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REGULAR ARTICLE

PRODUCTION OF ERGOSTEROL BY *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

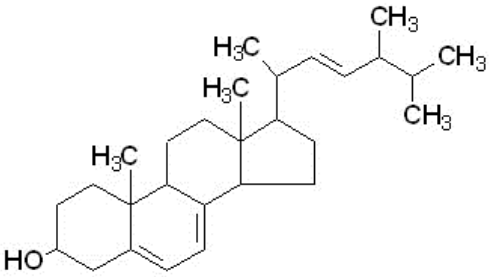
Ergosterol is an essential component of yeast cells that maintains the integrity of the membrane. In this study the production of ergosterol by yeast *Saccharomyces cerevisiae* strains Kolín, Gyöng and 612 was investigated. Ergosterol was isolated by multilevel extraction associated with saponification and analyzed by reverse phase high performance liquid chromatography with PDA detector. It was found that the highest content of ergosterol (7055.53 $\mu g.g^{-1}$ d.w.) was reached after 52 hours of strain Gyöng cultivation.

Keywords: ergosterol, *Saccharomyces cerevisiae*, sterols

INTRODUCTION

Sterols are essential components of all eukaryotic cells and are present in two forms as free sterols and steryl esters. Free sterols are located mainly in the plasma membrane, where they are very important for the fluidity and permeability of the membrane and have

various effects on the activities of membrane-bound proteins. The steryl esters are sequestered in cytosolic lipid particles, where they play an important role in sterol homeostasis **(Lamačka and Šajbidor, 1997; Shobayashi *et al.*, 2005; Yuan *et al.*, 2006).** The predominant sterol in *Saccharomyces cerevisiae* is ergosterol (Picture 1), which is an economically important metabolite.



Picture 1 Ergosterol

(http://themedicalbiochemistrypage.org/images/ergosterol.jpg)

Ergosterol is a fungal specific sterol a precursor of vitamin D_2 (ergocalciferol) which can be transformed into vitamin D_2 by ultraviolet irradiation **(Bhosle *et al.*, 2011)**. Vitamin D_2 has important role in the body to absorb Ca^{2+}, PO_4^{3-} and in preventing rickets and osteoporosis. Ergosterol also plays an important role in physiological function, what is the reason for study yeast, especially *Saccharomyces* sp. as a source of ergosterol **(He *et al.*, 2000; Rychtera *et al.*, 2010; Wu *et al.*, 2012)**.

MATERIAL AND METHODS

Microorganisms and cultivation conditions

Experiments were carried out with yeasts *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strains Kolín, Gyöng and 612, which were obtained from distillery Slovenské liehovary a likérky, a.s. Leopoldov, Slovakia. The yeast were conserved in Malt Extract agar for microbiology in darkness at 4 °C and was grown on YPD (Yeast Peptone Dextrose) medium containing 10 $g.L^{-1}$ yeast extract, 20 $g.L^{-1}$ peptone and 35 $g.L^{-1}$ glucose. Yeast cells were grown under shaking (Yellow line, RS 10 Basic, SRN) at 30 °C in dark and at initial cell densities of 0.5 x 10^6 $cells.ml^{-1}$ **(Šillerová *et al.*, 2010)**.

Sample preparation for ergosterol determination

Yeast cells were collected by centrifugation (Sigma Laborzentrifugen, SRN) at 5000 x g for 10 minutes and suspended in 50 ml of acetone. Cells were disrupted in suspension of sea sand and 50 ml 10 % alcoholic KOH was added. Saponification of the mixture was carried out by heating at 90 °C for 30 minutes. The mixture was cooled to room temperature, sterols were extracted with 50 ml of diethyl ether and evaporated at 50 °C to dryness (Vacuum evaporator RV 06 IKA, SRN). Sterols were dissolved in 2 ml ethanol for HPLC and the mixture was centrifuged, filtered (filter for HPLC, PRE-CUT 0.45 μm, Alltech GB) and then injected into RP - HPLC (Photo Diode Array detector, Surveyor Plus, Thermo Finnigan, USA) **(Márová *et al.*, 2010)**.

Determination of the ergosterol content

Ergosterol was separated on column Kinetex C18, 2.6 μm, 150 x 4.6 mm, Phenomenex. The analysis was performed at 45° C and fotometric detection of ergosterol was done at 285 nm. Isocratic elution was carried out by methanol for HPLC with flow rate of 1 $ml.min^{-1}$. Data processing of analyses was assessed using Xcalibur 1.3 software (Thermo Finnigan, USA).

RESULTS AND DISCUSSION

In this work ergosterol production of three strains yeast *Saccharomyces cerevisiae* during the 0 – 76th hour cultivation was determined (Figures 1, 2, 3). At the beginning of cultivation the highest ergosterol content was reached by strain Kolín (Figure 1). The highest content of ergosterol (1139 $\mu g.g^{-1}$ d.w.) was obtained after 28th hour cultivation and the prolongation of cultivation time (more than 32 hours) effected the decreasing of ergosterol production.

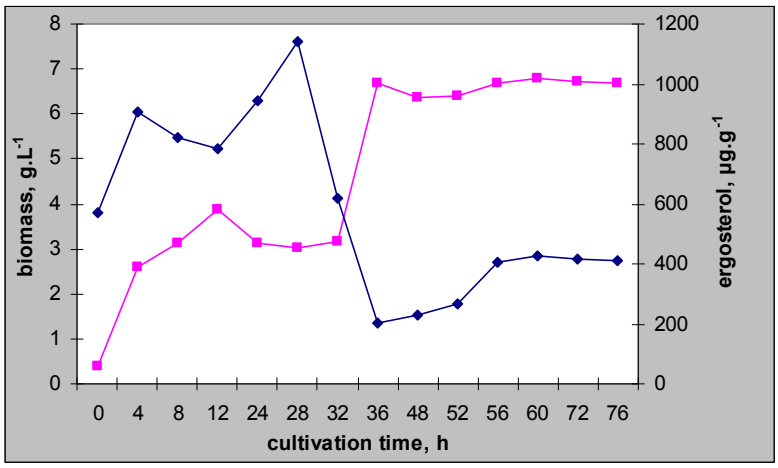


Figure 1 Biomass and ergosterol production during the yeast *Saccharomyces cerevisiae* strain Kolín cultivation. Ergosterol content (♦), biomass (■).

Ergosterol production by the strain Gyöng (Figure 2) was in first growth phase lower in the comparison to the strain Kolín, but in 52nd hour of cultivation the production of sterols increased to 7055.53 µg.g^{-1} d.w., which means the highest value of ergosterol content in all tested yeast strains.

Shobayashi *et al.* (2005) tested effects of culture conditions on ergosterol biosynthesis by *Saccharomyces cerevisiae*. Ergosterol was investigated as an important factor in the ethanol tolerance of yeast cells and the total production ranged from 5800 to 16500 µg.g^{-1} d.w. depencence of glucose concentration in cultivation medium.

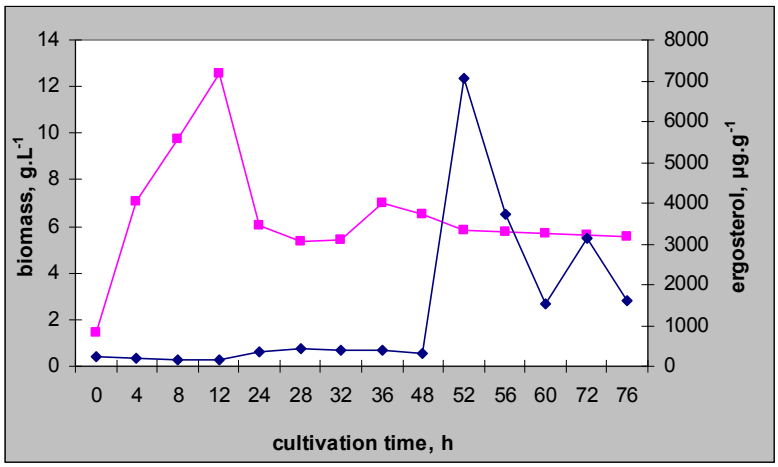


Figure 2 Biomass and ergosterol production during the yeast *Saccharomyces cerevisiae* strain Gyöng cultivation. Ergosterol content (♦), biomass (■).

The highest concentration of ergosterol in strain 612 (Figure 3) was produced between 28th and 52nd hour of cultivation and the maximal ergosterol production was in 48th hour of cultivation (2822.38 $\mu g.g^{-1}$ d.w.) compared with **Lopez *et al.* (2010)** reported that ergosterol content in *Saccharomyces cerevisiae* in 48th hour of cultivation was 210 $\mu g.g^{-1}$ d.w. From our results it follows that *Saccharomyces cerevisiae* strain 612 can be very important producer of such interesting substance as is ergosterol.

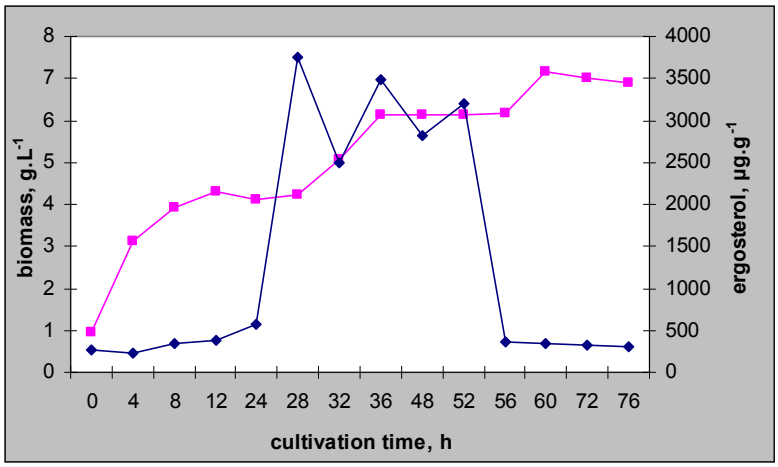


Figure 3 Biomass and ergosterol production during the yeast *Saccharomyces cerevisiae* strain 612 cultivation. Ergosterol content (♦), biomass (■).

Landolfo *et al.* (2010) studied the effects of lipid nutrition on wine yeast oxidative stress response in two strains of *Saccharomyces cerevisiae*. Biomarkers of oxidative stress, oxidative damage and antioxidant response were evaluated together with viability and acetic acid production during fermentation of a synthetic must lacking lipid nutrients as compared to added oleic acid and ergosterol. The results show that the availability of lipid nutrients causes a significant reduction in the intracellular content of reactive oxygen species and in the oxidative damage to membranes and proteins. The supplementation of lipid nutrients mitigates oxidative stress and oxidative damage in wine strains of *Saccharomyces cerevisiae* during growth under unfavourable conditions. In previous work **Šillerová *et al.* (2011)** described strains Kolín, Gyöng and 612 as a source of biogenic substances, especially vitamin B_2. Based on these facts and according to our results we can conclude that biomass of all tested strains of yeast *Saccharomyces cerevisie* could be used as a part of food supplement in human nutrition.

CONCLUSION

This study confirmed the suitability of specific yeast strains as a potential nutrition supplement for their health-promoting attributes, especially high content of ergosterol. Based on the results it was shown that all three strains of yeast *Saccharomyces cerevisiae* produced relatively hight values of ergosterol in particular phases of cultivation depending on strain specificity. From the results mentioned above it is evident that *S. cerevisiae* yeast biomass could be applied as a complex source of ergosterol widely used as provitamin D.

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