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ACTIVITY OF SUPEROXIDE DISMUTASE ENZYME IN YEAST *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Reactive oxygen species (ROS) with reactive nitrogen species (RNS) are known to play dual role in biological systems, they can be harmful or beneficial to living systems. ROS can be important mediators of damage to cell structures, including proteins, lipids and nucleic acids termed as oxidative stress. The antioxidant enzymes protect the organism against the oxidative damage caused by active oxygen forms. The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical, produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. In this study, SOD activity of three yeast strains *Saccharomyces cerevisiae* was determined. It was found that SOD activity was the highest (23.7 $U.mg^{-1}$ protein) in strain 612 after 28 hours of cultivation. The lowest SOD activity from all tested strains was found after 56 hours of cultivation of strain Gyöng (0.7 $U.mg^{-1}$ protein).

Keywords: superoxide dismutase, *Saccharomyces cerevisiae*, oxidative stress, antioxidative enzymes

INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($OH^{\cdot}$) arise normally during aerobic metabolism (with an estimated 2 % of oxygen utilized by the yeast cell being converted into the superoxide anion). ROS have the potential to cause oxidative damage to proteins, nucleic acids and other macromolecules, which can severely compromise cell health and viability **(Shanmuganathan *et al.*, 2004)**.
Superoxide dismutase (SOD, EC 1.15.1.1) is a class of metalloproteins which catalyze the dismutation of superoxide radicals ($O_2^{\cdot-}$) to oxygen and hydrogen peroxide. SOD provides a vital defense mechanism against the formation of deleterious oxygen species. The presence of SOD in all aerobic organisms protects cells against oxidative stress **(Šeatović *et al.*, 2003)**.
There are four types of SOD according to their catalytic metal cofactor: copper-zinc type (Cu-ZnSOD), manganese type (MnSOD), iron type (FeSOD) and nickel type (NiSOD) **(Djalali *et al.*, 2005)**. Cu-ZnSOD is located mainly in the cytosol of the eukaryotic cells, in the cytoplasm and chloroplasts of higher plants; FeSOD is typical of the bacterial cell matrix and plant organelles, while MnSOD is predominantly present in the mitochondrial matrix of eukaryotic cells and in prokaryotes **(Guo *et al.*, 2008)**. *Saccharomyces cerevisiae*, like most other eucaryotes, contains Cu-ZnSOD (product of the *SOD1* gene) in the cytosol and MnSOD (product of the *SOD2* gene) in the mitochondria. These enzymes together with small molecule antioxidants, such as glutathione and ascorbate; other antioxidant enzymes, such as catalase and peroxidase; and metal chelating proteins, such as metallothionein, allow aerobes to survive under O_2, presumably by minimizing oxidative damage **(Longo *et al.*, 1996)**.
The objectives of this study were (1) to determine superoxide dismutase activity of yeast *Saccharomyces cerevisiae* strains 612, Gyöng and Kolín during the growth and (2) to choose one of three tested yeast strains which had the highest SOD activity and can be used as supplement with antioxidant activity in human nutrition.

MATERIAL AND METHODS

Microorganisms and inoculum preparation

Experiments were carried out with yeasts *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strains 612, Gyöng and Kolín. The yeast strains were obtained from distillery Slovenské liehovary a likérky, a.s. Leopoldov, Slovakia. The yeasts were individually maintained at 4 °C on malt extract agar plates and subcultured at monthly intervals. For inoculum preparation 5 loops of cells from an isolated colony on malt extract agar plate were aseptically transferred to 100 mL of liquid YPD (Yeast Peptone Dextrose) medium containing 10 $g.L^{-1}$ yeast extract, 20 $g.L^{-1}$ peptone and 35 $g.L^{-1}$glucose. The culture was incubated at 30 °C with shaking at 280 rpm. Then 20 mL of this over-night culture was transferred into 100 mL fresh YPD with the same composition. Yeast cells were grown under shaking (280 rpm) at 30 °C in dark. The yeasts were collected from 0 to 76 hours of incubation at 4 hours intervals.

Cell-free extract preparation

Cell wall disruption was carried out by French Press (Stansted Fluid Power LTD, UK). Cells from 0 hour to 76 hours of cultivation were harvested by centrifugation at 5000 x g for 10 minutes and washed twice with distilled H_2O. Cells were suspended in 10 mL 0.01 $mol.L^{-1}$ phosphate buffer pH 7,0 and crushed at 18,000 Psi pressure, maintaining temperature at 0 - 4 °C. This was followed by centrifugation at 4 °C, 5000 x g for 10 minutes. The residue was discarded and the supernatant was used for determination of enzymatic activity.

Assay of superoxide dismutase activity

In this study the diagnostic Ransod set (RANDOX, Great Britain) was used for the determination of superoxide dismutase activity. The principle of the method was based on the xanthine and xanthine oxidase that produce superoxide radicals reacting with tetrazolium salt to red formasan. SOD activity is determined as a degree of inhibition of this reaction which occurs at 37 °C. Following preparation was identical both for the prepared yeast samples and standards from which the calibration curve was constructed **(Březinová Belcredi *et al.*, 2010)**.
The sample (0.05 mL) and the substrate (1.7 mL) were added into a cuvette and the mixture was carefully blended. Reaction was started by addition of xanthine oxidase (0.25 mL). The cuvette was placed into the spectrophotometer and an absorbance of 505 nm was measured. The first absorbance was measured after 30 seconds (A_1) and the second after 3 minutes (A_2). The result was converting to SOD $units.mg^{-1}$ protein.

Protein concentration

Proteins concentration was determined by the method of **Bradford (1976)** in which bovine serum albumin was used as standard.
Optical density (OD) at 600 nm was the criterion of yeast cells growth.

RESULTS AND DISCUSSION

Nowadays the study of antioxidant enzymes activity is one of the most important topics in food research. *Saccharomyces cerevisiae* is extensively exploited organism and it follows from our results that yeast can be also the source of antioxidative substances.
SOD have been detected and isolated from a number of microorganisms, plants and animals **(Donnelly *et al.*, 1989)** and it has been used as an antioxidant in the food industry **(Orozco *et al.*, 1998)**. It is isolated commercially using human or bovine erythrocytes, bovine liver **(Huber and Schulte, 1973)**, or microorganisms **(Johansen, 1983; Scott *et al.*, 1987)**. **Karadag and Bilgin (2010)** reported that activity of superoxide dismutase in human erythrocytes is 7.68 U.mg^{-1} protein. It is important to find new sources of this enzyme.
In the present study we investigated SOD activity of three strains *Saccharomyces cerevisiae* during cultivation (Figures 1, 2, 3). Based on the results it was shown, that there were some differences between SOD activity of different yeast strains.

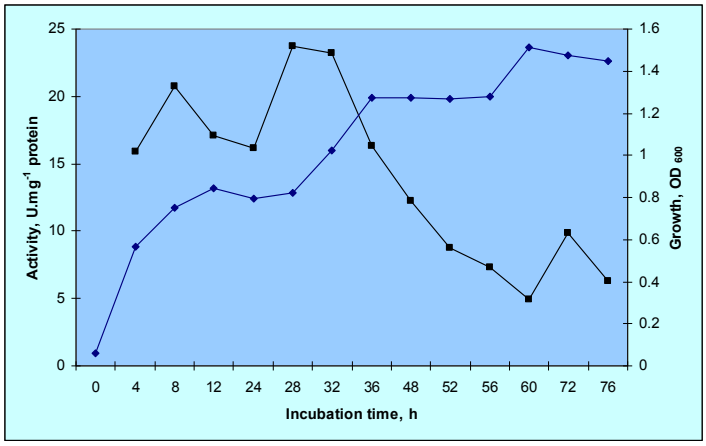

Figure 1 SOD activity during the cultivation of yeast *Saccharomyces cerevisiae* strain 612. Optical Density (OD) of yeast biomass at 600 nm (♦), activity of SOD (■)

The highest SOD activity (23.7 U.mg^{-1} protein) was found in strain 612 (Figure 1) after 28 hours of cultivation. This result confirm the finding of **Nedeva *et al.* (2004)** who observed SOD activity in the range 18.4 - 19.9 U.mg^{-1} protein in the yeast *Saccharomyces cerevisiae* depending on strain specificity. During following cultivation the SOD activity decreased very rapidly. The lowest value of SOD activity was found after 60 hours of cultivation (5.0 U.mg^{-1} protein).

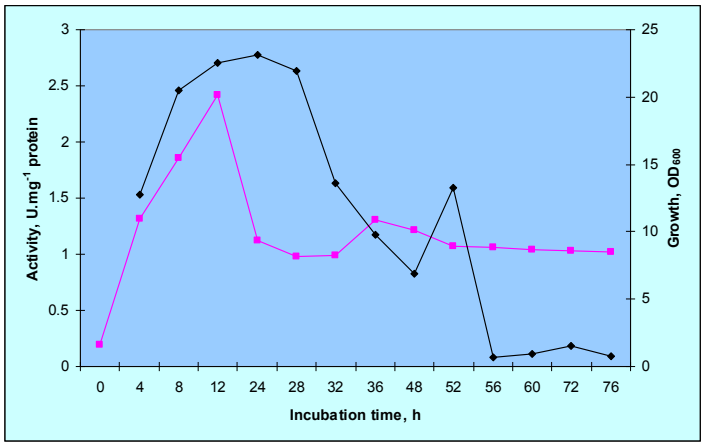

Figure 2 SOD activity during the cultivation of yeast *Saccharomyces cerevisiae* strain Gyöng. Optical Density (OD) of yeast biomass at 600 nm (■), activity of SOD (♦)

According to the data in Figure 2, the SOD activity of strain Gyöng (23.2 U.mg^{-1} protein) reached after 24 hours of incubation, is higher than reported **Kumar and Malhotra (2008)** in ber fruit (6.9 U.mg^{-1} protein). Based on our result we can conclude relatively high SOD activity in the yeast. The lowest SOD activity was found after 56 hours of cultivation (0.7 U.mg^{-1} protein) what means the lowest value of SOD activity of all tested yeast strains.

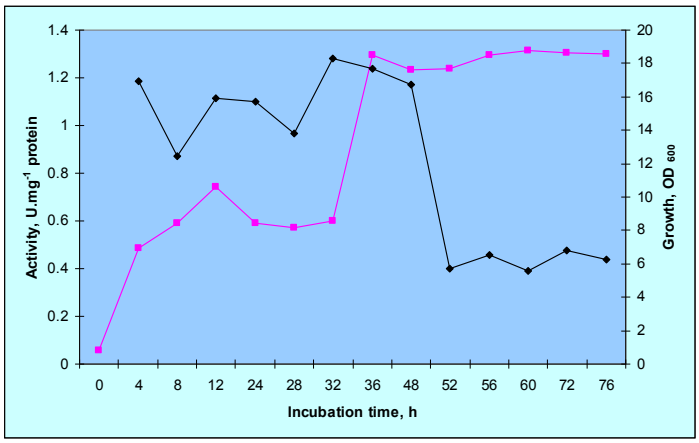

Figure 3 SOD activity during the cultivation of yeast *Saccharomyces cerevisiae* strain Kolín. Optical Density (OD) of yeast biomass at 600 nm (■), activity of SOD (♦)

During the cultivation of strain Kolín (Figure 3) the SOD activity was higher in first part of yeast growth, that means between 0 - 48 hours of cultivation. The maximum value of SOD activity (18.3 U.mg^{-1} protein) was obtained after 32 hours of cultivation. It is comparable to finding of **Guo *et al.* (2007)** who detected the SOD activity in *Thermoascus aurantiacus* of 19.2 U.mg^{-1} protein. The prolongation of cultivation time (after 52 hours) caused decreasing of SOD activity. The lowest value of SOD activity was reached in 60th hour of cultivation (5.5 U.mg^{-1} protein).
From the results mentioned above it is evident that *Saccharomyces cerevisiae* yeast biomass could be applied as a source of antioxidants in nutrition. This was confirmed also in previous study, when was found high total antioxidant activity of yeast strain 612, Gyöng and Kolín **(Lavová and Urminská, 2013)**.

CONCLUSION

This study confirmed the suitability of yeast as a potential supplement in nutrition for their health-promoting attributes, especially relatively high SOD activity. It was confirmed that all three strains of yeast *Saccharomyces cerevisiae* contain high SOD activity in particular phases of cultivation depending on strain specificity and that the highest SOD activity produced strain 612 (23.7 U.mg^{-1} protein) after 28 hours of cultivation.

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