THE NEW PROCEDURE FOR OBTAINING AND BIOCHEMICAL CHARACTERIZATION OF THE MANNOPROTEIN PREPARATION DERIVED FROM SEDIMENTS OF WINE YEAST

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ABSTRACT:

This study presents a procedure for obtaining a new mannoproteic preparation using yeast biomass extracted from waste generated by the wine industry. The preparation was analyzed and found to contain a high content amount of essential amino acids, proteogens, immunoactive and pigments in particular, anthocyanin. Additionally, has been established the preparation to exhibit high activity of the antioxidant enzymes superoxide dismutase and catalase, which is attributed to its unique composition.

Based on these findings, it can be concluded that the preparation has high biological value and significant potential for use in agriculture, particularly in animal husbandry, food and cosmetic industries.

KEYWORDS:

wine yeasts waste, mannoprotein preparation, amino acids, anthocyanins, enzymatic activity

1. Introduction

Currently, scientific studies are increasingly focused on finding ways to reuse industrial waste. Especially for biotechnology, yeast sediments from the wine industry are of interest. After fermentation, these sediments can be utilized in various ways, one of which is to extract mannoproteins from the outermost layer of the cell wall. Mannoproteins can constitute up to 50% of the dry mass of

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Saccharomyces cerevisiae (Klis et al., 2002; Guadalupe et al., 2007), but the amount that can be obtained depends on the yeast strain and the conditions of winemaking and production (Ribereau-Gayon et al., 2006; Rosi et.al., 2000). The structure of mannoproteins in the yeast of wines has been described in several studies essentially, they are composed of many small polysaccharide chains linked to proteins and peptides by covalent and non-covalent bonds (Waters et al., 1994; Guadalupe et al., 2010). Depending on the method of obtaining, mannoproteins can also register a high antioxidant activity that contributes to widening the fields of implementation (Križková et al., 2001; Drábiková et al., 2009).

The unique molecular and structural properties of mannoproteins make them attractive for a wide range of applications. For example, biopreparations containing mannoproteins can be developed for use in animal husbandry, providing nutritional benefits and protecting health (Hatoum et al., 2012; Pintilie et al., 2011). Animal feed costs account for 65-70% of production expenses, so reducing costs and providing adequate nutrition is crucial for increasing production (Suzzi et al., 1995). Mannoprotein preparations can also be used as a food additive in the food industry, improving the stability and texture of products such as dairy, bakery, sauces and meat products (De Iseppi et al., 2019).

Additionally, mannoproteins are increasingly used in the cosmetic industry as an ingredient in skin and hair care products, providing moisturizing and soothing properties, improving texture, and enhancing stability (Li et al., 2019; Reis et al., 2021).

Therefore, further research is needed to develop new technologies to reduce the impact of agro-industrial residues on the environment and to make new mannoprotein preparations that provide benefits and additional sources of income.

Taking the above into account, the aim of the research is to develop a new process for obtaining the mannoprotein preparation from the biomass of the waste yeasts of the wine industry.

2. Material and methods

The object of study was the yeast biomass (*Saccharomyces cerevisiae*) from the production of the *Merlot* wine that was kindly provided by the Cricova winery.

The procedure for obtaining the mannoprotein preparation

Initially, the sediments of *Merlot* red wine that were brought from the winery were centrifuged to remove the remaining liquid and frozen at -18°C for storage. The thawed wine yeast biomass was mixed with a 1:1 ratio of sodium phosphate buffer, pH 7.8, and subjected to autolysis at a temperature of +45°C for 8 hours with periodic stirring. After the autolysis process, the suspension was centrifuged at 3500 rpm for 15 minutes.

To obtain a higher amount of anthocyanins in the mannoprotein preparation after autolysis, the cell walls were treated with a 50% ethyl alcohol solution at a ratio of 1:3. The suspension was centrifuged at 3500 rpm for 15 minutes. After centrifugation, the cell walls were treated with a 1:5 ratio of 1N NaOH solution and hydrolyzed at 80±5°C for 2 hours. The alkaline suspension was separated by centrifugation at 3500 rpm for 15 minutes.

The obtained alkaline supernatants were sedimented with 96% ethyl alcohol in a volume of 1:2, forming beige-reddish flakes with a viscous consistency, which represented the mannoprotein fraction. The liquid and solid phases were separated by centrifugation at 2000 rpm for 5 minutes. The sediment was repeatedly washed with 96% ethyl alcohol, and the phases were separated by centrifugation.

The mannoprotein fraction was dissolved in distilled water and standardized to a concentration of 10 mg/ml active substance, pH was adjusted with acetic acid to 7.0-7.5. To avoid compromising the biochemical parameters, antioxidant and enzymatic activities of the preparation, and to ensure the inactivation of concomitant microflora, sterilization was performed by drying through three series of heating at +55°C followed by successive cooling.

Methods of achieving research. 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 et al., 1995). The content of amino acids in the preparation was determined by the chromatographic method described by Garaeva et al. (2009). The concentration of β-carotene in the sample was measured using 96% ethyl alcohol at room temperature. The sample was stirred for 30 minutes, and the resulting extract was separated by centrifugation.

The concentration of β-carotene in the extract was determined by measuring its absorbance at a wavelength of 450 nm. This method of measurement has been described in previous studies by Cepoi (2014).

Catalase (CAT) activity 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

An important parameter for evaluating the quality and efficiency of mannoprotein preparations is the determination of their amino acid content. The results presented in Figure no. 1 demonstrate that the mannoprotein preparation contains 13.70 mg/100 ml of essential amino acids, 13.87 mg/100 ml of immunoactive amino acids, and 28.58 mg/100 ml of proteinogenic amino acids. The quantitative analysis of the amino acid groups shows that the proteogenic ones predominate. According to the data obtained, it was established that aspartic acid prevails 3.27 mg/100 mg, glycine 1.05 mg/100 mg and serine 1.0 mg/ 100 mg. From the range of essential amino acids, 7 were identified, plus 2 semi-essential amino acids histidine and arginine. From the group of essential amino acids in the mannoprotein preparation in major quantities are lysine 2.15 mg/100 ml, leucine 1.93 mg/100 ml, valine 1.18 mg/100 ml and threonine 1.14 mg/100 ml (Figure no. 2).

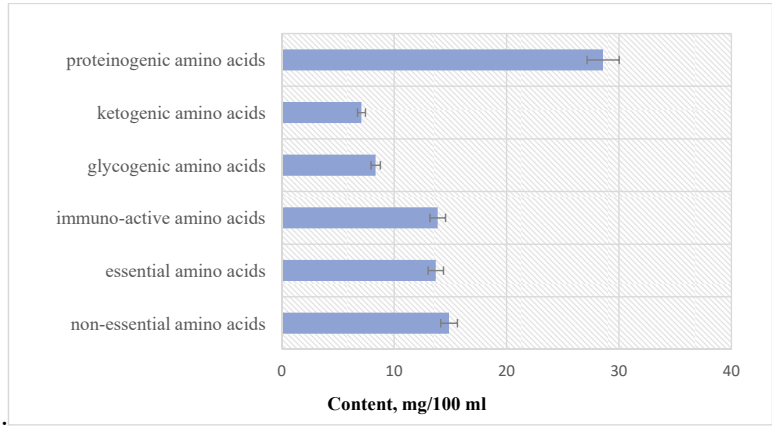

Figure no. 1. The amino acid content in the mannoprotein preparation obtained from wine yeast waste sediments

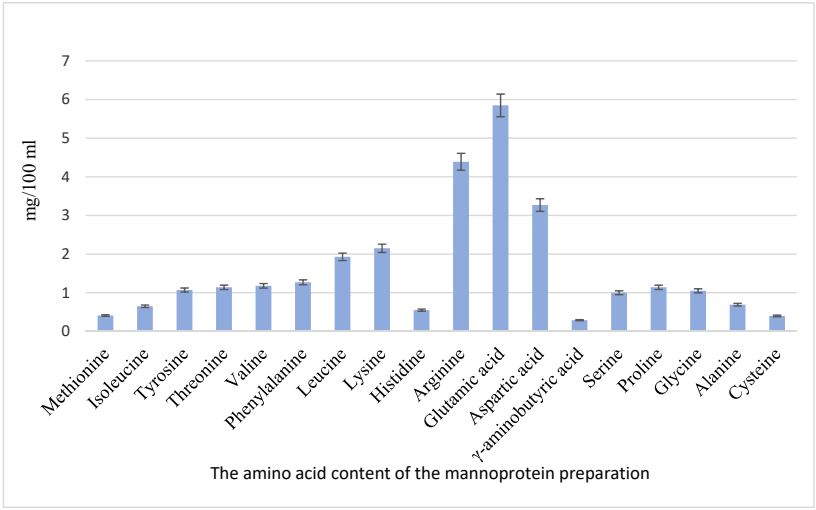

Figure no. 2. Quantitative and qualitative amino acid composition in the mannoprotein preparation

Regarding the composition of the amino acids present in the mannoproteins extracts, the results obtained are consistent with other studies in the specialized literature, which state that the mannoprotein fraction contains in its composition up to 17 types of amino acids,

with a spectrum similar to that established (Wang, 2005). Also from the results presented by Klis et al., the content of glutamic acid, aspartic acid and serine prevails in the mannoprotein extracts. And from the group of essential amino acids, a predominance of valine, leucine, threonine and tyrosine content was established (Silva et al., 2014; Klis et al., 2002). Studies have also reported varying amounts of amino acids in mannoprotein preparations obtained from different sources. A study by Smith et al. (2015) found that the mannoprotein preparation extracted from yeast cells contained 10.25 mg/100 ml of essential amino acids, 12.60 mg/100 ml of immunoactive amino acids and 25.40 mg/100 ml of proteinogenic amino acids. In another study, the mannoprotein preparation had 15.30 mg/100 ml of essential amino acids, 11.80 mg/100 ml of immunoactive amino acids, and 26.90 mg/100 ml of proteinogenic amino acids (Lee et al. 2018). Compared to these studies, the mannoprotein preparation obtained of wine sediments contains higher levels of essential and proteinogenic amino acids and similar levels of immunoactive amino acids.

At the next stage, in order to improve the antioxidant properties of the mannoprotein preparation, the supplementation was carried out with other compounds from the yeast waste composition, including anthocyanins and *β-carotene*. This step was achieved by processing the cell walls with 50% ethyl alcohol, placing in a shaker at 200 rpm. per minute. for 30 minutes at room temperature and centrifugation at 3500 revolutions per minute for 15 minutes.

In the result, it was established that the content of anthocyanins is 12.42±0.08 mg/g and the content of β-carotene 0.433±0.0003 mg/100g. In the experimental variants in which this step was not carried out, the anthocyanin content is significantly lower than 1.16 mg/g (Table no. 1). The results obtained are consistent with other studies obtained by other authors that revealed the ability of mannoproteins to absorb different phenolic compounds from wine (Guadalupe et al., 2007; Mazauric et al., 2006). Also, the presence of anthocyanins contributed to obtaining a higher enzyme activity.

Thus, at the next stage, the activity of the antioxidant enzymes catalase and superoxide dismutase was evaluated, which serve to protect cells from toxic effects and are indispensable in many biochemical processes, including intracellular signaling and cell

defense (Zamocky et al., 1999; Weydert et al., 2010). As a result, it was established that the activity of CAT enzymes is 525±3.1 mmol/min. per mg protein and SOD of 263±7.0 U/mg protein.

Table no. 1. Biochemical and enzymatic compositions of mannoprotein preparation obtained from wine yeast waste sediments

Parameter	*Value*
β-carotene, mg/100g	0.433±0.0003
Anthocyanins, mg/g	12.42±0.08
CAT activity, mmol/min. per mg protein	525±3.1
SOD activity, U/mg protein	263±7.0

The results indicate that the preparation has high enzymatic activity, which is attributed to its biochemical composition, specifically the content of amino acids and anthocyanins. In general, preparations with antioxidant activity of microbial origin are considered to have greater activity and stability than those of plant origin or chemically synthesized.

Based on the aforementioned, the proposed process in this study offers significant advantages from both an economic and environmental standpoint. It helps reduce energy consumption, eliminates the need for cultivation and the application of many high-cost stimulation factors, and reduces expenses associated with industrial waste management. Moreover, this process yields higher amounts of amino acids than other methods and is characterized by higher activity of SOD, which is a stable enzyme that is minimally influenced by external factors and remains consistent over time. Another argument supporting this process is that the traditional method of obtaining and extracting anthocyanins involves the use of biochemical components that can harm the environment and personnel, expensive equipment, and a longer extraction time. Conversely, the procedure for obtaining anthocyanins in the mannoprotein preparation was specifically tailored to the research object and implemented using the most effective and non-toxic method.

4. Conclusions

The new process for obtaining biologically active mannoproteins preparation from wine yeast sediments has demonstrated promising prospects for various applications in agriculture, food industry, and cosmetics due to its economic and ecological efficiency. The preparation contains a high content of essential, immunoactive, and proteogenic amino acids and also a balanced content of anthocyanins and *β-carotene* which has its properties. Additionally, the preparation has high activity of antioxidant enzymes catalase and superoxide dismutase, indicating its potential as a natural antioxidant.

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