Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 1, 2023
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

AMINO ACID COMPOSITION AND ANTIOXIDANT ACTIVITY OF BIOLOGICALLY ACTIVE PREPARATIONS OBTAINED FROM WINE YEAST SEDIMENTS

Oleg CHISELIȚA, Alina BEȘLIU, Natalia CHISELIȚA, Nadejda EFREMOVA, Elena TOFAN

Institute of Microbiology and Biotechnology of Technical University of Moldova,
1 Academiei Street, Chisinau, Republic of Moldova

Corresponding author email: alina.besliu@imb.utm.md

Abstract

In this study, 4 biologically active preparations obtained from wine yeast sediments are characterized according to their amino acid content and total antioxidant activity.
As a result, it has been established that the preparations contain the full range of essential and immunoactive amino acids, the content of essential amino acids varies between 13.7-287.662 mg/100 ml and immunoactive 13.9-447.33 mg/100 ml. The study of the total antioxidant activity in the biologically active preparations obtained is within the limits of 33.4 ± 0.3-85.9 ± 0.9% inhibition, the maximum being obtained in the amino acid protein preparation SRM-AAP. In conclusion, we can mention that the obtained results indicate that the biologically active preparations obtained from the sediments of wine yeasts are rich in essential and immunoactive amino acids and have a high antioxidant activity, being attractive for implementation in agriculture, especially in animal husbandry, food and cosmetic industry.

Key words*:* amino acid, antioxidant activity, biologically active preparations, wine yeast.

INTRODUCTION

Currently, scientific research is increasingly oriented towards studies that address the problem of industrial waste and that aim to reuse it efficiently and sustainably.
In the context of the Republic of Moldova, the wine industry is an important sector of the economy that has an estimated production of around 11.5 million hectoliters per year and that has recorded constant increases in production in recent years. Following the winemaking process, huge amounts of sediments are obtained that are formed during the fermentation, filtration, centrifugation and maturation stages, which are finally thrown away as waste that pollutes the environment (Devesa-rey et al., 2011).
The main components of wine sediments are yeasts that contain a rich variety of biologically active compounds, including pigments, proteins and amino acids (Baik et al., 2003; Kim et al., 2011). One of the main directions of waste reduction in the wine industry is the development of progressive technological processes for processing and obtaining biologically active preparations with beneficial effects on human and animal health (Tao et al., 2017). To assess the quality, effectiveness and potential benefits of biologically active preparations obtained from wine yeast sediments, the study of amino acid composition and total antioxidant activity is important (Belda et al., 2017; Fidelis et al., 2020).
Research has shown that the amino acid composition of yeast sediments can vary depending on various factors, such as the type of wine, the fermentation process and the strain of yeast used. A study by Li et al. (2015) who analyzed the amino acid composition of yeast sediments from different types of wine found significant differences in their profiles.
Some amino acids were also found to be particularly abundant in yeast sediments, such as glutamic acid, aspartic acid, and alanine (Sarubbo et al., 2015).
These amino acids are known to have antioxidant properties and are involved in various metabolic processes in the body. Research has shown that preparations derived from yeast sediments can have important antioxidant, antimicrobial and anticancer activities (Wu, 2013; Callejón et al., 2014; Zheng et al., 2019).

Based on the above, the relevance of this direction highlights the need to solve the problem of industrial waste that contaminates the environment and remains unused and the prospect of using yeasts in biotechnology for the production of biopreparations with high biological value.

Therefore, this study aimed to determine the amino acid composition and antioxidant activity of biologically active preparations obtained from wine yeast sediments

MATERIALS 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.

Process for obtaining biologically active preparations from wine yeast sediments

The amino acid protein preparation SRM-AAP was obtained through yeast biomass autolysis using a sodium phosphate buffer (ratio 1: 1) at temperature 45°C for 8 hours. The liquid phase was then separated from the solid residue, and sterilization by tyndalization was performed. The solid residue left over after obtaining SRM-AAP was used to prepare the anthocyanin SRM-AN preparation. The extract was obtained by treating the cell walls with 50% ethyl alcohol, followed by agitation on a shaker at 200 rpm for 30 minutes at room temperature. The resulting mixture was then centrifuged for 15 minutes and sterilization.

The mannoprotein preparation, known as SRM-MP, was obtained from the solid sediment that remained after obtaining the anthocyanin preparation. This was achieved through hydrolysis in a 1N NaOH solution (ratio 1: 5) at a temperature of 80 ± 5°C for 2 hours. The resulting mixture was then sedimented with 96% (v/v) ethyl alcohol at a ratio of 1: 2. Finally, the sediment was dissolved in distilled water, the pH was adjusted to 7.0-7.8, and sterilization by tyndalization was performed (Beșliu et al., 2022).

Finally, from solid residue it was obtained the β-glucan fraction SRM-GL insoluble in alkalis and acids, which was treated with 0.5N acetic acid (at a ratio of 1: 5) at temperature 75 ± 5°C for 1 hour. After the acid hydrolysis, the resulting suspension was centrifuged at 3500 rpm for 15 minutes to separate the phases. The β-glucan fraction was then washed three times with distilled water.

Methods of achieving research

The content of amino acids in the preparations was determined by ion exchange chromatography method described by Garaeva et al. (2009).

The antioxidant activity in the preparations was determined by the spectrophotometric method using the radical cation 2,2-azinobis 3-ethylbenzothiazoline-6- sulfonic acid (ABTS) (Re et al., 1999).

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 DISCUSSIONS

Following the research carried out by fractional extraction, 4 biologically active preparations were obtained from the same volume of processed yeast sediments. Thus, tests to determine the content of amino acids in the amino acid protein preparation revealed that it contains the entire range of essential and immunoactive amino acids. The total amount of essential amino acids (lysine, threonine, phenylalanine, isoleucine, leucine, valine, histidine and methionine) was found to be 287.662 mg/100 ml, while the immunoactive amino acids (glycine, glutamic acid, serine, alanine, arginine and cysteine) was 447.33 mg/100 ml (Figure 1). Among the essential amino acids, lysine was the most abundant with 84.4 mg/100 ml, followed by threonine with 69.0 mg/100 ml, valine with 34.4 mg/100 ml and phenylalanine with 34.3 mg/100 ml. From the range of immunoactive amino acids, maximum amounts of glycine, alanine and serine were determined (Figure 1).

Similar results regarding the amino acid content of autolyzed wine yeast extracts were also presented in the study by Osés et al. (2019) who obtained high levels of essential and non-essential amino acids, with lysine, proline, leucine and glutamic acid being the most abundant (Osés et al., 2019).

The study of amino acid content in the SRM-AN preparation showed lower values compared

to that determined in the amino acid protein preparation. Thus, the sum of the essential amino acids is 168.0 mg/100 ml, and the immunoactive ones are 258.7 mg/100 ml (Figure 2). It was found that the essential amino acids are represented by important amounts of lysine 46.5 mg/100 ml, threonine 35.8 mg/100 ml and valine 20.8 mg/100 ml, and the immunoactive ones by proline 484.6 mg/100 ml, glutamic acid 79.0 mg/100 ml and aspartic acid 38.6 mg/100 ml.

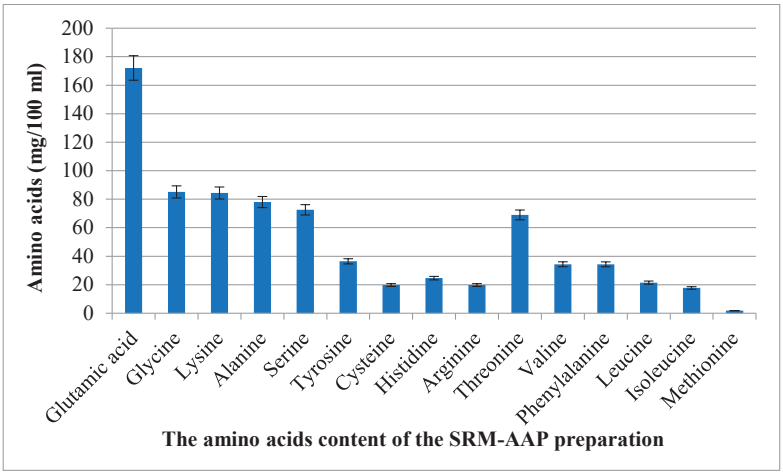

Figure 1. The amino acid content in the SRM-AAP preparation obtained from wine yeast waste sediments

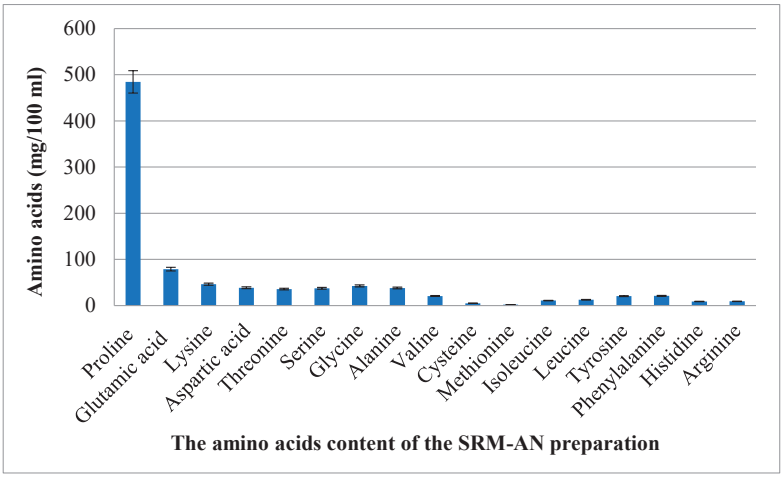

Figure 2. The amino acid content in the SRM-AN preparation obtained from wine yeast waste sediments

Next, to ensure consistent quality and effectiveness, the content of amino acids in the mannoprotein preparation was evaluated. The results are shown in Figure 3. According to the results obtained, it was established that the content of essential and immunoactive amino acids is 13.7 mg/100 ml and, respectively, 13.9 mg/100 ml. Quantitative analysis of essential amino acids established that lysine, leucine, valine and threonine prevail among them. It is also characterized by a high arginine content of 4.39 mg/100 ml, aspartic acid 3.27 mg/100 ml and phenylalanine 1.28 mg/100 ml.

The obtained results indicate that the quantitative composition of amino acids in mannoprotein preparations obtained from wine yeasts can vary depending on the extraction and purification methods used. A similar spectrum of essential amino acids was also obtained in the study by Vivar-Quintana et al. (2004) in which the content of lysine, leucine and valine recorded the highest levels. In contrast, the preparation in the current study had higher levels for arginine only.

These differences highlight the importance of carefully characterizing biologically active preparations to ensure consistent quality and efficacy (Vivar-Quintana et al., 2004).

Finally, the amount of amino acids in the SRM-GL preparation was analyzed.

The findings are presented in Figure 4.

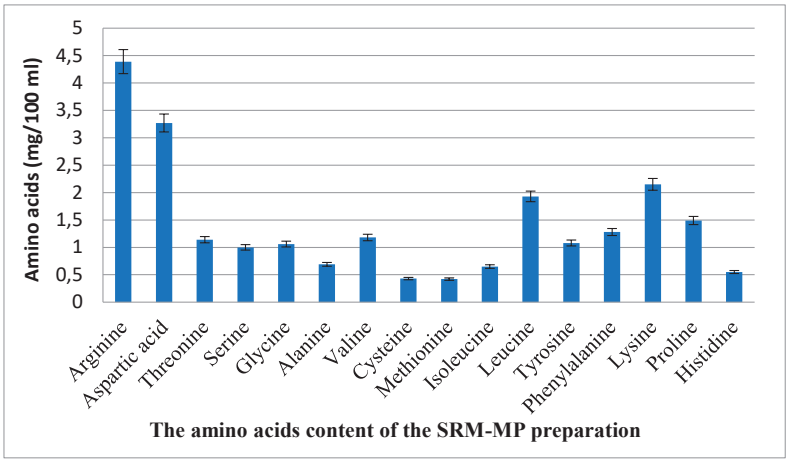

Figure 3. The amino acid content in the SRM-MP preparation obtained from wine yeast waste sediments

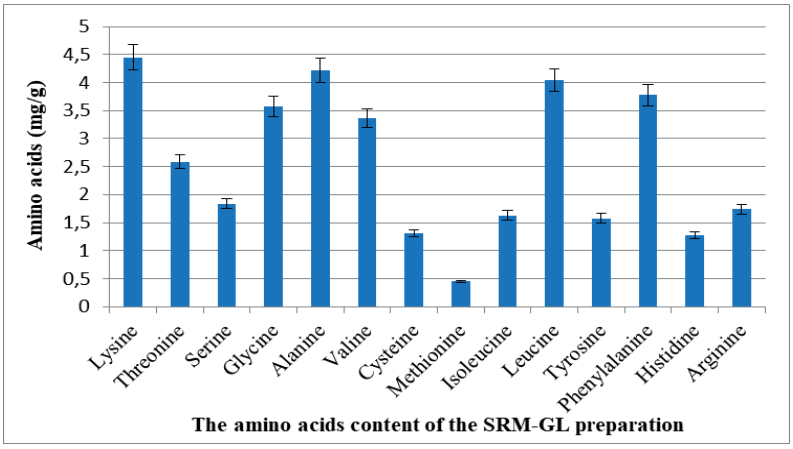

Figure 4. The amino acid content in the SRM-GL preparation obtained from wine yeast waste sediments

It was determined that the preparation contains 51.2 mg/g of proteinogenic amino acids, of which 23.3 mg/g are essential and 27.9 mg/g are immunoactive. Lysine, leucine, phenylalanine and valine are the most abundant essential amino acids, while alanine, serine and glycine prevail among the immunoactive ones. These results are consistent with previous studies in the literature, which report that the total content of essential amino acids in β-

glucans ranges from 22-39 mg/g, with lysine, leucine, and valine being the most abundant (Gobbetti et al., 2010; Barros et al., 2014).
In the later phase of the study, due to the substantial composition of essential amino acids in the preparations, the total antioxidant activity was evaluated. According to the obtained results, it was established that the SRM-AAP preparation is characterized by a very high total antioxidant activity equal to 85.9 ± 0.9% inhibition. Whereas, the activity in the other preparations was within the limits of 33.4-52.2% inhibition (Figure 5).

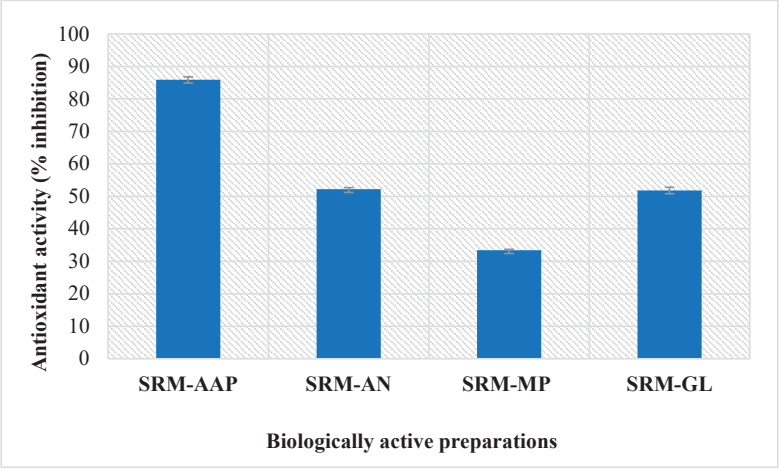

Figure 5. Antioxidant activity in biologically active preparations obtained from wine yeast sediments

Similar results exist in several studies that investigated the antioxidant activity of biologically active preparations obtained from wine sediment waste. Protein and mannoprotein extracts obtained from yeasts were reported to have strong antioxidant activity in vitro, which was attributed to their high content of amino acids and polysaccharides (Kim et al. 2007). Another study by Yao et al. (2020) demonstrates that mannoproteins isolated from wine yeast possess antioxidant activity in vitro and suggested that they could be used as natural antioxidants.
The study by Zhou et al. (2016) found that anthocyanin extracts from waste wine sediments have strong antioxidant activity, which was attributed to their high phenolic content (Zhou et al. 2016).

CONCLUSIONS

The biologically active preparations derived from wine yeast sediments exhibit a rich biochemical composition of essential, proteogens and immunoactive amino acids. Based on the biochemical composition, it can be observed that the preparations also exhibit high levels of antioxidant activity. The antioxidant activity values range from 33.4 ± 0.3-85.9 ± 0.9% inhibition, with the SRM-AAP preparation displaying the highest value.
These preparations have demonstrated a variety of beneficial properties, including nutrition and antioxidant effects, rendering them potentially valuable for a range of applications in several sectors, including animal husbandry, cosmetics and the food industry.

ACKNOWLEDGEMENTS

The results were obtained within the Project 20.80009.5107.16. "New biologically active microbial preparations for increasing the reproductive and productive potential of animals of zootechnical interest", funded by the National Agency for Research and Development.

REFERENCES

Baik, O. D., Kim, Y. J., Kim, J. H., Kim, S. J., & Suh, H. J. (2003). Production and characterization of pigments from sedimented yeast cells of *Candida utilis*. *Journal of microbiology and biotechnology*, *13*(1), 83-88.

Barros, L., Baptista, P., & Ferreira, I. C. F. R. (2014). Beta-glucans' extraction from different sources and their use in the food industry: A review. *Food and Bioproducts Processing, 95,* 16-28.

Belda, I., Ruiz, J., Navascués, E., Marquina, D., Santos, A., & Calderón, F. (2017). Biotechnological potential of non-Saccharomyces yeasts isolated during spontaneous fermentations of Malvar (*Vitis vinifera* cv. L.). International *Journal of Food Microbiology, 241,* 120-126.

Beshliu, A., Chiselitsa, N., Chiselita, O., Efremova, N., Tofan, E., Sprincean, A., & Danilis, M. (2022). Biochemical composition and antioxidant activity of the mannoprotein preparation obtained yeast biomass from wine industry waste. *Notulae Scientia Biologicae, 14*(2), 1-9.

Callejón, R. M., Espinosa, J. C., & García, A. M. (2014). Analytical methods for the characterization and identification of bioactive peptides in food products: A review. *Journal of Agricultural and Food Chemistry, 62*(28), 6495-6508.

Devesa-Rey R, Vecino X, Varela-Alende J, Barral M, Cruz J, & Moldes A. (2011). Valorization of winery waste vs. the costs of not recycling. *Waste Management 31*(11):2327-35.

Fidelis, M., Delamare, A. P. L., & Tessaro, I. C. (2020). Wine yeast sediment: characterization and applications in the food industry. *Food Research International, 131*, 109042.

Garaeva, S.N., Redkozubova, G.V., & Postolati, G.V. (2009). Amino acids in living organisms. Academy of Sciences of Moldova. Institute of Physiology and Sanocreatology, 552.

Gobbetti, M., Rizzello, C. G., Di Cagno, R., & De Angelis, M. (2010). How the sourdough may affect the functional features of leavened baked goods. *Food Microbiology, 27*(6), 1071-1079.

Kim, J. S., Kwon, C. S., & Son, K. H. (2007). Antioxidant activities of various solvent extracts from *Allomyrina dichotoma* (Arthropoda: Insecta) larvae. *Journal of Food Science, 72*(7), S511-S515.

Kim, J. Y., Choi, G. W., & Kang, D. H. (2011). Characterization of amino acid composition and antioxidant activity of sedimented yeast from makgeolli. *Journal of the Korean Society of Food Science and Nutrition, 40*(11), 1604-1609.

Li, H., Liu, Y., & Li, P. (2015). Amino acid composition and antioxidant activities of protein hydrolysates from different wine grape pomaces. *Food Science and Biotechnology, 24*(1), 93-99.

Osés, J., Bartolomé, B., & Gómez-Cordovés, C. (2019). Amino acid profile of a hydrolysed wine yeast extract by high-performance liquid chromatography-tandem mass spectrometry. *Food Chemistry, 284*, 17-24.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*(9/10), 1231-1237.

Sarubbo, L. A., Farias, C. B., de Luna, J. M., de Oliveira, D., Borges, A. C., & Campos-Takaki, G. M. (2015). Antioxidant and antimicrobial activities of single cell protein in solid state fermentation from pineapple waste. *African Journal of Biotechnology, 14*(4), 246-253.

Tao, Y., Zhang, Y., & Liu, Y. (2017). Purification and antioxidant activity of peptides from defatted walnut (*Juglans regia* L.) meal hydrolysate. *Journal of Food and Nutrition Research, 5*(6), 435-441.

Vivar-Quintana, A. M., Polo-Díez, L. M., González-Fernández, E., Álvarez-Rivera, G., Fernández-González, C., & González-Rodríguez, M. V. (2004). Analysis of the amino acid composition of mannoproteins in wine by reversed-phase high-performance liquid chromatography and precolumn OPA derivatization. *Journal of Agricultural and Food Chemistry, 52*(13), 4082-4087.

Wu, G. (2013). Functional amino acids in nutrition and health. *Amino acids, 45*(3), 407-411.

Yao, Y., Zhang, L., Huang, X., & Chen, J. (2020). Antioxidant activity of mannoproteins isolated from wine yeast in vitro. *Journal of Food Science and Technology, 57*(1), 152-159.

Zheng, H., Yu, Z., Wang, Y., Zhang, B., & Zeng, X. (2019). Purification and characterization of an antioxidant peptide from yeast sediment. International *Journal of Biological Macromolecules, 131*, 774-779.

Zhou, H., Liu, Y., He, C., Yan, J., & Shi, J. (2016). Antioxidant activities of anthocyanins extracted from waste wine sediments. *Molecules, 21*(9), 1179.