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REGULAR ARTICLE

COPPER ENRICHED YEAST *SACCHAROMYCES CEREVISIAE* AS A POTENTIAL SUPPLEMENT IN NUTRITION

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ABSTRACT

Copper is very important microelement found in all organs and tissues of the human body. Our aim was to investigate the influence of different Cu^{2+} concentrations on the yield of yeast biomass and the ability of the strain 612 of *Saccharomyces cerevisiae* to absorb different salts of copper. Metal content in yeast biomass was determined by AAS method. The highest increasing of copper amount, i.e. 11.74 $mg.g^{-1}$ was detected after 48 hours of cultivation on YPD medium with addition of 150 $mg.100\ ml^{-1}$ in the form of copper sulphate.

Keywords: Copper, Yeast, *Saccharomyces cerevisiae*

INTRODUCTION

The uptake of trace elements by the yeast *Saccharomyces cerevisiae* is the object of very important research in recent years **(Mapolelo and Torto, 2004; Stehlik-Tomas et al.,**

2004; Wang and Chen, 2006). Yeast biomass as well as biomass enriched with trace minerals has been demonstrated to be non-conventional alternative protein sources and useful in improving animal health and growth performance **(Fernandes et al., 1998; Stehlik-Tomas et al., 2009)** and represents a valuable supplement to human diet because of high content of proteins and vitamins, too. It is known for its ability to accumulate metal ions and to form organically bounded microelements **(Stehlik-Tomas et al., 2003; Mrvčić et al., 2007; Šillerová et al., 2010)**. Biogenic microelements, i.e. Zn, Cu, Mg, Se, Fe play an important role primarily as cofactors for a large number of enzymes. Copper is essential element for cuproenzymes in which is bound to specific amino acid residues in an active site **(Prohaska and Gybina, 2004; Stehlik-Tomas et al., 2004)**. All living organisms require copper for growth and development, it is necessary for bone formation, energy metabolism, nerve transmission, pigmentation of the skin, normal hair growth and red blood cell production **(Pang et al., 2001; Kuo et al., 2006)**. Copper is actively absorbed from the blood in the upper small intestine and bound to albumin **(Skalická et al., 2009)**. The adult human body contains about 70 – 100 mg of copper, muscles contain about 50 % of total body copper **(Bhagavan, 2002)**.

MATERIAL AND METHODS

Biological material

Saccharomyces cerevisiae Meyen ex E.C. Hansen strain 612 was obtained from the distillery Slovenské liehovary a likérky, a. s. Leopoldov, Slovakia. The yeast was maintained on Malt Extract agar for microbiology (Merck, Germany) and was grown on Yeast Peptone Dextrose medium (YPD) **(Šillerová et al., 2010)**.

The conditions of cultivation

According to **Shanmuganathan et al. (2004) and Barbulescu et al. (2010)** the stock solutions were prepared in the forms of $Cu(NO_3)_2 . 3 H_2O$ (POCH, Poland), $CuSO_4 . 5 H_2O$ and $CuCl_2 . 2 H_2O$ (Lachema, Czech Republic) in deionised water. The concentration of the solutions was 10 % w/w with pH of 3.2 for copper nitrate, pH of 3.5 for copper sulphate and pH of 2.8 for copper chloride. Cu^{2+} was added to the required final concentrations of 30, 60, 90, 120, 150 and 180 mg.100 ml^{-1} of YPD medium which was inoculated with initial cell densities of 0.5 x 10^6 cells.ml^{-1}. Yeast were cultivated by submersed cultivation under aerobic conditions on an orbital shaker (MEZ, Czechoslovakia; 280 rpm) at 30 ˚C in dark for 48 h. Biomass was harvested by centrifugation (K 70 D, Engelsdorf/Leipzig; 1509 x g, 30 min), rinsed twice with distilled water and lyophilized (LYOVAC GT 2, AMSCO/FINN-AQUA, Germany).

Sample preparation and AAS determination

Enriched biomass of yeast was boiled with glass beads in concentrated nitric acid and hydrogen peroxide; after than filtrated using Filter Paper MN 615 ¼ (210 mm). Copper cell content in yeast biomass was measured by AAS method (Varian Spectr AA100), using an air-acetylene flame, OD = 324.7 nm with copper lamp current of 4 mA.

Statistical evaluation

The software STATISTICA **(StatSoft, Inc. (2005). STATISTICA Cz, version 7.1.)**, one-way analysis of variance (ANOVA) for data analysis was used. Results for cellular copper content in yeast and the influence of copper on the yield of biomass were performed with the Fisher's LSD test at P = 0.05. Data are presented as means ± SD.

RESULTS AND DISCUSSION

The yeast cells were cultivated in the medium with increasing addition of three different copper salts and the effect to biomass yield and content of copper in the yeast biomass was studied (Fig 1 - 2). Based on results it was shown that the differences in the yield of yeast biomass in all three used copper forms (Fig 1) were significant (P = 0.000). It was obtained up to 1.2 g of dry yeast biomass. Higher concentration, i.e. 150 mg.100 ml^{-1} YPD in the form of copper chloride caused the death of yeast cells. **Silóniz et al. (2002)** reported that higher concentrations of Cu^{2+} affected both morphology and physiological parameters of the viable yeast and markedly decreased yeast cell growth rate **(Mrvčić et al., 2007)**. The higher concentrations of Cu^{2+} in all forms (Fig 2) caused significantly higher content of Cu^{2+} in the yeast cells (P = 0.000). The highest value of Cu^{2+}, i.e. 11.74 mg.g^{-1} of dry yeast biomass was reached in medium contained 150 mg of copper sulphate. **Stehlik-Tomas et al. (2004)** reported that the highest value of copper ions in dry matter was 1.1 $mg.g^{-1}$ and **Mrvčić et al. (2007)** found that the maximum amount was 1.16 $mg.g^{-1}$.

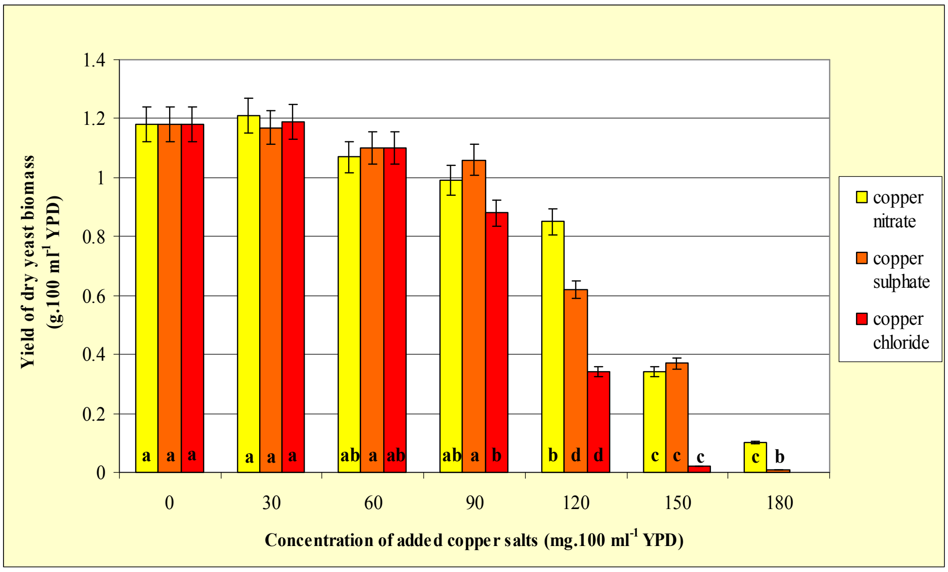

Figure 1 The influence of three copper salts on the yield of yeast biomass, $P < 0.05$, values are means ± SD, n = 6.

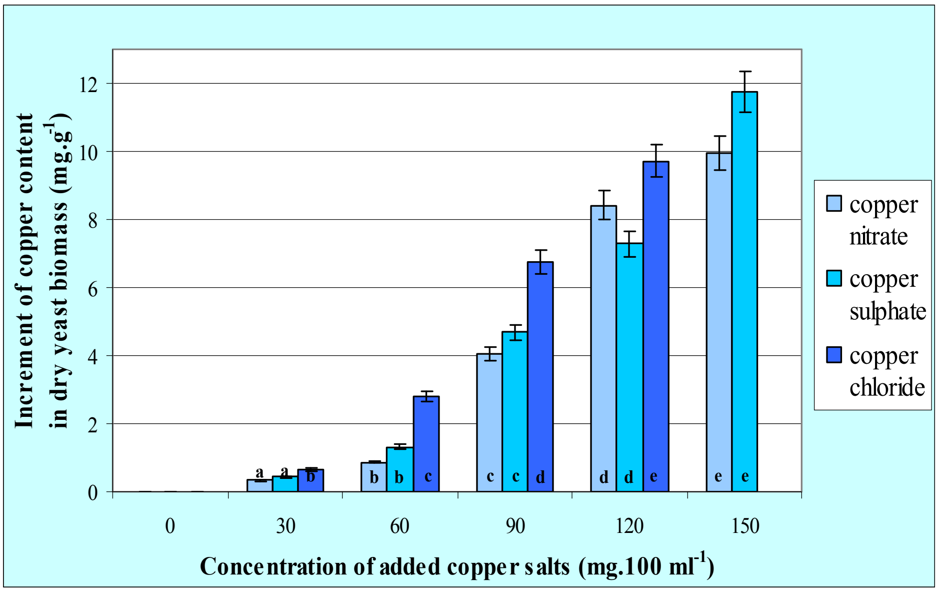

Figure 2 Influence of added copper salts on the copper content in yeast biomass, $P < 0.05$, values are means ± SD, n = 3.

CONCLUSION

According to the research carried out, it was shown that tested concentrations of all copper forms influenced the yield of cultivated biomass as well as the content of Cu^{2+} in the studied biomass. Copper enriched biomass can be potentially used as a supplement of this important microelement in feed and/or food nutrition in natural form.

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