BIOCHEMICAL CHARACTERIZATION OF THE YEAST BIOMASS RESULTING FROM THE WINEMAKING PROCESSES

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Abstract

Valorization of winemaking wasteis an actual ecological problem that needs to be solved. The current study was carried out to evaluate biochemical composition of *biomass* of *wine sedimentary yeasts*. According to obtained results,yeast sediments resulting in natural white and red wine making present a good source of protein as well, as of essential and immunoactive amino acids. The studied types of sediments possess high antioxidant activity and activity of antioxidant enzymes catalase and superoxid dismutase, the values varying, depending on the type of wine. So, yeast sediments of wine production can be proposed for the further development of new technologies for the production of bioactive extracts with antioxidants properties.

Keywords: *Saccharomyces cerevisiae*, vinification wastes, antioxidants, protein, enzyme.

Introduction

During the last years winemaking has become the most dynamically developing branches of the Russian agro-industrial complex. According to a study, in 2022 world wine production amounted to 258 mhl, with a decrease of 3 mhl (-1%), compared to 2021. In Moldova vinified production is estimated at 1.4 mhl (OIV 2022). Waste recycling contributes to obtaining of valuable products needed for a large number of economical directions. Over the last decade, the intensive study of reutilization of wastes is important (Ericson 2022, Rodrigues 2014, Otles et al. 2015). Yeasts from wine production can serve as prime source for food and feed additives with high biological value.

Waste of wine production can be used for the production of natural cosmetics (Hiba et al. 2021). It is known that polyphenols - ingredients with high antioxidant properties are subsequently obtained from the extracts on the base of wine yeasts (Ky et al. 2014).

The wide variety of biochemical parameters of yeasts remained from wine production, might led to obtaining of the different bio preparations such as enzymatic, protein, mannoprotein preparations.

Disposal of production waste that pollutes the human environment is one of the most important environmental and economic problems of society. A lot of waste is generated during the production of wine. Complex processing of secondary raw materials of winemaking is recognized not only as necessary and useful from the point of view of environmental protection and recreational activities, as it helps to reduce environmental pollution, but also as a highly efficient type of commercial activity. The use of yeast as a source of bioactive substances and complexes is one of the important areas of modern biotechnology(Leon-Gonzales 2018). It is

Received 24 February 2024; Reviewed 10 July 2024; Accepted 29 July 2024; Published Online 29 July 2024; Published xx September 2024

known that yeasts can serve as a source of enzyme extracts that could be used also in food industry and save the value of food raw matter. According to recent studies, enzymes with pronounced antioxidant properties, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) have an important role in protection of live organisms from the negative consequences of free radicals and reactive oxygen species (Ighodaro and Akinloye 2018).

Bio-waste is defined as a result of technological food production. The development of technologies for the production of feed protein and other biologically active substances, based on waste from the wine industry, is relevant, both in terms of the safe use of this raw material and the elimination of environmental risks (Iuga and Mironeasa 2020, Garcia-Lomillo et al. 2014).Thus, the research goal was to evaluate biochemical composition of yeasts biomass obtained from the processes of wine production..

Wine production is one of the most important fields of food industry (Stopka et al. 2008). According to recent researchers, winery waste is characterized by a high concentration of organic substances, such as proteins, lipids, polyphenols, carbohydrates (Ferrer-Galego and Silva 2022, Minusi et al. 2003, Zhang et al. 2017).

Untreated it can harm the environment, including the soil, water, plants. Wine industry produces a large amount of wastewater and organic waste that must bere-used with the aim to avoid contaminating. Regrettably, only a few of these by-products are used for livestock sector and food industry. *Furthermore*, by-products of wine industry can be used for valorization of bioactive preparations, pharmaceutical, food, and cosmetic ingredients (Gurev et al. 2022). The new preparation can be used, also, in the fields of animal husbandry and veterinary medicine.

Currently, *scientific* researchers are aimed at improving the technology of wine production. Due to the increase of the wineryindustry, the problem of utilization of wine waste has arisen (Musteață et al. 2021, Ye et al. 2015). During the preparation of wine, sedimentary yeasts are formed, which contain a complex of organic compounds with biological value, so it is advisable to introduce environmentally safe and effective technologies for processing these types of waste, taking into account their physiological properties.

Thus, there are several solutions to increase the value of these numerous wastes, namely yeast biomass as a food additive and feed or source of biologically active substances, which, however, have not been widely used. Therefore, the alternative of utilization and recycling of these wastes is of great interest.

It is necessary to develop procedures for the recovery and transformation of by-products with a certain degree of innovation. It can therefore be difficult to reduce or minimize waste production in wine production processes that are limited by infrastructures or human resources. As a consequence, it is important to develop valorization procedures, leading to the further implementation of wastes of the wine industry.

Materials and Methods

The object of research were samples of wine yeast selected at a winery in «Cricova» SA. The yeast sediments from wine were subjected to centrifugation to separate the remaining liquid. The commercial *Saccharomyces cerevisiae* strains were used for winemaking. The yeasts biomass (*Saccharomyces cerevisiae*)of lower fermentation from the production of red *Merlot* (RSM –red sediments Merlot) and *Cabernet Sauvignon* (RSC- red sediments Cabernet) and white *Rkatsiteli* wine (WSR – white sediments Rkatsiteli) were offered by the wine complex.

The ABTS radical decolorization assay was used to evaluate *total antioxidant* activity of sedimentary yeasts samples (Re et. al.1999). The absorbance was measured at 734 nm.

% Inhibition =(Control Abs-Sample Abs)/Control Abs*100.

Control Abs – the absorbance of ABTS radical solution,

Sample Abs – the absorbance of ABTS radical solution +sample.

Folin Ciocalteau reagent has been used for the determination of protein content in samples of yeasts biomass from wine sediments (Lowry et al. 1951). The method is based on reactions of the colour complex formation. For the calibration curve was used bovine serum albumin as atandard. Based on the coefficient determined from the calibration curve, was used the calculation formula: C = A750·K·ɳ·100/m,, where C - protein content in biomass (%); A_{750} - absorbance at 750 nm; K - recalculation coefficient (in mg protein/ml sample) determined from the calibration curve; ɳ - dilution; 100 - % coefficient; m - mass of test sample, mg.

The total carbohydrate content was measured spectrophotometrically by the anthrone method. *Anthrone method* is one of the most used *methods* for the *determination* of soluble *carbohydrates (Dey* and Harborn 1993*). Carbohydrates estimation is measured at* wavelength of 620 nm. Carbohydrates are dehydrated at the reaction with concentrated H_2SO_4 to form furfural- a blue-green complex. A calibration curve was constructed in successive dilutions of the glucose standard solution. According to hydrolysis with anthrone and cooling of the samples to room temperature, the absorbance was measured at a wavelength of 620 nm relative to the control sample. Using the QUANTITATIVE WORKSPACE application provided with spectrophotometer, the value for the coefficient K used in the quantitative calculation of carbohydrates was obtained. The calculation formula is K=C/A620, where C - concentration of glucose in samples, mg/ml, A - absorbance of the sample at 620 nm.

The superoxide dismutase activity was determined as the inhibition or reduction of Nitroblue tetrazolium (NBT) in the presence of *Tetramethylethylenediamine* (TEMED) (Titova and Subbotina 2012). Measurements were conducted at a wavelength of 560 nm. The presence of the reaction medium blue tetetrazolium allows to estimate the decrease in superoxide radical production per unit based on photometric detection of the formation of diformazane (a product of reduction of nitroblue tetrazolium by superoxide radicals). SOD activity was determined using the following formula:

SOD activity(Units/mg protein)=((Control Abs – Sample Abs)*100%)/C

Control Abs – the absorbance of control probe,

Sample Abs – the absorbance of sample probe,

C – concentration of protein (mg/l).

The catalase activity was measured according to the spectrophotometric assay that was *based* on the *ability* of *hydrogen peroxide* to *form salts* with *molybdenum stable* colored *complex (Komina et al. 2012)*. The absorbance was registered on spectrophotometer through the changes of optical density against blank at 410 nm. Catalase activity was determined by the following formula:

CAT activity = (Control Abs-Sample Abs)*V/v • t • C, where

Control Abs – absorbance of a blank probe,

Sample Abs – absorbance of a sample probe,

V – total volume of the probe,

v – sample volume,

t – incubation time,

C – extinction coefficient (22.2*103 $mmol^{-1}*cm^{-1}$).

The determination of amino acids content was assessed by ion-exchange chromatography method is reliable for determining of the free amino acid content of different types of yeasts biomass from wine yeasts. For the determination of the composition of amino acid 100 mg of the yeast biomass were dried at 60 °C and were subjected to hydrolysis at 120 °C for 15 min with concentrated hydrochloric acid. The sample was filtered and 0,5 ml of the liquid was adjusted to volume of 2 ml with buffer solution, Ph = 2,2 and was subjected to analysis. The amino acid analyzer AAA-339M (CZECH Republic) was used for the analysis(Garaeva et al. 2009).

Quantitative determination of macro and micro elements and heavy metals accumulated in yeasts biomass was evaluated by ICP-OES method. The digestion was carried out by the following procedure: 2.0 ml concentrated HNO_3 and 1.0 ml H_2O_2 was added to 50 mg of yeast biomass (sample). The digestion was carried out by the autoclave method. After digestion the samples were transferred to 50 ml volumetric flasks, and deionised water was added for the final volume. The macro and microelements s were determined by ICP-OES. The content of macro elements, microelements and heavy metals was determined by the spectrophotometric method, at Thermo Scientific iCAP 6200 Duo spectrometer Scientific, United Kingdom (U.S. EPA. 2007).

Ash content was determined by weight of inorganic matter remaining after the removing water and organic matter (Harris, Marshall 2017). The ash content was gravimetrically determined by muffle furnace ignition at 550° C.

The lipid content was determined gravimetrically by the method proposed by Bligh and Dyer (Bligh, 1959). For the lipid extraction to 1,0 g of yeast biomass the mixture 1:2:0,8 (v/v/v) cloroform -ethanol - water was used. Extraction is carried out at room temperature by continuous stirring for 60 min. Chloroform is removed by distillation in a vacuum evaporator at 40^0C. The lipid mass obtained is dried at temperature 105 ± 20C to a stable mass, for which at least 3 times. Using the ratio of the amount of lipid obtained to the biomass used for extraction is determined is determined by the following formula.

Lipids content, $\%=m_{Lipids}*100/m_{sample}$, where

m_{lipids} – dry weight of lipids obtained by the extraction procedure,

m_{sample} -Dry weight of yeast biomass sample.

Statystical analysis. The obtained data were analyzed using one-way Analysis of variance (ANOVA). The mean and standard deviation was calculated to demonstrate quantative variables. P – values less than 0.05 were considered significant.

Results and discussion

Because, in order to evaluate the prospect of use and the spectrum of possible products, it is necessary to know the biochemical composition of the yeast biomass. At the initial stage the total content of proteins (including amino acids), carbohydrates, lipids, macro-, micro-, trace elements, heavy metals, total antioxidant activity and activity of antioxidant enzyme such as catalase and superoxide dismutase activity was studied in the three types of yeast biomass. These types were obtained from red, white wine production, as well as red wine production with perlite.

The results of the study of biochemical composition are presented below (Figure 1).

It can be mentioned that yeast biomass from the production of red wine Merlot and Cabernet contains significant quantities of proteins 65.97±0.45 and 64.74%±0.27% per yeast dry weight and have the minimum amount of lipides 2.47±0.03 and 4.22±0.14 % per yeast dry weight, respectively. While yeast biomass from the production of white wine *Rkatsiteli* contains proteins - 32.0 % per yeast dry weight and 11.00±1.0 %. per yeast dry weight . The maximum content of carbohydrates was found in the yeast biomass RSM. Thus, the biochemical analysis has demonstrated significant differences in the total protein content and lipid content among the studied wine wastes.

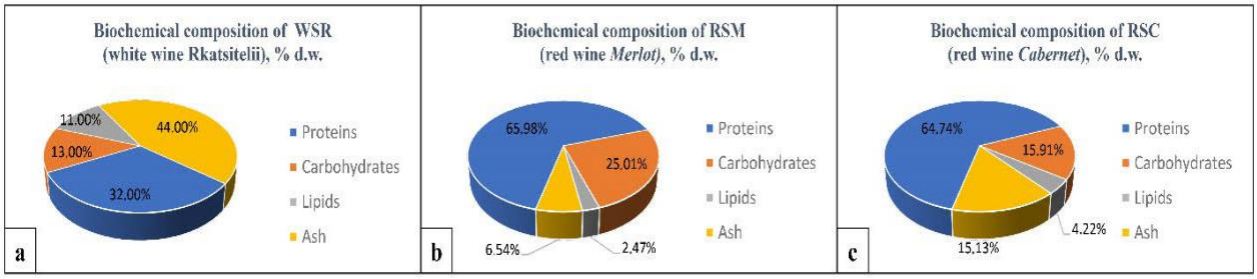

Figure 1 (a), (b), (c). Biochemical composition of yeast biomass from the wine waste, WSR– **a)**, RSM - **b,** RSC– **c)**.

In addition to the fact that wine yeasts contain significant amounts of proteins and biologically active polysaccharides, they are used a source of antioxidant substances. For these reasons, total antioxidant activity and the activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were further evaluated in the wastes of wine production (Table 1).

Thus, it has been established that all sediments possess antioxidant activity, but the values vary from case to case, depending on the type of wine. *Antioxidant activity* was measured by *ABTS* radical scavenging *assay.* For example, RSM has highest total antioxidant activity equivalent to 61.5±2.0% inhibition, while CAT activity is the lowest of all and constitutes 222.04±10.28 mmol/min. per mg protein. The total antioxidant activity of WSR and RSC is lower than that of SRM and constitutes respectively 47.2±3.8 and 49.9±0.3% inhibition, while CAT activity in both cases is high - 859.61±38.05 and 739.23±5.51 mmol/min. mg protein respectively. SOD activity, in all three sediment types, is comparable and constitutes from 171.0±5.8 to 227.0±2.52 U/mg protein.

Table 1. Antioxidant activity and activity of CAT, SOD enzymes in yeast biomass from the production of dry white and red wines from the wine complex "Cricova" SA.

Indices	**WSR**	**RSM**	**RSC**
Catalase activity (CAT), mmol/min. mg protein	859.61±22.0	222.04±5.9	739.23±3.18
Superoxid dismutase activity (SOD),U/mg protein	171.0±5.8	227.0±1.5	211.0±5.5
ABTS, % inhibition	47.2±3.8	61.5±2.0	49.9±0.3

Amino acids are used as active ingredients in the production of food supplements or in the pharmaceutical industry. The study of the amino acid content of yeast biomass from the wine waste has revealed that the sediments from white and red winemaking contain the full range of proteinogenic amino acids(cysteine, aspartic acid, threonine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine), but their content differs depending on the type of wine (Figure 2).

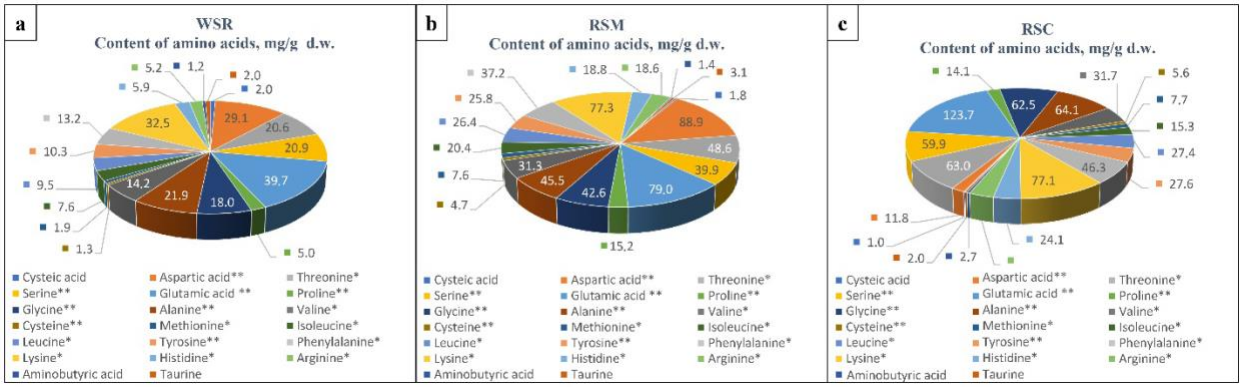

Figure 2 (a), (b), (c). Amino acid content of yeasts biomass from the wine waste, WSR (**a)**, red RSM (**b),** RSC (**c).**

Thus, the sum of amino acids detected in WSR - 256.61 mg/g dry weight was lower, compared to the same indices of RSM and RSC - 627.52 and 683.64 mg/g dry weight. Considerable amounts of essential amino acids, such as phenylalanine, histidine, isoleucine were determined in yeast biomass of the studied variants. Similar results have been reported previously according to which, high content of histidine and phenylalanine was revealed in wine by-products (Buzzanka et al. 2024) and wine samples fermented with yeast strains (Taran and Antohi 2014).

It is necessary to mentione the high content of essential and immunoactive amino acids in red wine sediments, the sum of which was 286.1-314.45 and 400.33-446.83 mg/g dry weight, respectively, that indicates the biological and nutritional value of wine yeast biomass (Figure 3).

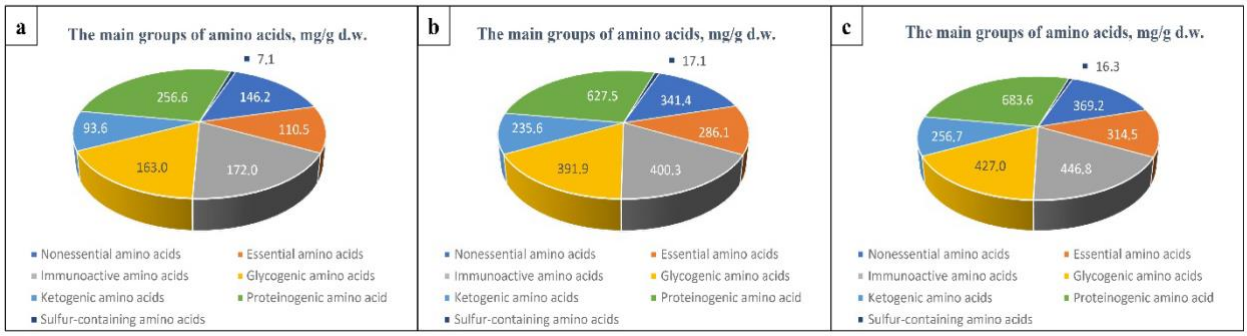

Figure 3 (a), (b), (c). The main groups of amino acids of yeasts biomass from the wine waste, WSR– (**a)**, RSM – (**b),** RSC – (**c).**

The study has assessed the *content of macro*, micro, and heavy metals elements in wine sediments from the production of dry white and red wines (Figure 4-5).

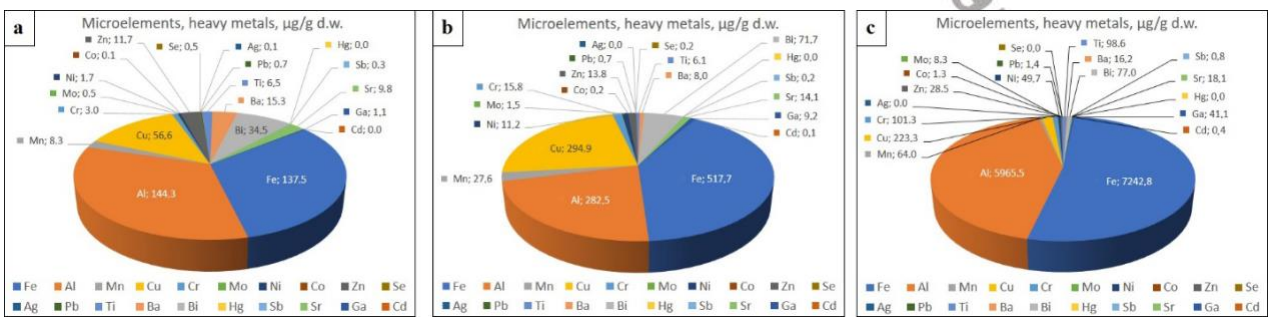

Figure 4 (a), (b), (c). The content of microelements and heavy metals in sediments from the wine waste, WSR **-(a**), RSM–(**b),** RSC– (**c)** from the wine complex «Cricova» SA.

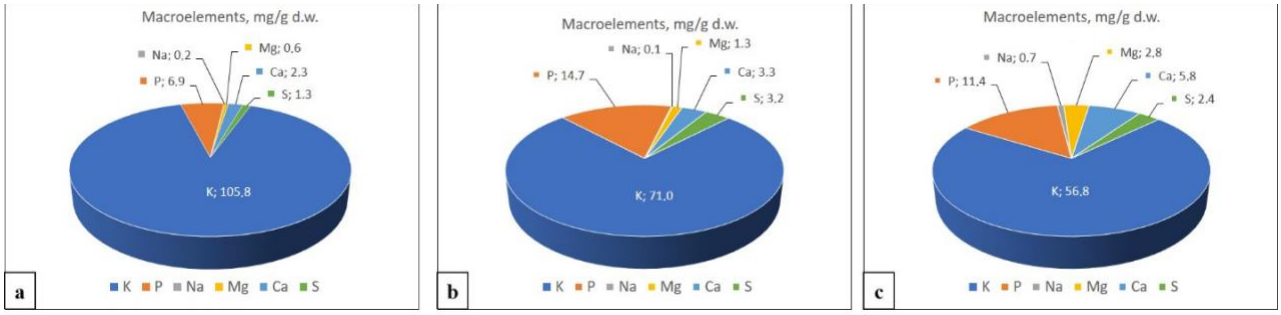

Figure 5 (a), (b), (c). The content of macroelements in sediments from the production of dry white and red wines, WSR **-(a),** RSM – (**b),** RSC– (**c)** from the wine complex «Cricova» SA.

Thus, it was established that yeasts sediments contain macroelements: K 56.8-105.8 mg/g dry weight; P 6.9-14.7 mg/g dry weight; Na 0.1-0.7 mg/g dry weight; Mg 0.6-2.8 mg/g dry weight; Ca 2.3-5.8 mg/g dry weight; S 1.3-3.2 mg/g dry weight. It should be mentioned the very wide spectrum of trace elements in the yeasts from wine sediments: Fe; Al; Mn; With; Cr; Mo; Us; Co; Zn; Se; Ag; Li; V; B; Rb in, the concentration of which varies within very wide limits. According to the literature data, yeast strains might affect the content of Co, Cu, Mg, Na, Pb, Sr and Zn in the wine. (Nicolini 2003).

According to the obtained results, yeast sediments from white and red winemaking might serve as source of protein, the nutritional value of which is expressed by the high content of essential and immunoactive amino acids. Wine sediment yeasts can be used for the development of nutritional supplements and feed additives.

CONCLUSION

Given the increased interest in wine-making, there is an increasing necessity to reduce wine wastes through the development of new technologies for the production of bioactive extracts on the base of wine making yeasts. Yeast sediments used in natural white and red winemaking represent a good source of protein, the nutritional value of which is expressed by the high content of essential and immunoactive amino acids, a source of lipids, macro- and microelements polysaccharides, also, possess high antioxidant activity and activity CAT and SOD enzymes and can serve as a basis for the development of food supplements, feed additives and biologically active antioxidant preparations for increasing of the reproductive potential of livestock animals.Yeast preparations derived from sedimented wine yeasts may be used in the animal husbandry, food and cosmetic industries.The use of winemaking industry by-products could reduce the environmental impact and elaborate a promising way of its application in different areas of modern economy.

Acknowledgments: The results were obtained within the Project 20.80009.5107.16. funded by the NARD of Moldova

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