

EXTRACTION PROCESSES AND BIOLOGICALLY ACTIVE PREPARATIONS OBTAINED FROM BREWER'S YEAST SEDIMENTS FOR USE IN THE ANIMAL HUSBANDRY

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ABSTRACT:

The purpose of this research was to develop extraction processes and obtain biologically active preparations based on the sediments of brewer's yeast for application in the livestock sector. Following the fractional extraction, 2 ecological preparations with valuable biochemical composition were obtained. The amino acid-protein preparation LB-AAP is characterized by a high content of proteins rich in essential amino acids and the mannoprotein preparation-LB-MP which contains balanced amounts of protein, carbohydrates and has a high activity of antioxidant enzymes. It was found that supplementing the basic fodder of the sows with the mannoprotein preparation contributes to the regulation of the reproductive function and the efficient preservation of the valuable pig genofond. The extraction processes and the biochemical composition of the preparations highlight the high biological activity and the enormous potential for use in animal husbandry.

KEYWORDS:

yeasts waste, biologically active preparations, proteins, carbohydrates, enzymatic activity

1. Introduction

Of particular importance today is research into the use of by-products of the alcoholic beverage industry, especially yeast sediment, obtained in huge quantities as a result of the industrial manufacturing process. Brewer's yeast sediments form on average after five fermentative production processes after which they are no longer useful and must be removed. It is estimated that 15 to 18 tons of surplus yeast are produced per 10,000 hL of finished beer (Kunze, 1999).

But the yeast biomass (*Saccharomyces*) has a varied and valuable biochemical composition, which has enormous potential and can be successfully exploited even after the completion of the process of fermentation and production of beer. According to studies in the literature, the residual yeast biomass contains from 30 to 70 % DM of protein, carbohydrates, it is also a rich source of B vitamins and fiber that could be applicable in both food and non-food products (Podpora et al., 2016). This necessitates the search for alternatives to use this by-product to mitigate the impact on the environment and, consequently, to reduce the costs of waste treatment in the industry.

Due to the specific properties and rich content of biologically active substances, a current field is the use of yeast waste after the completion of the fermentation process as a raw material in biotechnology for the production of natural preparations with high biological activity. Of particular interest is the obtaining of protein extracts from the remaining yeast biomass, rich in essential amino acids and enzymes (Kwiatkowski et al., 2012). Compounds such as mannoproteins, which are a major structural element of the yeast cell wall, and can also be obtained from the process of autolysis in yeast biomass are of interest due to their specific biochemical and enzymatic composition because they have a balanced content of proteins, carbohydrates, and antioxidant enzymes (Araújo et al., 2014; Kwiatkowski et al., 2012; Bzducha-Wróbel et al., 2013).

The results of the studies obtained so far have found that protein and mannoprotein preparations obtained from the yeast cell wall have a high potential for application in the livestock sector. Due to their biochemical composition they can be applied for nutrition, stimulation of reproduction, productivity, protection of animal health and also

contribute to the reduction of costs per unit of production (Ząbek et al., 2014; Sokolenko et al., 2015).

Thus, the purpose of this research was to develop extraction processes and obtain biologically active preparations based on brewer's yeast sediments for application in the animal husbandry.

2. Material and methods

The object of study was the yeast biomass (*Saccharomyces cerevisiae*) from the production of the beer *Lager*, that was kindly provided by the Kellers brewery (Budești).

The determination of the dry weight (d. w.) was performed gravimetrically according to the usual method – by drying the sample in oven at +105 °C till constant mass with further calculation of the dry weight (Egorov et al., 1995).

The protein was determined spectrophotometrically according to the Lowry method (Lowry et al., 1951), with crystalline albumin from bovine serum as the standard.

The content of amino acids in the preparations was determined by the chromatographic method (Garaeva et al., 2009) with the help of the Institute of Physiology and Sanocreatology of Moldova.

The total carbohydrate content was determined spectrophotometrically with the use of antron reagent and D-glucose as standard (Dey et al., 1993). The absorption was recorded on the PG T60 VIS Spectrophotometer at 620 nm.

Catalase activity (CAT) was determined by the spectrophotometric method, which is based on the ability of hydrogen peroxide to interact with molybdenum salts to form a stable colored complex (Komina et al., 2012).

Superoxide dismutase (SOD) activity was determined spectrophotometrically. The method is based on inhibiting the reduction of tetrazolium-nitroblue salt in the presence of TEMED and riboflavin (Nekrasova et al., 2008).

Statistical processing of the results was performed using the MO Excel and Statistics 9.0 software suite. The results were expressed by calculating the mean, standard deviation and confidence interval for an average of three repetitions. All differences were considered statistically significant for $P \leq 0.05$.

3. Results and discussions

Methods for obtaining biologically active preparations from brewer's yeast sediments and establishing the biochemical composition play a very important role for practical application. Thus, in the experiments presented in this study, the procedures for obtaining biologically active preparations by fractional extraction were initially evaluated (Figure 1).

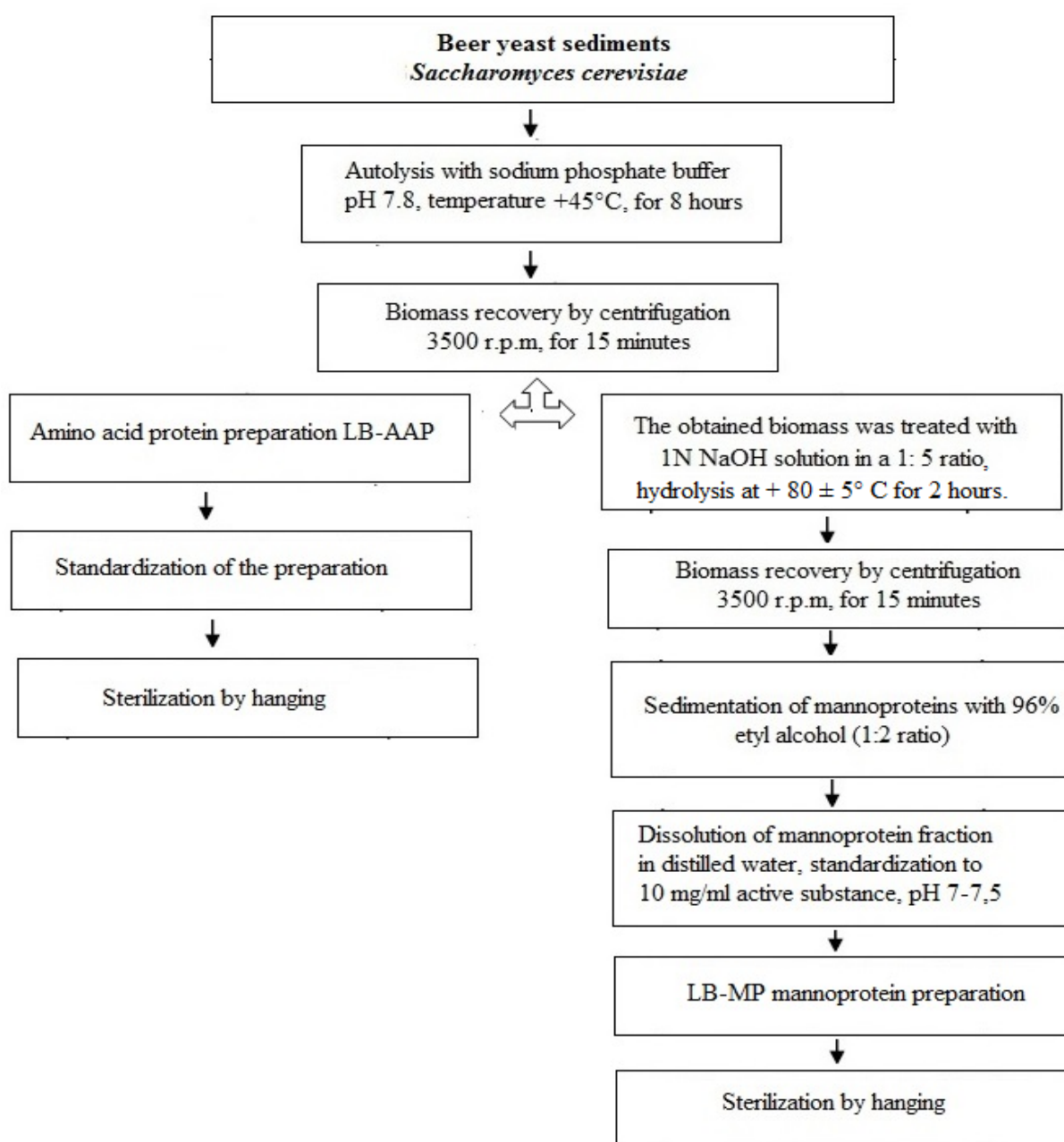


Fig. 1. The general scheme for obtaining biologically active preparations from beer yeast sediments

The procedure for obtaining the preparations is as follows:

Step 1. Initially, the beer sediments that were brought from the factory were centrifuged to remove the remaining liquid and frozen at $-18\text{ }^{\circ}\text{C}$ for storage.

Step 2. Subsequently, the biomass of thawed brewer's yeast is mixed with sodium phosphate buffer, pH-7.8 (1: 1 ratio). The obtained suspension is subjected to autolysis at $+45\text{ }^{\circ}\text{C}$ for 8 hours, with periodic stirring to destroy the cell wall. At the end of the autolysis process, the suspensions were centrifuged at 3500 rpm. for 15 minutes.

Step 3. The amino acid-protein preparation-LB-AAP is obtained from the liquid fraction. This is followed by standardization of the preparation and sterilization by typing in order not to compromise the biochemical parameters and inactivation of the concomitant microflora.

Step 4. To obtain the mannoprotein preparation, the cell walls after centrifugation were treated with 1N NaOH solution (1:5 ratio) and subjected to hydrolysis at a temperature of $+80\pm 5\text{ }^{\circ}\text{C}$ for 2 hours, centrifugation at 3500 rpm. for 15 minutes.

Step 5. The obtained alkaline supernatants are mixed with 96 % ethyl alcohol in a volume of 1: 2 to sediment the mannoprotein in the form of white-beige flakes with a viscous consistency. The separation of the liquid and solid phases are followed by centrifugation for 5 minutes at 2000 rpm. Repeated washing of the sediment with 96 % ethyl alcohol, separation of the phases by centrifugation and, obtaining mannoproteins.

Step 6. Subsequently, the mannoproteins are dissolved in distilled water, the LB-MP mannoprotein preparation is obtained, the standardization up to the concentration of 10 mg/ml active substance (preparation of the required concentration can be obtained), the pH is adjusted to neutral values 7.0-7.5 and hanging sterilization.

The obtained results showed that the lysis of the rigid cell wall of the yeasts with sodium phosphate buffer solution at a temperature of $+45\text{ }^{\circ}\text{C}$, for 8 hours increases the effectiveness and as a result 2 biologically active preparations with valuable biochemical composition are obtained from the same biomass. Also, the extraction process has been adapted to lower temperatures to avoid protein denaturation and preserve the antioxidant and enzymatic activity of the preparations,

compared to other processes in which heat treatment is used (Yu et al., 2012). Both the proposed process is more advantageous because it uses industrial yeast waste from beer production that does not require cultivation and the application of many stimulating factors with high cost, compared to the processes for obtaining preparations in which strains are used for production (Usatii et al., 2016). At the same time, the process reduces the autolysis temperature, the time, and the amount of ethyl alcohol used for the sedimentation of mannoproteins being more efficient from an economic point of view.

The evaluation of the quality and nutritional value of biologically active preparations obtained from yeast biomass largely depends on their biochemical composition, especially the total content of proteins and carbohydrates (Costa et al., 2012; Li et al., 2018, 2019).

Thus, as a result of the research it was established that the amino acid-protein preparation LB-AAP is characterized by high protein content of 64.6 ± 2.6 % d.w. and essential amino acids 55.7 g/100 g protein, also contains minimal amounts of carbohydrates 11.7 ± 2.2 % and lipids 0.13 ± 0.02 % (Figure 2).

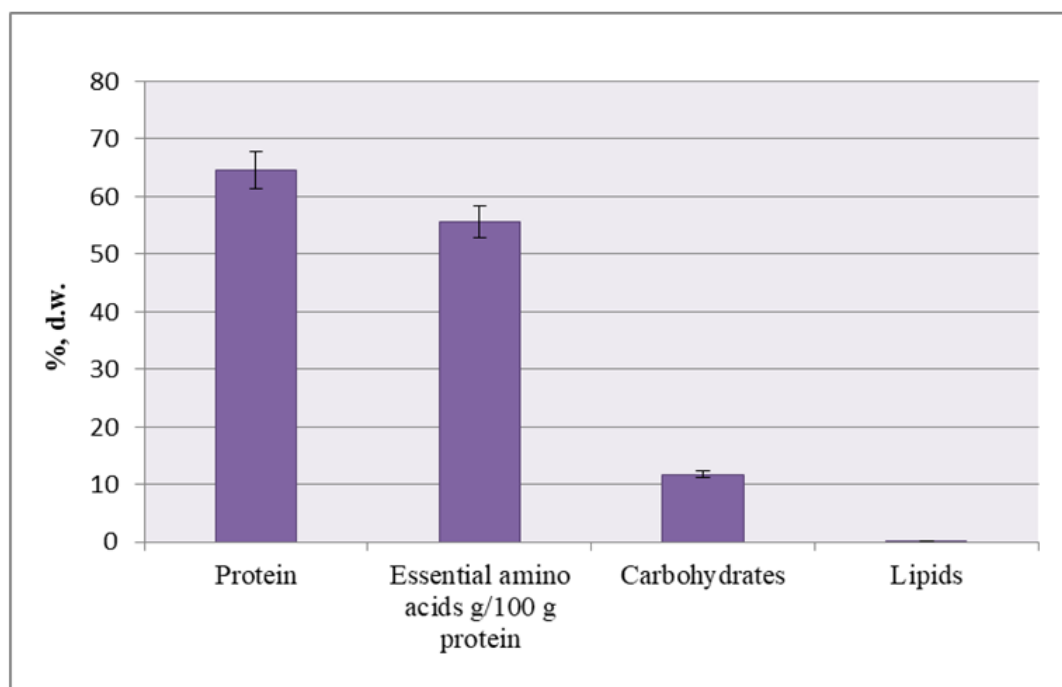


Fig. 2. Biochemical composition of amino acid protein preparation LB-AAP obtained from beer yeast waste sediments

The data obtained on the biochemical composition of the amino acid-protein preparation LB-AAP are comparable to other studies in the literature. According to the data presented by Pinto et al., 2013 residual yeast autolysates have a protein content of 35-60 % (Pinto et al., 2013). Podpora et al. obtained from brewer's yeast an autolysate containing – 62.5-63.8 % d.w. protein and 2.9 % d.w. carbohydrates (Podpora et al., 2016), and the extracts obtained Vieira et al. recorded content of 64.1 ± 0.2 % d.w. protein and 12.9 ± 0.1 % d.w. carbohydrates (Vieira et al., 2016).

Next, the biochemical composition of the mannoprotein preparation was evaluated – LB-MP was established that the preparation contains balanced amounts of protein 36.6 ± 0.58 % d.w. and carbohydrates 40.9 ± 3.04 % d.w. Also due to the specific biochemical composition is characterized by the activity of the antioxidant enzymes catalase and superoxide dismutase, the results are shown in Table 1.

Table 1.
Biochemical and enzymatic compositions of LB-MP preparation obtained from beer yeast waste sediments

| <i>Parameter</i> | <i>Value</i> |
|------------------|---|
| Proteins | $36,6 \pm 0,58$ % d.w. |
| Carbohydrates | $40,9 \pm 3,04$ % d.w. |
| CAT activity | $741,2 \pm 44,8$ mmol/min. per mg protein |
| SOD activity | $66,2 \pm 2,9$ U/mg protein |

Comparable results were also obtained by Costa et al., the mannoprotein extract obtained by heat treatment of biomass at a temperature of $+85$ °C for 7 hours and constant stirring was characterized by a content of 25.9 % carbohydrates and 51,4 % protein (Costa et al., 2012). Meanwhile, the mannoprotein preparation-LB-MP due to the reduction of temperature in the process of obtaining in addition to the content of proteins and carbohydrates, also possesses the activity of the antioxidant enzymes SOD and CAT.

The results obtained indicate that the biochemical composition of mannoprotein preparations obtained from yeast biomass of *Saccharomyces* varies according to its origin, the species used, and the method of obtaining the preparation.

Subsequently, the mannoprotein preparation – LB-MP was also tested in the procedure for regulating the reproductive function of sows. It was established that feeding sows with basic feed, supplemented with biologically active mannoprotein preparation in the concentration of 1 g / 100 ml contributes to the increase of the number of live piglets born at calving by 1.6 heads, the decrease of the number of dead piglets born by 1.8 heads, increase the live weight of the nest by 1.19 kg and at 21 days by 0.85 kg.

The obtained results ensure the rational use of the domestic and imported genetic fund of pigs and the obtaining of an increased number of viable offspring with high genetic potential, the increase of meat production per sow / year, the increase of the daily average of piglets during their fattening period.

The results are consistent with other studies in which yeast biomass preparations, rich in carbohydrate and protein compounds, improve and significantly increase productivity and fertility rates in different groups of animals, improve the health of growing pigs, and can increase disease resistance (Li et al., 2014).

4. Conclusions

The newly developed process for obtaining biologically active preparations from brewer's yeast sediments is more economically and environmentally efficient, as it contributes to reducing energy consumption, additional costs for the management of industrial waste from beer production and has promising prospects for application in the livestock sector. The amino acid-protein preparation LB-AAP is characterized by a high content of proteins, essential amino acids and contains minimal amounts of carbohydrates and lipids. While the mannoprotein preparation-LB-MP contains balanced amounts of protein, carbohydrates and is characterized by high activity of the antioxidant enzymes catalase and superoxide dismutase. The high biological activity of the mannoprotein preparation-LB-MP

contributes to the regulation of the reproductive function of sows, obtaining an increased number of viable offspring with high genetic potential and increasing meat production.

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