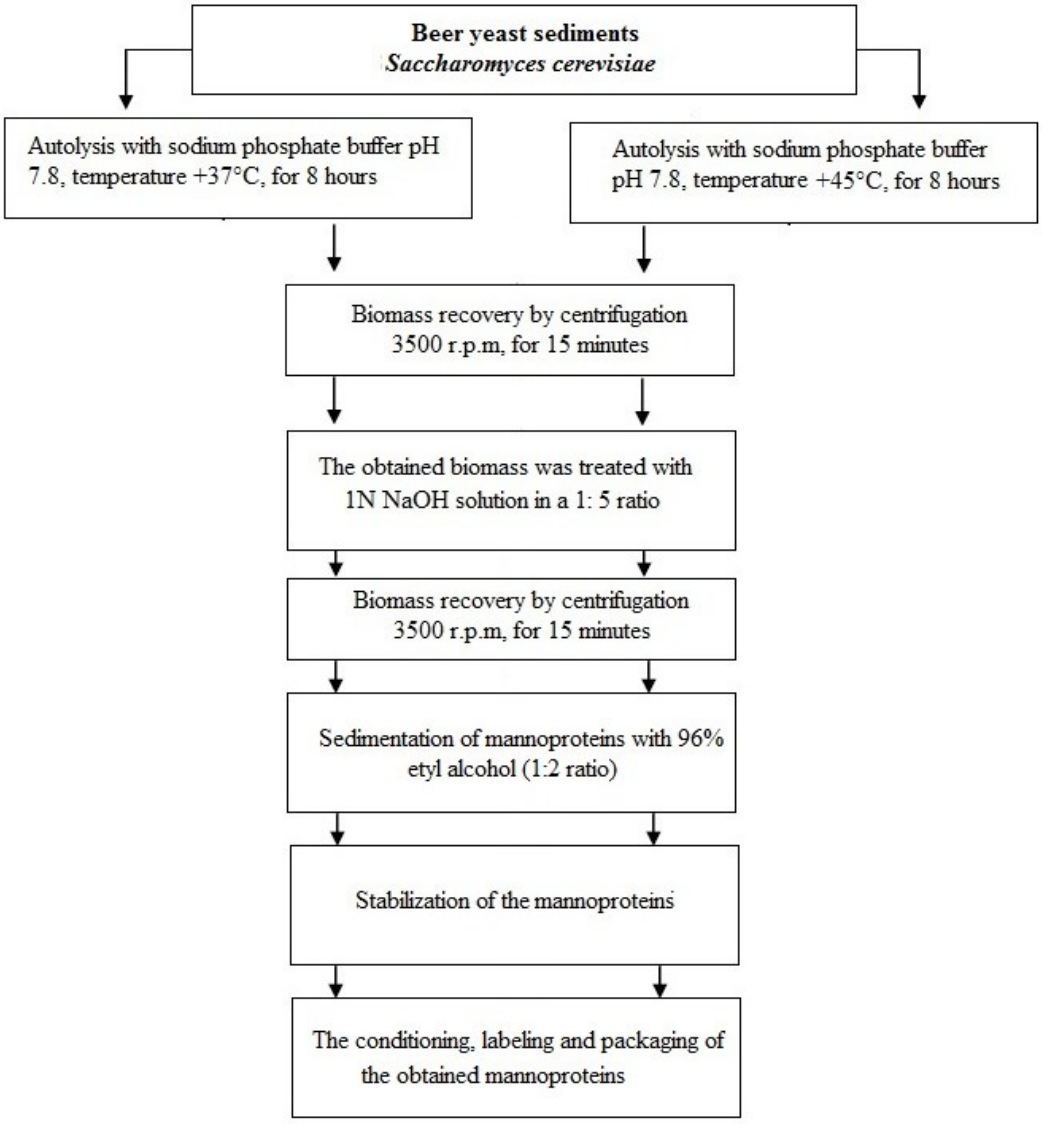

Figure 2. *The general scheme for obtaining mannoproteins from beer yeast sediments*

Following the application of the described procedures, it was established that the mannoprotein content constitutes 41.34 % dry biomass and 44.85 % dry biomass respectively, from the cell walls of yeasts, the results are shown in Table 2. The new extraction processes allow to obtain with 7 - 16 % more mannoproteins compared to the closest solution, they are also more advantageous because they use waste that pollutes the environment, reduce the autolysis time, temperature and amount of used ethyl alcohol.

Similar data in this regard have been reported by other researchers, they have mentioned that mannoproteins constituted average 35 - 40 % dry biomass from cell walls [5].

Table 2. *Content of mannoproteins obtained from yeast waste sediments from beer production*

Experimental variant	Mannoproteins %, dry biomass	%, control
Control	38.5	100
Sodium phosphate buffer, temperature +45 °C	41.34±0.58	107
Sodium phosphate buffer, temperature +37 °C	44.85±0.67	116

Subsequently, in order to establish the quality of the obtained mannoproteins, the biochemical composition the content of proteins and carbohydrates were studied. According to the obtained results, it was determined that the extracted mannoproteins according to the described procedures contain 54 - 63 mg·mL^{-1} dry matter, of which the values of the protein content vary from 64.5 ± 0.05 % at 67.7 ± 0.31 % dry biomass and the carbohydrate content is 17.2 ± 0.70 - 21.8 ± 0.57 % dry biomass.

The obtained results are also confirmed by the data from the specialized literature in which the protein content in mannoprotein extracts varies from 5 - 60.13 %, and that of carbohydrates is up to 25.9 ± 0.1 % dry biomass [3, 18].

Next, the enzymatic activity of catalase and superoxide dismutase in the obtained mannoproteins was evaluated. As a result, it was established that the catalase activity in the experimental samples is 46.22 ± 2.67 - 51.74 ± 4.0 µkat·L^{-1}, and the superoxide dismutase activity is 63.06 ± 0.0 - 80.96 ± 1.43 U·mg^{-1} protein (Figure 3).

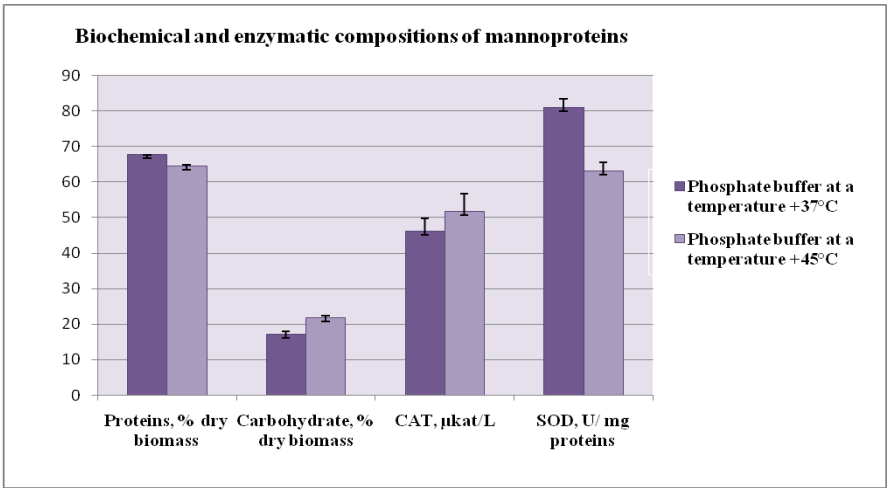

Figure 3. *Biochemical and enzymatic compositions of mannoproteins obtained from beer yeast waste sediments*

Finally, we can conclude that presented results confirm the efficiency of using the processes of extraction of mannoproteins from the sediments of yeast waste from beer production.

CONCLUSIONS

Generalizing obtained results in this study it can be mentioned that two new ecological and less expensive processes for obtaining mannoproteins from yeast waste sediments from beer production have been developed.
Processes are with the application of autolysis with sodium phosphate buffer at temperatures of +37 °C or +45 °C for 8 hours and reduction of the amount of alcohol, increase by 7 - 16 % the extracted mannoproteins, compared to the control sample.
The obtained mannoproteins contain 17.2 ± 0.70 - 21.8 ± 0.57 % dry biomass carbohydrates and 64.5 ± 0.05 - 67.7 ± 0.31 % protein and high catalase and superoxide dismutase activities. The presented results provide conclusive evidence that the elaborated processes have a high potential for use in the zootechnics, medicine, food, cosmetology and agriculture sectors.

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