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BIOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF DIFFERENT PREPARATIONS FROM MICROBIAL WASTE OF THE BEER INDUSTRY

– Short communication –

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Abstract: The purpose of this research was to evaluate the biochemical composition and antioxidant activity of different natural preparations obtained from the yeast biomass of beer industry waste. It was found that the preparations had a valuable biochemical composition, high antioxidant activities, a broad spectrum of immunoactive and essential amino acids, and that their protein and carbohydrate contents varied depending on the extraction stage and solvent. In conclusion, the varied biochemical composition, significant antioxidant and enzymatic activities, the innocuousness of *Saccharomyces* yeasts for living organisms, suggest that the obtained preparations can be an excellent source of biologically active substances and that their high biological activity presents a considerable potential for animal husbandry, food industry and cosmetics.

Keywords: yeasts, waste, biologically active preparations, proteins, carbohydrates, antioxidant and enzymatic activity.

INTRODUCTION

At present much attention is paid to the possibility of utilization of the industrial by-products or residual materials from the food industry, obtained in large quantities (Mironescu, 2011). One example is those of residues resulted from the brewing and wine-making processes. The importance of this direction is conditioned by the need to solve the problem of yeast sediment accumulation, and by the possibility of obtaining natural preparations with high biological value for economy.

The correct and efficient use of the microbial waste from the alcoholic beverage industry would prevent negative impacts on the environment, reduce the costs of waste management, and offer natural and harmless biologically active products for humans, animals and birds. To obtain such preparations from the brewery yeast it is important first to determine the biochemical composition of the biomass. Yeasts are known as a valuable source of proteins, lipids, which can be used in the food and cosmetic industry, animal husbandry and in other fields vitamins, trace elements, antioxidants, etc., (Pinho et al., 2010; Shahat et al., 2017).

Biologically active substances in the microbial biomass can possess antiviral and antibacterial properties, immunomodulatory and immuno-stimulatory properties, antioxidant activity, as well as mycotoxin and heavy metal biosorption capacity (Fakruddin et al., 2017).

Current studies were published regarding the use of mannoprotein and β-glucan preparations from *Saccharomyces* yeasts in food products. It was shown that they could be used as stabilizers and thickeners in food emulsions like mayonnaise, lettuce sauce and skimmed milk yogurt (De Iseppi et al., 2019; Chirsanova et al., 2021). It was also demonstrated, that they could be used for stimulating animal reproduction and productivity, for cost reduction in obtaining eco-friendly products of animal origin that are safe for human health (Ząbek et al., 2014; Sokolenko et al., 2015).

In this context, our previous research was focused on evaluating the possibility of utilizing the beer

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yeast from the waste of the Kellers brewery in the village of Budești, Chisinau. We determined the biochemical composition of the yeast biomass. It was found that the yeast contained significant amounts of proteins, including essential and immunoactives amino acids, carbohydrates, especially proteomannans and β-glucans, lipids, phospholipids, sterols, waxes, various trace elements, and had a high antioxidant activity. The results demonstrated that the yeast was an excellent source of biologically active substances and could be used for obtaining biologically active preparations (Beșliu et al., 2020; Chiseliţa et al., 2020; Tofan et al., 2021).

Thus, the purpose of this research was to evaluate the biochemical composition and antioxidant activity of the different preparations, obtained from the yeast biomass of the beer industry waste.

MATERIALS 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 (Budeşti).

Initially, the semi-liquid biomass from the brewery was centrifuged to remove the remaining liquid, and the sediment was frozen at -18°C for storage. Since according to some experts the residual ethanol in the yeast cream does not affect the extraction and determination of the cellular constituents (Costa et al., 2012), the biomass was not washed with water. Before obtaining the preparations, the yeast biomass was thawed at room temperature. Fractional extraction from the beer yeast biomass based on preparation of water, alkaline and acid solutions, filtration, sedimentation, purification and drying was used to obtain three preparations: amino acid protein-LB-AAP, mannoprotein-LB-MP and β-glucanic-LB-GL.

The LB-AAP preparation was obtained based on the yeast biomass autolysis procedure, using sodium phosphate buffer at +45°C for 8 hours and separating the liquid phase from the solid one as described in Beșliu et al., 2020.

The solid residue remaining after obtaining LB-AAP was used to obtain the LB-MP preparation - by treatment with 1N NaOH solution (1:5 ratio) and hydrolysis at +80±5°C for 2 hours. After hydrolysis the mixture was centrifuged at 3500 rpm for 15 minutes to separate the liquid phase from the solid phase. From the obtained supernatant the mannoproteins (in the form of white-beige flakes with viscous consistency) were sedimented by 96% ethyl alcohol added in the ratio of 1:2. After sedimentation the mannoproteins were purified by ethyl alcohol (Beșliu et al, 2021).

The solid residue remaining from the LB-MP preparation was used to obtain the LB-GL preparation - by removing the lipids by a mixture of ethanol, chloroform and 10% acetic acid (5:1:1) at +35-40°C for 20 min., repeated extraction with 45 ml of chloroform under the same temperature and duration conditions, and treating with 0,5N acetic acid (1:5 ratio) at +75±5°C for 1 hour. After the acid hydrolysis, the suspension was centrifuged at 3500 rpm for 15 minutes to separate the phases. The supernatant was removed and the sediment, which constituted the β-glucan fraction insoluble in alkalis and acids, was washed 3 times by distilled water and dried at +50±5°C to constant mass.

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 futher calculation of the dry weight (Egorov, 1995).

The total carbohydrate content was determined spectrophotometrically by using the antron reagent and D-glucose as the standards (Dey et al., 1993). The absorption was measured by the PG T60 VIS Spectrophotometer at 620 nm.

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.

Total antioxidant activity was determined spectrophotometrically using the cation radical 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Re et al., 1999).

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

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

Statistical analysis was done using the MO Excel and Statistics 9.0 software. The results were expressed as the mean, standard deviation and confidence interval at $P \leq 0.05$ from three repetitions.

RESULTS AND DISCUSSION

It was found that the obtained preparations contained different amounts of protein and carbohydrates. Thus, LB-AAP contained 64.6±2.6% (d. w.) proteins and 11.7±2.2% (d. w.) carbohydrates, indicating a high nutritional value of the preparation, comparable to those reported in the literature (Figure 1). For example, Vieira et al. obtained (from brewery yeast) an autolysate containing 64.1±0.2% (d. w.) protein and 12.9±0.1% (d. w.) carbohydrates (Vieira et al., 2016), and the extracts obtained by Podpora et al. - 62.5-63.8% (d. w.) protein and 2.9% (d. w.) carbohydrates (Podpora et al., 2016). The authors suggested their preparations for production of new functional and nutraceutical foods.

The LB-MP mannoprotein preparation contained 36.6±0.58% (d. w.) protein and 40.9± 3.04% (d. w.) carbohydrates (Figure 1). In this regard, several researchers reported that the biochemical composition of the mannoprotein extracts from *Saccharomyces* biomass varied depending on their origin, the species used and the extraction method. Thus, the mannoprotein extract obtained by Araújo et al. from *S. uvarum* brewery yeast (by extraction at +121°C for 4 hours in an autoclave) contained 39% (d. w.) carbohydrates and 58% (d. w.) protein (Araújo et al., 2014), and the one obtained by Costa et al. (by heat treatment of the biomass at +85°C for 7 hours with constant stirring) contained 25.9% carbohydrates and 51.4% protein (Costa et al., 2020). According to Barriga et al., mannoproteins containing 30 to 50% protein correspond to the class of mannoproteins with enzymatic functions, consisting of short, non-phosphorylated oligomannose chains attached to proteins, especially by threonine or serine residues by type O linkages (Barriga et al., 1999). This fits the rather high content of these two amino acids in the LB-MP preparation comparing to the other essential and immunoactive amino acids (Table 1).

The LB-GL preparation was of a polysaccharide nature, consisting mainly of β-glucans insoluble in alkalis and acids. It contained minimum amounts of protein 8.9±0.6% (d. w.) and a lot of carbohydrates – 75.2±0.8% (d. w.) (Figure 1). β-glucans are among the basic polysaccharides of the yeast cell wall, and they are known for the immunostimulatory, immunomodulatory, anticancer and antioxidant properties. They are widely used in various fields.

The biological activity of the β-glucan preparations and their use largely depend on the extraction and purification methods (Avramia et al., 2021; Liepins et al., 2015). The results obtained in this research were comparable to those in the literature. Thus, Thammakiti et al. obtained from the biomass of the brewery yeast (by extraction with alkalis and acid) an extract containing 65.2% (d. w.) carbohydrates and 6.5% (d. w.) protein (Thammakiti et al., 2004). Using the same method in combination with repeated purification of the β-glucanic preparation from mannoproteins and autoclaving in citrate buffer solution, the carbohydrate content was increased up to 93.0% (d. w.), and the protein content was reduced to 3.9% (d. w.) (Petravic-Tominac et al., 2011).

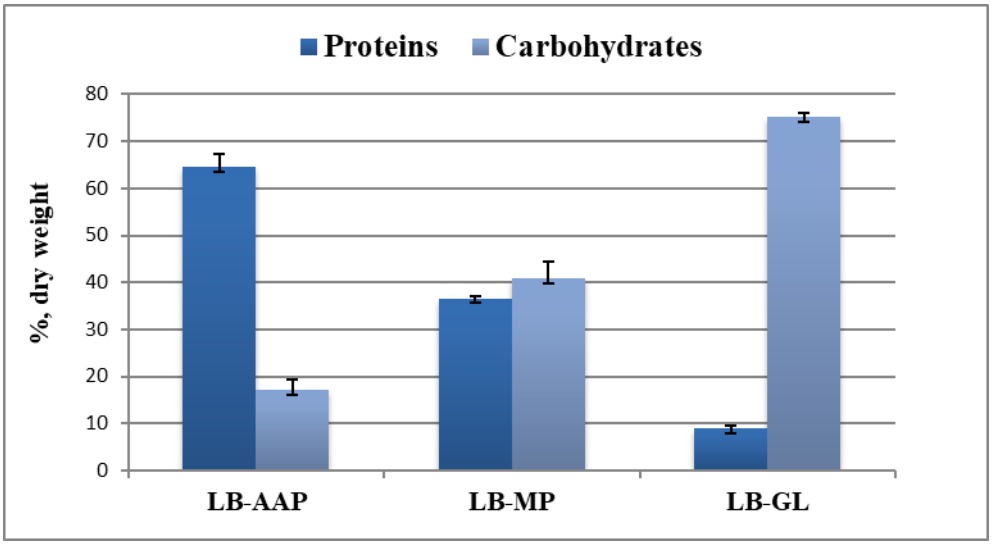


Figure 1. The protein and carbohydrate contents of the yeast biomass preparations

Next, the spectrum and the quantitative content of the amino acids in the obtained preparations were determined. As a result, it was determined that the preparations, although in different quantities, contained the full spectrum of essential and non-essential amino acids (Table 1). Thus, the amino acid protein preparation LB-AAP contained significant amounts of non-essential amino acids - 469.8 mg/g and of the essential - 360.0 mg/g, and the sum of immunoactive amino acids was 526.4 mg/g, which indicated its high nutritional value. Yeast proteins are characterized by low content of methionine and cysteine (INRA., 2004; Vieira et al., 2016), and this is exactly what was found in our case (table 1) - the content of these two amino acids was 3.2 and 3.0 mg/g respectively and that was significantly less comparing to the other amino acids.

A rather high content of phenylalanine, threonine and valine was measured in the LB-AAP preparation, which was not characteristic for the yeast proteins, but rather for the vegetable proteins, especially the barley ones (Barczak et al., 2008). Also, in the LB-AAP preparation relatively higher amounts of glutamic acid, proline and arginine were observed comparing to what is usually observable for the pure yeast biomass (Yamada et al., 2005). Also, the lysine and isoleucine contents were higher than the ones reported in the standards (FAO/WHO., 1991). The relatively high content of amino acids in the preparation could be due to the fact that it was obtained not from the pure yeasts, but from the must suspension, remaining after brewing, which was rich in protein, peptides and free amino acids, derived from barley malt, hops and others additives used in the beer production.

In the other two preparations, LB-MP and LB-GL, the qualitative spectrum of the detected amino acids was practically identical, although the quantitatities differed. Thus, if the LB-MP preparation contained, respectively, 23.6 and 33.8 mg/g (d. w.) of essential and non-essential amino acids, the LB-GL had, respectively, only 7.8 and 12.6 mg/g (d. w.). *S. cerevisiae* yeasts are known as an important source of antioxidant enzyme systems that include superoxide dismutase and catalase (Lavová et al., 2013).

Table 1. The amino acid content of the preparations obtained from the beer yeast biomass, mg/g (d. w.)

Amino acids	Preparations		
	LB-AAP	LB-MP	LB-GL
Cysteine acid	1.63	0.37	-
Aspartic acid**	49.1	4.77	1.93
Threonine*	50	5.47	0.42
Serin**	40.6	2.23	0.22
Glutamic acid**	161.9	13.19	4.03
Proline **	74.6	6.68	3.17
Glycine **	70.6	3.68	1.86
Alanine**	65.2	2.84	1.12
Valine*	40.8	3.32	1.38
Cysteine**	3.0	0.11	0.08
Methionine*	3.2	0.11	0.09
Izoleucine*	40.3	1.88	0.73
Leucine*	91.8	4.07	2.16
Tyrosine**	4.8	0.30	0.23
Phenylalanine*	41.1	2.62	0.93
Lysine *	49.1	3.82	1.11
Histidine*	7.9	0.78	0.22
Arginine*	35.8	1.57	0.79
G-aminobutyric acid	9.4	0.52	0.04
Ornithine	10.1	1.99	-
Σ non-essential amino acids	469.8	33.8	12.64
Σ essential amino acids	360.0	23.6	7.83
Σ immunoactive amino acids	526.4	37.7	11.87
Σ glycogenic amino acids	602.7	44.8	15.31
Σ ketogenic amino acids	227.1	12.7	5.16
Σ amino acids proteinogenic	829.8	57.4	20.5
Σ amino acids with S	7.8	0.6	0.17

* - essential amino acids; ** - non-essential amino acids

Since the biological activity and practical usefulness of the microbial preparations largely depend on their antioxidant properties, at the next stage of the research the obtained preparations were tested for their antioxidant activity, and for the enzymes catalase (CAT) and superoxide dismutase (SOD).

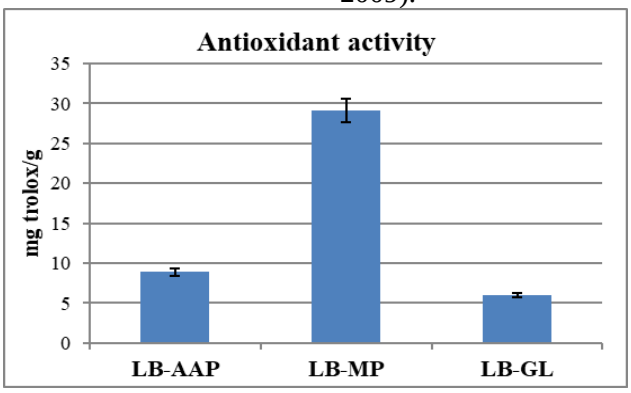
It was found that the amino acid protein and mannoprotein preparations had a high CAT enzyme activity of 595.5±9.1 and 741.2±44.8 mmol/min. per mg protein, SOD activity - 129.5±18.5 and 66.2±2.9 U/mg protein, and the antioxidant activity - 8.9±0.9 and 29.1±1.5 mg trolox/g (d. w.) respectively. The β-glucanic preparation had the antioxidant activity of 6.0±0.2 mg trolox/g (d. w.) and a lower catalase activity - 490.9±0.53 mmol/min. per mg protein, which were, nevertheless, comparable to the other preparations. Their SOD activity was significantly lower - only 12.96±0.04 U/mg protein (fig. 2).

The relatively low antioxidant activity of the β-glucans in the yeast cell wall fraction (comparing to the protein fraction) was observed by other authors too. Their conclusion was that proteins plaid the decisive role in the antioxidant activity of the cell wall components due to their lateral aromatic chains and thiol groups (Jaehrig et al., 2007; Jaehrig et al., 2008).

Comparing to the results reported in the literature, the obtained preparations had a higher antioxidant activity and more of the CAT and SOD antioxidant enzymes. Thus, the CAT and SOD activities of the extracts obtained by others from the yeast biomass grown on various nutrient media were clearly lower than in this research. For example, the amino acid extracts obtained by Lavova B. and Urminska D. from the biomass of *S. cerevisiae*, grown on YPD medium had a maximum SOD activity of 23.7 U/mg protein (Lavová et al., 2014), and the extracts obtained by Kujumdzieva et al. had the CAT activity ranging from 6.97 to 10.90 DE/min/mg protein, depending on the strain from which they were obtained (Kujumdzieva et al., 2002).

The significant antioxidant activity of the yeast biomass remaining after the beer fermentation can be explained by high antioxidant activity of the polyphenolic compounds contained in the hops used in beer production, and by their impact on the biotechnological and biochemical properties of the yeast (Klindukhov et al., 2009). During the alcoholic fermentation of the wort and the conditioning of the beer, the concentration of the polyphenolic compounds in the final product is reduced due to their adsorption by the yeast (Dima, 2009).

a.



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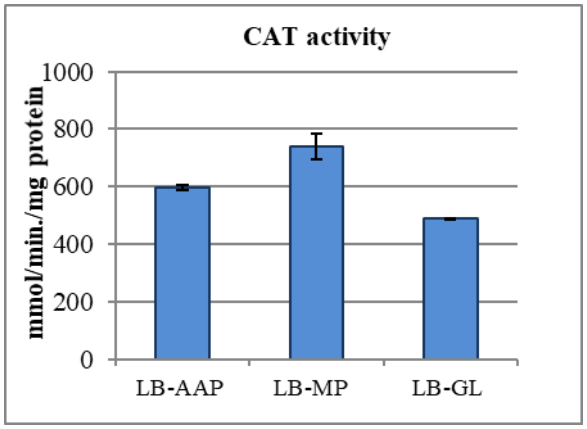

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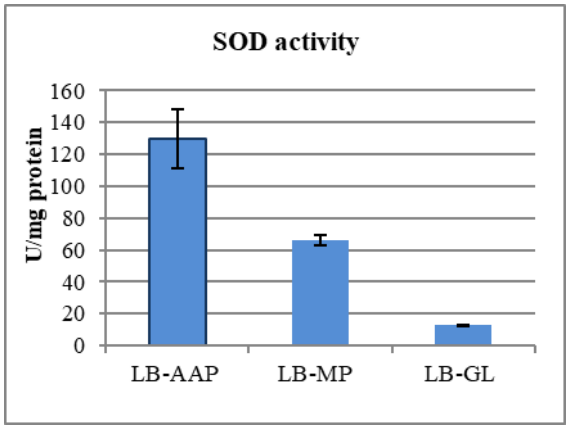

Figure 2. The antioxidant activity (a), CAT (b) and SOD (c) enzymes of the preparations obtained from brewery yeast biomass

CONCLUSIONS

The preparations obtained from the yeast biomass of beer industry waste had a valuable biochemical composition, were characterized by a high antioxidant activity and wide range of immunoactive and essential amino acids. Their protein and carbohydrate contents varied depending on the extraction stage and the used solvent.

The varied biochemical composition, the significant antioxidant and enzymatic activities of the products, and the innocuousness of *Saccharomyces* yeasts for living organisms demonstrated that the obtained preparations can be an excellent source of biologically active substances and that their high biological activity presents a considerable potential for animal husbandry, food industry and cosmetics.

Utilization of the by-products of the beer industry, and of the yeast biomass in particular, will contribute to effective waste management and will reduce the negative impact on the environment.

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Conflicts of Interest: The authors declare no conflict of interest.

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