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REGULAR ARTICLE

TOTAL ANTIOXIDANT ACTIVITY OF YEAST *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Antioxidants are health beneficial compounds that can protect cells and macromolecules (e.g. fats, lipids, proteins and DNA) from the damage of reactive oxygen species (ROS). *Sacchamomyces cerevisiae* are know as organisms with very important antioxidative enzyme systems such as superoxide dismutase or catalase. The total antioxidant activity (mmol Trolox equivalent – TE.g^{-1} d.w.) of *Saccharomyces cerevisiae* was measured by 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) during the yeast cultivation. It was found that the total antioxidant activity was the highest (1.08 mmol TE.g^{-1} d.w.) in the strain Kolín after 32 hours of cultivation and the lowest (0.26 mmol TE.g^{-1} d.w.) in the strain Gyöng after 12 hours of cultivation.

Keywords: antioxidant activity, ABTS, *Saccharomyces cerevisiae*

INTRODUCTION

In the last two decades there has been an explosive interest in the role of oxygen-free radicals, more generally known as “reactive oxygen species” (ROS) and of “reactive nitrogen species” (RNS) in experimental and clinical medicine. ROS/RNS are known to play a dual

role in biological systems, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS at low concentrations involve roles in defence against infectious agents and in the function of a number of cellular signaling systems. In contrast, at high concentrations, ROS can be important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids termed as oxidative stress **(Paulová *et al.*, 2004; Valko *et al.*, 2006)**.

The chemical diversity of antioxidants makes it difficult to separate and quantify individual antioxidants of natural compounds in foods or biological systems. Moreover the total antioxidant power as an "integrated parameter of antioxidants present in a complex sample" is often more meaningful to evaluate health beneficial effects because of the cooperative action of antioxidants **(Çelik *et al.*, 2010; Floegel *et al.*, 2011)**.

One of the spectrophotometric methods that have been applied to measurement of the total antioxidant activity in biological systems is $ABTS^{\cdot+}$ (2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay. The radical cation is generated by oxidation of ABTS with potassium persulfate **(Re *et al.*, 1998; Ozgen *et al.*, 2006; Teow *et al.*, 2007)**. This technique is based on the inhibition of the absorbance of the free radical $ABTS^{\cdot+}$ by antioxidants and on the relation of this radical cation with the antioxidant scavenger Trolox, which is the synthetic analogue of Vitamine E **(Salomao *et al.*, 2006)**.

The objective of this study were (1) to determine the antioxidant activity of yeast *Saccharomyces cerevisiae* during the growth and (2) to selection one of three yeast strains, which show the highest antioxidant activity and can be potential used as supplement in human nutrition.

MATERIAL AND METHODS

Microorganisms and growth conditions

Experiments were carried out with yeasts *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strains Kolín, 612 and Gyöng, which were obtained from distillery Slovenské liehovary a likérky, a.s. Leopoldov, Slovakia. The yeast was conserved in Malt Extract agar for microbiology (Merck, Germany) and cultivated on YPD (Yeast Peptone Dextrose) medium containing 10 $g.L^{-1}$ yeast extract (Imuna Pharm, Slovakia), 20 $g.L^{-1}$ peptone (Imuna Pharm, Slovakia) and 35 $g.L^{-1}$ (Lachema, Czech Republic). Yeast cells were grown under

shaking (MEZ, Czechoslovakia; 280 rpm) at 30 °C in dark and at initial cell densities of 0.5 x 10^6 cells.ml^{-1}.

Cell-free extract preparation

Yeast cells from 0 – 72th hour of cultivation were harvested by centrifugation at 5000 x g for 10 minutes and washed twice with distilled H_2O. Cell wall disruption was carried out according to the procedure of **Huang *et al.* (2010)** with some modification. Cell debris was removed by centrifugation at 5000 x g at 4 °C for 10 minutes and the supernatants were used for analysis.

$ABTS^+$ assay

ABTS was dissolved in H_2O to a concentration of 7 mM. The radical cation of ABTS was obtained by reaction with 2.45 mM potassium persulfate and allowing the stock solution to stand in the dark at room temperature for at least 12 hours. Before use, the $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm at 30 °C. Next, 1 ml of this $ABTS^{\cdot+}$ solution was added to 0.01 ml of sample and the decrease in absorbance was recorded for 10 minutes. A calibration curve for the Trolox equivalent antioxidant activity was built by plotting different concentrations of Trolox (mmol.L^{-1}) versus its total equivalent antioxidant activity. The antioxidant activity was calculated as the Trolox equivalent antioxidant capacity (TEAC) and expressed as microgram Trolox per millilitre of sample **(Thaipong *et al.*, 2006; Blanda *et al.*, 2008)**.

RESULTS AND DISCUSSION

In the present study we used a method that is able to assess the total antioxidant activity of biological samples as serum, tissues, fruit, microorganisms etc. In this work the total antioxidant activity of three strains *Saccharomyces cerevisiae* during cultivation was determined (Figures 1, 2, 3).

On the basis of results it was shown that at the beginning of cultivation there were some differences between antioxidant activity of yeast strains, the highest total antioxidant activity (0.71 mmol TE.g^{-1} d.w.) was found in strain Kolín (Figure 1) and the lowest (0.27 mmol TE.g^{-1} d.w.) in strain Gyöng (Figure 3).

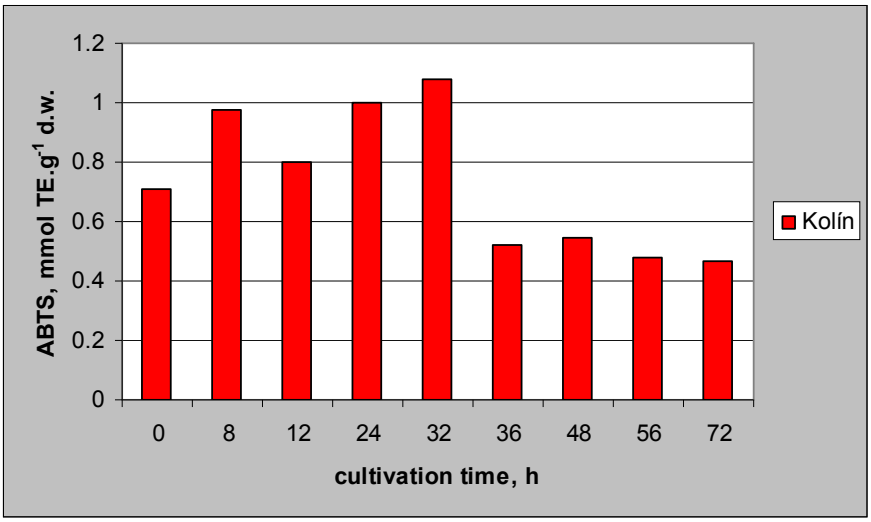

Figure 1 Changes of total antioxidant activity of *Saccharomyces cerevisiae* strain Kolín during cultivation

The highest antioxidant activity (1.08 mmol TE.g^{-1} d.w.) was obtained after 32 hours of cultivation of strain Kolín (Figure 1) and after next 4 hours it means in 36th hour of yeast cultivation the antioxidant activity decreased very rapidly to 0.52 mmol TE.g^{-1} d.w.

During the cultivation of strain 612 (Figure 2) the total antioxidant activity was higher to 24 hours of yeast growth. The maximum value of total antioxidant activity (0.81 mmol TE.g^{-1} d.w.) was obtained in 8th hour of cultivation and the prolongation of cultivation time (after 24 hours) caused decreasing of total antioxidant activity.

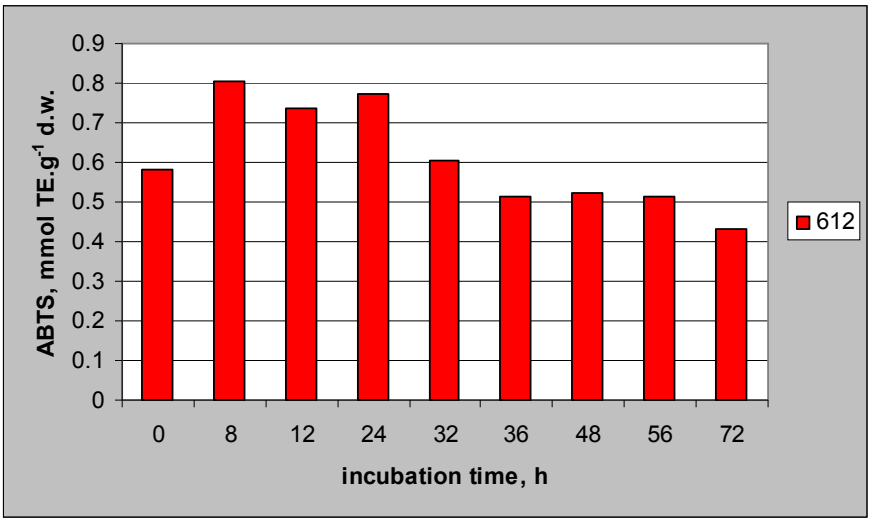

Figure 2 Changes of total antioxidant activity of *Saccharomyces cerevisiae* 612 during cultivation

In contrast with other analysed strains, total antioxidant activity of strain Gyöng (Figure 3) increased after 24 hours of cultivation. The lowest value of total antioxidant activity was reached in 12th hour of cultivation (0.26 mmol TE.g^{-1} d.w.), what means the lowest value of antioxidant activity in all tested yeast strains.

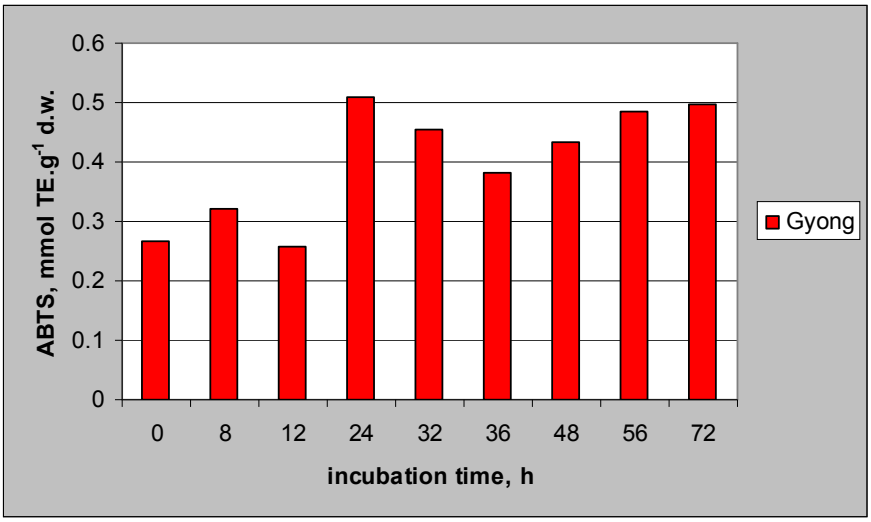

Figure 3 Changes of total antioxidant activity of *Saccharomyces cerevisiae* strain Gyöng during cultivation

Nowadays the study of total antioxidant activity is one of the most important topics in food research. *Saccharomyces cerevisiae* is extensively exploited organism and it follows from our results that this yeast can be also the source of antioxidative substances. **Teow *et al.* (2007)** reported that total antioxidant activity of sweet potatoes is between 0.1 – 1.3 mmol TE.g^{-1} f.w. and **Fidler and Kolářová (2009)** supposed total antioxidant activity of hop extract between 0.25 – 1.5 mmol TE.g^{-1} d.w. *Saccharomyces cerevisie*, especially strain Kolín in which is total antioxidant activity 1.08 mmol TE.g^{-1} d.w. could be used as food supplement in human nutrition not only for vitamine and protein content but also for high antioxidant activity.

CONCLUSION

This study confirmed the suitability of yeast as a supplement in nutrition for their nutraceutical or health-promoting attributes. Method based on radical-scavenging activity was used to determination of antioxidative status of *Saccharomyces cerevisiae*. It was confirmed that all three strains of yeast *Saccharomyces cerevisiae* show hight antioxidant activity. From

the results mentioned above it is evident that the hightest antioxidant activity shows strain Kolín (1.08 mmol TE.g^{-1} d.w.) after 32 hours of cultivation.

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