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Biochemical composition and antioxidant activity of the mannoprotein preparation obtained yeast biomass from wine industry waste

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Abstract

In this study, the procedure of obtaining new biologically active mannoprotein preparation based on the yeast biomass from the residues of the wine industry, namely the production of *Merlot* dry red wine is presented. The process for obtaining of the preparation includes lysis of the yeast cell wall with sodium phosphate buffer, extraction of anthocyanins by treatment with 50% alcohol solution, hydrolysis with 1N NaOH, sedimentation of mannoproteins with ethyl alcohol, centrifugation and dissolution of purified sediment in distilled water, standardization of the preparation obtained up to a concentration of 10 mg/ml. As a result, it has been established that the mannoprotein preparation possesses a valuable biochemical composition characterized by a high content of proteins, carbohydrates, anthocyanins, and a wide range of minerals, macro, microelements, and does not practically contain heavy metals. Due to its biochemical composition, the preparation, also, possessed antioxidant activities and high catalase and superoxide dismutase activity. In conclusion, we can mention that the varied biochemical composition, antioxidant, and enzymatic activity highlight the high biological value of the mannoprotein preparation and the enormous potential for implementation in various fields, especially in animal husbandry and viticulture, food and cosmetic industry.

Keywords: antioxidant and enzymatic activities; carbohydrates; mannoprotein preparation; proteins; waste; yeasts

Introduction

Of particular importance today is research into the use of industrial by-products from the production of wine, especially wine sediments, obtained in huge quantities as a result of the industrial manufacturing process. Sediment is formed during fermentation, filtration, centrifugation, and maturation stages which are eventually discarded as waste that pollutes the environment (Devesa-Rey *et al.*, 2011). Wine sediments consist mainly of yeast, tartaric acid, ethanol, phenols and pigments (Nerantzis *et al.*, 2006; Dimopoulou *et al.*, 2017).

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Of all the mentioned compounds, the use of yeast waste for the production of biologically active preparations with beneficial effects on human and animal health is of practical interest.

The important components of the yeast cell wall structure the mannoproteins released by *Saccharomyces cerevisia*e strains either or during fermentation when they are in the phase of inactive growth or after the autolysis process (Guadalupe *et al.*, 2010). Mannoproteins are the second most abundant class of polysaccharides found in wine sediments that are located in the outermost layer of the cell wall and can account for up to 50% of the dry mass of the cell wall of *Saccharomyces cerevisiae* (Klis *et al.,* 2002; Guadalupe *et al.*, 2007). However, the amount of mannoproteins depends on the strain and the vinification conditions (Rosi *et al.*, 2000; Ribereau-Gayon *et al.*, 2006). The structure of mannoproteins obtained from wines has been described in several studies, in essence, they consist of many small chains with one to four residues of D-mannose in α- (1→2) or (1→3), which are linked to polypeptide chains on serine or threonine residues (Waters *et al.*, 1994). Mannoproteins are often highly glycosylated and, are composed of carbohydrates consisting mainly of mannose glucose and proteins (Guadalupe *et al.*, 2010). According to studies, due to its biochemical composition, it also has high antioxidant activity (Križková *et al.*, 2001; Drábiková *et al.*, 2009).

The molecular and structural properties of mannoproteins make them attractive for the development of bio preparations for use in animal husbandry, for animal nutrition and health protection (Pintilie *et al.*, 2011; Hatoum *et al.*, 2012), feed costs are 65-70% on average. Thus, reducing of costs, replacing of biologically active components and organizing an adequate diet play a major role in increasing production (Suzzi *et al.*, 1995). Therefore, further research is needed to develop new technologies to reduce the environmental impact of agro-industrial waste and to make new preparations that provide additional sources of income in the livestock sector.

Mannoprotein preparations can also be applied in the food industry as stabilizers in food emulsions, such as mayonnaise and salad dressing (De Iseppi *et al.*, 2019). In addition, they can be applied to improve wine technology and organoleptic properties. According to studies presented by Dupin during the alcoholic fermentation and the aging of wine, mannoproteins have shown a positive effect on the stability, reduction of tartrate and keep color in sparkling wines (Dupin *et al.*, 2000).

Taking into account the above, the purpose of the research was to evaluate the biochemical composition and antioxidant activity of the mannoprotein preparation obtained from the yeast biomass from the waste of the wine industry.

Materials and Methods

Material and research methods

The yeast biomass (*Saccharomyces cerevisiae*) of lower fermentation from the production of *Merlot* wine, offered by the Cricova winery, was used as research material.

The determination of the dry biomass was performed gravimetrically according to the usual method by drying the biomass in an oven at a temperature of +105 °C to constant mass and recalculating the dry weight (d. w.) (Egorov, 1995). 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 (Dey *et al.*, 1993). The protein was determined spectrophotometrically according to the Lowry method (Lowry *et al.*, 1951), using crystalline albumin from bovine serum as a standard. The anthocyanin content was determined spectrophotometrically at 535 nm wavelength, the method is based on the extraction of anthocyanins using polar solvents (Lima *et al.*, 2011).

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

The activity of the enzyme 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 coloured complex (Komina *et al.*, 2012). The activity of superoxide dismutase (SOD) was determined spectrophotometrically, the method is based on inhibiting the reduction of the tetrazolium-nitroblue salt in the presence of TEMED and riboflavin. The content of macroelements, microelements, and heavy metals was determined by the spectrophotometric method, the principle of the method is based on the simultaneous multi-elemental quantification of the content of macro-, micro-and traces of toxic elements by ICP-OES spectrometry at Thermo Scientific iCAP 6200 Duo spectrometer Scientific, United Kingdom).

Procedure for obtaining the RSM-MP preparation

The biologically active mannoprotein preparation RSM-MP (Red sediment of *Merlot*) obtained from *Merlot* wine yeast sediments was prepared as follows: 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. Subsequently, the biomass of thawed wine yeasts is mixed with sodium phosphate buffer, pH-7.8 (1:1 ratio). The obtained suspension is subjected to autolysis at +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.

Furthermore, to obtain anthocyanins in the mannoprotein preparation after autolysis, the cell walls were treated with a 50% ethyl alcohol solution in a ratio of 1:3. Due to this step, a higher amount of anthocyanins was obtained in the preparation.

After centrifugation, the cell walls were treated with 1N NaOH solution (1:5 ratio) and hydrolysed at +80±5 °C for 2 hours, alkaline suspension separated by centrifugation at 3500 rpm for 15 minutes. The obtained alkaline supernatants are sedimented with 96% ethyl alcohol in a volume of 1:2, upon sedimentation with alcohol, beige-reddish flakes are formed with a viscous consistency which represents the mannoprotein fraction. The separation of the liquid and solid phases is followed by centrifugation for 5 minutes at 2000 rpm. Repeated washing of the sediment with 96% ethyl alcohol and separation of the phases by centrifugation. Dissolve the purified sediment (mannoprotein fraction) in distilled water. Standardization of the preparation obtained up to a concentration of 10 mg/ml active substance.

Sterilization

Sterilization by drying (3 series of heating at a temperature of +55 °C and successive cooling) in order not to compromise the biochemical parameters, the antioxidant and enzymatic activities of the preparation, and the inactivation of the concomitant microflora.

Statistical analysis

Statistical processing of the results was performed using the MO Excel and Statistics 9.0 software suite. The obtained results of the 3 repetitions were expressed by calculating the mean, standard deviation, and confidence interval for a mean. All differences were considered statistically significant for $P \leq 0.05$.

Results and Discussion

Important parameters for evaluating the quality and nutritional value of mannoprotein preparations are determination of the total content of carbohydrates and proteins (Costa *et al.*, 2012; Li *et al.*, 2018, 2019). According biochemical to the tests performed, it was established that the mannoprotein preparation RSM-MP obtained from the waste of the wine industry contains 57.84±0.9% (d. w.) protein and 31.40±2.65% (d. w.) carbohydrates (Figure 1).

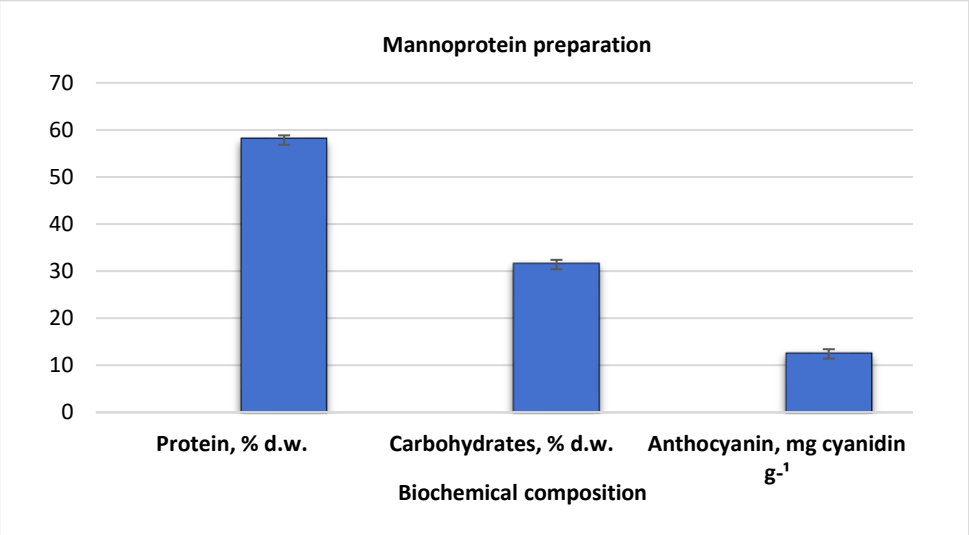

Figure 1. The biochemical composition in the mannoprotein preparation RSM-M

Similar results regarding the carbohydrate and protein contents were obtained by other researchers, who established that the carbohydrate content in mannoprotein extracts obtained from red wine is up to 35% (Vidal *et al.*, 2003) and the protein content varies from 30% to 50% (Barriga *et al.*, 1999; Lukondeh *et al.*, 2003).

Another basic component of the wine sediments is phenolic compounds, which include phenolic acids, tannins and anthocyanins (Perez *et al.*, 2019). Of all the phenolic compounds, anthocyanins are of interest because they have many beneficial effects on human and animal health. *In vitro*, animal and human studies by other authors have shown the biological potential of these molecules and have shown that they can counteract oxidative stress, act as antimicrobials, and counteract the onset and progression of many non-contagious diseases, such as neurodegenerative, cardiovascular, metabolic diseases and cancer (Khoo *et al.*, 2017). They are also well known for protecting visual function along with vitamin A and carotenoids (Khoo *et al.*, 2019). The activities of anthocyanins have been attributed to their ability to capture free radicals and their action on a range of enzymes such as cyclooxygenase and mitogen-activated protein kinase and the signaling of inflammatory cytokines. No adverse effects of anthocyanin derivatives have been reported, even after ingestion of very high doses (Mattioli *et al.*, 2020).

Furthermore, to obtain anthocyanins in the after autolysis, the cell walls were treated with a 50% alcohol solution in a ratio of 1:3, due to this step, a higher amount of anthocyanins was obtained in the preparation. According to the results obtained, it was established that the RSM-MP preparation contains 12.42±0.08 mg cyanidin g^{-1} anthocyanins. The results are consistent with other studies by other authors that have shown the ability of mannoproteins to absorb various phenolic compounds from wine (Mazauric *et al.*, 2006; Guadalupe *et al.*, 2007).

The composition of the macroelements, microelements, and heavy metals in the RSM-MP preparation was further established. The obtained results showed that the preparation contains a very varied spectrum of macroelemets and microelements which is composed of K, P, Na, Ca, S, Fe, Al, Mn, Cu, Cr, Mo, Ni, Co, Zn, Ag, Li, As, V, B, Rb, Be, In in different concentrations (Table 1). It is important to note that the RSM-MP preparation obtained from *Merlot* wine yeast sediments contains tiny amounts of small heavy metals and does not contain Hg, Sb, Sn, Ge and Te. The obtained results are confirmed by other research describing that mannoprotein extracts obtained from yeast *Saccharomyces cerevisiae* are rich in minerals, macro and microelements (Yamada *et al.*, 2003).

Table 1. Content of macroelements, macroelements, trace elements and heavy metals in the RSM-MP preparation

Macroelements	Value
K	0.127±0.005
P	0.042±0.008
Na	0.459±0.006
Mg	0.0009±0.002
Ca	0.007±0.000009
S	0.004±0.000006
Microelements, µg/g (d. w.)	
Fe	1.17±0.03
Al	1.77±0.05
Mn	0.025±0.0003
Cu	0.88±0.2
Cr	0.032±0.001
Mo	0.017±0.002
Ni	0.021±0.0009
Co	0.004±0.0007
Zn	0.084±0.001
Se	-
Ag	0.05±0.02
Li	0.0085±0.0015
As	0.010±0.01
V	0.053±0.01
B	0.46±0.28
Rb	3.63±0.006
Be	0.038±0.001
In	0.03±0.009
Heavy metals, µg/g (d. w.)	
Pb	-
Ti	0.015±0.007
Ba	0.03±0.0005
Bi	0.064±0.015
Hg	-
Sb	-
Sr	0.099±0.024
Ga	0.090±0.01
Ge	-
Cd	0.004±0.00001
Sn	-
Te	-
Tl	0.08±0.01

At the next stage, the total antioxidant activity and the CAT and SOD activities were was evaluated (Table 2). As a result, it was established that the mannoprotein preparation RSM-MP possesses equivalent antioxidant activity of 33.4±0% inhibition and the activity of CAT enzyme is 525±3.1 mmol/min. per mg protein and SOD of 263±4.04 U/mg protein.

Table 2. Antioxidant activity, CAT and SOD enzymes of mannoprotein preparation RSM-MP

Parameter	Value
CAT activity, mmol/min. per mg protein	525±3.1
SOD activity, U/mg protein	263±4.04
Antioxidant activity, % inhibition	33,4±0.3

According to the obtained results, it was established that the RSM-MP preparation has a high antioxidant activity which is due to its composition, especially namely the content of carbohydrates, proteins and anthocyanins. According, those reported in the literature, carbohydrates are an important source of antioxidants (Ramarathnam *et al.*, 1995). There is also research that states that most of the antioxidant activity of carbohydrates is manifested at the combination with several components (Wang *et al.*, 2007; Chen *et al.*, 2008). The study, presented by Siu *et al.*, suggests that yeast carbohydrates that are combined with phenols and proteins have high antioxidant activity (Siu *et al.*, 2014). High antioxidant activity in mannoprotein preparations is also recorded due to its high protein content. This is because the protein is composed of peptides made up of amino acids, which act as antioxidants due to the formation of phenol groups (Qian *et al.*, 2008). Due to its antioxidant activity, mannoprotein extracts contribute to the reduction of reactive oxygen species and exert a high antimutagenic activity (Križková *et al.*, 2001; Drábiková *et al.*, 2009).

Another important property of RSM-MP is the antioxidant activity of CAT and SOD enzymes. Catalase serves to protect cells from the toxic effects of hydrogen peroxide by catalyzing the breakdown of H_2O_2 into water and oxygen. And superoxide dismutase catalyzes the reduction of superoxide anions of hydrogen peroxide and protects cells from dangerous levels of reactive oxygen. Superoxide dismutase is indispensable in many biochemical processes, including intracellular signaling, defense, and cellular functions (Zamocky and Koller, 1999; Weydert and Cullen, 2010). In general, preparations of microbial origin with antioxidant activity possess higher activity and stability than those of plant and animal origin (Lukondeh *et al.*, 2003).

Finally, we can conclude that the use of by-products of the wine industry, especially yeast biomass in the elaboration of mannoprotein preparations with valuable biochemical composition, contributes to significant efficiency of waste management, reducing their negative impact on the environment and highlighting the prospect of implementation in various fields of economy.

Conclusions

The RSM-MP mannoprotein preparation obtained from the yeast sediments of the wine industry contains a varied biochemical composition rich in proteins, carbohydrates and anthocyanins. Taking into account the biochemical composition, the preparation also has a high antioxidant and enzymatic activities. The values of the antioxidant activity are 33.4±0.3% inhibition and the activity of CAT enzymes is 525±3.1 mmol/min. per mg protein and SOD 263±4.04 U/mg protein. Due to its valuable biochemical composition and antioxidant properties, the RSM-MP preparation has a relevant potential for application in animal husbandry, food industry, cosmetic and agriculture sector.

Authors' Contributions

Investigation: A.B, N.C., N.E., E.T., A.L., M.D.; review: A.B.; data curation and editing A.B, N.C., N. E., O.C.; conceptualization, supervision and research resources: O.C. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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