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ORIGINAL PAPER

INFLUENCE OF INCUBATION CONDITIONS ON BAKER'S YEAST ENRICHMENT IN SELENIUM

Beata Szulc-Musioł[1], Barbara Dolińska[1], Florian Ryszka[2]

[1]Department of Applied Pharmacy
School of Pharmacy and the Division of Laboratory Medicine
Medical University of Silesia
[2]Pharmaceutical Research and Production Plant „Biochefa", Sosnowiec

Abstract

Selenium has been recognized as an essential nutrient for human health; however, the gap between toxic and essential levels of selenium is narrow. The recommended daily selenium dose for adult humans ranges from 50-100 µg and it should not exceed 400 µg. The capability of baker's yeast to bind with Se^{+4} ions which are present in the yeast-culture media has provided an opportunity to obtain natural bioplexes of this microelement. The influence of incubation conditions on efficiency of selenium enrichment in baker's yeast was studied. The study process factors were as follow: factor 1: glucose concentration (0.055, 0.166, 0.277 mol dm^{-3}); factor 2: incubation temperature (25, 30, and 35°C); factor 3: selenium concentration (1, 4, 7 10^{-4} mol dm^{-3}). The yeast cell enrichment with selenium was conducted for 24 hrs. Data were analysed by using the Pearson correlation coefficient. The selenium bioaccumulation values ranged from 141.67 to 1136.69 mg kg^{-1} of dried baker's yeast. It was shown that the content of total selenium (r = 0.99, p = 0.0001) and organic selenium (r = 0.99, p = 0.0001) in yeast biomass correlated positively with its concentration in the culture medium, while selenium concentration in the medium had significantly negative correlation with yeast biomass (r = -0.92, p = 0.0001). The Pearson correlation coefficient showed a significant correlation between glucose concentration and biomass yield (r = 0.27, p = 0.048). Temperature (r = 0.44, p = 0.002) and the concentration of glucose in culture medium (r = 0.80, p = 0.0001) correlated positively with total process efficiency. The highest amount of selenium (1136.69 mg kg^{-1}) incorporation was obtained in variant 8: with sugar concentration of 0.277 mol dm^{-3} and selenium concentration of 7 10^{-4} mol dm^{-3} in the medium, at the temperature of 35°C.

Keywords: baker's yeast, sodium selenite, selenium enrichment, incubation conditions.

dr Beata Szulc-Musioł, Department of Applied Pharmacy, School of Pharmacy and Division of Laboratory Medicine, Medical University of Silesia, Kasztanowa Street 3, 41-200 Sosnowiec, Poland, e-mail: bszulc@sum.edu.pl

INTRODUCTION

Selenium is an essential trace element indispensable for proper nutrition of humans and animals alike, although it may produce toxic effects in situations when its dose exceeds the physiological demand for it. Selenium is required in microgram amounts; the recommended daily allowance (RDA) is 55 μg per day, and it has a narrow margin of safety, the upper tolerable intake limit is 400 μg per day (Hawkes et al. 2008).

Inadequate supply of selenium has been associated with predisposition to or manifestation of various human diseases such as Keshan and Kashin-Beck disease, cancer, impaired immune function, neurodegenerative and age-related disorders and disturbances of the thyroid hormone axis (Kafai, Ganji 2003, Stehlik 2003, Duntas 2006, Boosalis 2008, Facompre, El-Bayoumy 2009).

The recent identification of various distinct selenocysteine-containing proteins, encoded by 25 human genes, provides information on the molecular and biochemical basis of beneficial and possible adverse effects of this trace element (Papp et al. 2007).

Indirectly through these selenoenzymes, this trace element is a cofactor of several metabolic pathways, including thyroid hormone metabolism, antioxidant defence systems and immune function (El-Bayoumy et al. 2002, Negro 2008, Ravn-Haren et al. 2008).

Selenium could occur in pharmaceuticals as organic compounds (selenomethionine, selenocysteine) or inorganic compounds (selenate, selenite). A popular form of supplements is selenium yeast (Rayman 2004). Selenium administered in the form of a bioplex is absorbed more efficiently than in the form of its inorganic salts simply because in combination with proteins it permeates through the hydrophobic biological membranes without any difficulty (Rayman 2004, Fairweather-Tait et al. 2010).

The present study is a continuation of the research conducted by our Department on microelement incorporation into baker's yeast cells. Our former studies already demonstrated the high capability of the yeast to bind with microelements such as copper, iron, manganese, chromium, zinc, iodine and selenium (Dolińska et al. 2009, 2011, 2012). In the present study, baker's yeast biomass has been enriched with selenium in the course of the bioabsorption process, owing to which it constitutes a carrier for this microelement both in human diet supplementation and in domestic animal nutrition. This way of selenium administration enables us to avoid the possible danger of toxic concentration of this microelement (Griffiths et al. 2006, Schrauzer 2006). Another advantage is that it allows us to obtain waste-free production during selenium incorporation into the yeast cells. In laboratory conditions, the effects of the selected factors, such as glucose and Se^{+4} salts concentrations as well as the incubation temperature upon the efficiency of baker's yeast enrichment with selenium were determined.

MATERIAL AND METHODS

Chemicals

Fresh *Saccharomyces cerevisiae* was purchased from the company Lesaffre, Wołczyn, Poland. The yeast contained 33% dry matter and had a soluble protein content of 26 mg g^{-1} wet matter and 78 mg g^{-1} dry matter. Anhydrous glucose, pure for analysis, was purchased from Pliva, Cracow, Poland; sodium selenite was purchased from Aldrich, USA. A Hydrolab water purification system was used to obtain the water used in the experiment.

Experiment Procedure

Crumbled yeast (30.4 g of fresh, 10 g of dry mass) was added to 0.2 dm^{-3} of the aqueous solutions of glucose of the final concentrations of 0.055 and 0.277 mol dm^{-3}, respectively, and then stirred up for 10 minutes, using a magnetic stirrer. Next, selenium solution in the form of sodium selenite was added until the respective concentrations were 1, and 7 10^{-4} mol dm^{-3}. The whole mixture was again stirred for 10 min. until homogenous suspension was obtained. In culture control, the concentration of glucose was 0.166 mol dm^{-3} and selenium was 4 10^{-4} mol dm^{-3}.

The cultures were incubated for 24 hrs in a rotary shaker (KS-15-Controler Edmunt Buchler GmbH) at 120 rpm, at frequency of 120 cycles per minute and temperatures of 20, and 35°C, respectively. The incubation temperature for the control was 30°C. Following the incubation, the yeast suspension was centrifuged (approximately 1500 x g, for 15 minutes, using MPW-360 centrifuge, Poland). The supernatant was decanted while the sediment was twice suspended in 100 ml deionized water, stirred up for about 5 minutes and then centrifuged again. The obtained wet sediment was initially dried at the temperature of 60°C for 2 hours. Next, the process of drying up was continued at 105°C, until the solid mass was obtained. For measure of inorganic selenium, the suspension of biomass in ultrapure water was extracted in a boiling bath for 1 h and made to a constant volume. Next, the mixture was centrifuged at 8500 x g for 15 minutes. The design of the experiment is shown in Table 1. All the experiments were repeated five times.

Selenium assay procedure

The content of selenium in the yeast was determined by the fluorometric method according to Watkinson, using a Perkin-Elmer Model LS-30 fluorescence spectrofluorometer setting, excitation and emission wavelengths 356 and 520 nm, respectively, as previously described (Danch, Drożdż 1996).

Statistical analysis

The results were calculated as mean values ($x \pm$ SD) of five replicates. The statistical analysis was carried out using the Statistica (StatSoft Inc.)

Table 1

Desing of experiment

Variant	X1	X2	X3	X1- glucose concentration (mol dm^{-3})	X2 - incubation temperature (°C)	X3 - selenium concentration (mol dm^{-3})
1	-1	-1	-1	0.055	25	0.1
2	-1	-1	+1	0.055	25	0.7
3	-1	+1	-1	0.055	35	0.1
4	-1	+1	+1	0.055	35	0.7
5	+1	-1	-1	0.277	25	0.1
6	+1	-1	+1	0.277	25	0.7
7	+1	+1	-1	0.277	35	0.1
8	+1	+1	+1	0.277	35	0.7
9	0	0	0	0.166	30	0.4

software. Correlations between the parameters were examined by the Pearson correlation coefficient (r). The statistical significance of the differences between the means was analysed using the Fisher-Snedecor test. A level of $p \leq 0.05$ was adopted to indicate statistical significance.

Total process efficiency of the obtained yeast biomass was calculated according to the following formula:

$$\text{Total process efficiency (\%)} = \frac{\text{selenium content in culture medium (mg)}}{\text{the amount of built-in selenium in of dry biomass (mg kg}^{-1}\text{)}} \cdot 100\%$$

RESULTS AND DISCUSSION

The *Saccharomyces cerevisiae* yeast is a unicellular organism belonging to *Ascomycetes* fungi. The knowledge of its genetics, biochemistry and molecular biology has enabled the use of *Saccharomyces cerevisiae* on a large scale in industry. Particular attention should be drawn to their high capability of forming large populations of cells as well as to the fact that they are non-pathogenic for humans, and therefore a large scale synthesis of foods and pharmaceutics free of toxic contaminants is possible. In addition, baker's yeast contains many valuable components like vitamin B (Varga, Maráz 2002), and demonstrates the capability of natural binding of the elements present in the environment. Metal ions adsorbed on the cell's surface may be then a subject of intracellular bioacummulation. The research on obtaining yeast biomass enriched with organic compounds of selenium has been carried out. Such studies were conducted in Poland among others by Danch and Chmielowski (1985), Diowksz et al. (1999), Tuszyński and Pasternakiewicz (2000), Achremowicz et al. (2002).

Both efficiency and speed of the product manufacture depend on many factors such as tolerance to varying concentrations of the yeast-culture me-

dium constituents and nutritional and temperature demands in the technological process. Also, other process parameters such as – pH, viscosity, shaking speed, aeration, and foaming properties of the yeast culture should be taken into consideration.

For the production of selenium enriched yeast the following culture systems are applied: batch, fed-batch, and continuous culture, of which but batch strategy seems to be most common. Depending on the incubation conditions, the amount of selenium that could be incorporated into yeast cells may range between 500 to 3000 ppm (Demirci, Pometto 1999). The biosorption mechanism by which the selenium is accumulated in yeast cells depends on the oxidation state. The results obtained by Perez-corona et al. (1997) indicated that Se(VI) showed low affinity for yeast cells and remained in solution while Se (IV) was accumulated into the cell. Similar conclusions were reached by Demirci and Pometto (1999). They described a continuous fermentation method for incorporation of sodium selenite or sodium selenate into *Sacharomyces cerevisiae* biomass. Lower biomass and less selenium content were produced in a medium with sodium selenite compared to fermentation with sodium selenite.

The method of sodium selenite addition to the nutrient medium also has an effect on accumulation of selenium in yeast cells. Ponce de Leon et al. (2002) revealed that the amounts of selenium incorporated in seeded yeast are approximately four times higher than the amount of incorporation by single enrichment in the growth phase. However, the growth phase enrichment ultimately yields more L-selenomethionine. Suhajda et al. 2000 observed that adding of sodium selenite (30 μg ml^{-1} in 6 parts) during the exponential growth phase resulted in selenium-accumulation in 2050 μg g^{-1} dried baker's yeast and 50% of inorganic selenium content in the yeast. While addition of sodium selenite after the phase of logarithmic growth resulted in 1500 μg g^{-1} dried baker's yeast and 27% of inorganic selenium content in the yeast.

Exposure of *Saccharomyces cerevisiae* to the effect pulse electric field (PEF) resulted in increase of selenium accumulation in relation to control culture without PEF. PEF exerts its effect by causing the formulation of pores in the membrane of treated cells and increasing permeability for macromolecules in its membrane (Pankiewicz, Jamroz 2007).

Baker's yeast has been successfully enriched with different trace elements (Hegoczki et al. 1997, Tuszyński, Pasternakiewicz 2000, Stehlik-Tomas et al. 2004, Philpott 2006, Osterc et al. 2009, Gaensly et al. 2014). The concept of yeast biomass enrichment with microelements through their bioaccumulation and bioabsorption starts to be implemented in the industrial practice.

The present study has determined the effects of glucose and selenium concentrations in the yeast-culture medium as well as incubation temperature upon efficiency of the process of obtaining selenium yeast biomass. The effect of incubation conditions on the process of selenium incorporation into the *Saccharomyces cerevisiae* yeast is shown in Table 2. It can be seen

Table 2

The influence of incubation conditions on process parameters of selenium-enriched yeast

Variant	Dried yeast biomass (g)	Total selenium accumulation (mg kg^{-1})	Organic selenium formation (mg kg^{-1})	Total process efficiency (%)
1	7.654 ± 0.087	162.23 ± 7.888	141.67 ± 6.430	68.593 ± 3.526
2	5.702 ± 0.044	1013.47 ±11.154	971.05 ± 11.777	70.063 ± 1.994
3	8.580 ± 0,130	167.25 ± 9.134	155.20 ± 4.923	84.279 ± 4.440
4	6.354 ± 0.088	1018.31 ± 10.575	961.34 ± 12.257	77.272 ± 1.519
5	8.740 ± 0.109	176.51 ± 15.494	160.23 ± 6.881	88.634 ± 2.300
6	6.638 ±0.079	1136.67 ± 19.765	1046.74 ± 15.429	87.979 ± 1.606
7	8.818 ± 0.188	184.85 ± 10.148	171.66 ± 3.439	95.825 ± 1.117
8	6.622 ± 0.074	1204.92 ± 37.370	1136.69 ± 10.445	95.252 ± 2.078
9	7.048 ± 0.045	643.38 ± 17.202	612.30 ± 11.498	91.044 ± 1.129

that the more selenium was added to the yeast medium, the higher the amount of selenium was incorporated into *S. cerevisiae* (it was circa six times higher after the amount of selenium added to the yeast was increased from 1 to 7 10^{-4} mol dm^{-3}). Depending on incubation conditions, the amount of in -built selenium into yeast cells varies from 141.67 to 1136.69 mg kg^{-1} dry mass which is respectively 57 to 88% of the selenium amount added to the culture medium. The highest amount of selenium (1136.69 mg kg^{-1}) incorporated was obtained in variant 8: with sugar concentration of 0.277 mol dm^{-3} and selenium concentration 7 10^{-4} mol dm^{-3} in the medium, at temperature of 35°C. The resultant selenium yeast biomass, regardless of the conditions under which the experiment was carried out, had a drab colour.

The results of the correlation analysis between incubation conditions and features of selenium baker's yeast (total selenium, organic selenium, biomass yield, total process efficiency) are summarized in Table 3. Selenium concen-

Table 3

Pearson correlation coefficients between incubation conditions and examined parameters of selenium yeast

Incubation conditions	Total selenium accumulation		Organic selenium formation		Biomass yield		Total process efficiency	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Glucose concentration	0.08	0.583	0.08	0.598	0.27	0.048	0.80	0.0001
Incubation temperature	0.01	0.924	0.03	0.849	0.18	0.242	0.44	0.002
Selenium concentration	0.99	0.0001	0.99	0.0001	-0.92	0.0001	-0.06	0.677

r – Pearson correlation coefficient, *p* – significance

tration in the medium was a factor that had significantly positive correlated with total selenium (r = 0.99, p = 0.0001) and organic selenium (r = 0.99, p = 0.0001) in yeast biomass. However, selenium concentration in the medium had a significant negative correlation with yeast biomass (r = -0.92, p = 0.0001).

Temperature and the concentration of glucose in the culture medium did not demonstrate any relation with the total selenium accumulation (r = 0.01, p = 0.924; r = 0.08, p = 0.583) or the organic selenium formulation (r = 0.03, p = 0.849; r = 0.08, p = 0.598). The concentration of glucose in the culture medium was a parameter that showed significantly positive correlation with yeast biomass (r = 0.27, p = 0.048). At a high level of glucose, the amount of biomass yield increased.

The study showed that total process efficiency was significantly correlated with temperature (r = 0.44, p = 0.002) and the concentration of glucose (r = 0.80, p = 0.0001). On the other hand, reverse but not significant correlation (r = -0.06, p = 0.677) was found between total process efficiency and selenium concentration in the culture medium.

Maximum percentage (ca 96%) of selenium built into the yeast cells was obtained in variants 7 and 8. In this case, nearly waste-free selenium yeast production, with the lowest amount (about 4%) of the remaining unincorporated selenium in the culture medium, was achieved.

It was estimated that in appropriate culture conditions a yeast cell can incorporate around 6000 ppm of selenium but full replacement of methionine by seleno-methionine is not possible (Schrauzer 2006). Cells do not absorb all ions present in the culture medium, even in the case of their low concentration. Thus, accumulation of metal ions probably depends on intracellular transportation systems and on their chelating strength by medium compounds and cellular substances (Tuszynski 2000).

CONCLUSIONS

Fermentations performed in the medium with higher sodium selenite concentrations produced lower biomass yeast and more accumulation of selenium in baker's yeast compared to fermentations with lower concentration of this compounds. Increased temperature and higher concentration of glucose in culture correlated positively with medium total process efficiency.

Regardless of the incubation conditions, selenium yeast biomass obtained in all the samples had a drab colour and the content of inorganic selenium in the finished product was approximately 7%.

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