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ORIGINAL RESEARCH PAPER

INDUSTRIAL NUTRIENT MEDIUM USE FOR YEAST SELENIUM PREPARATION

GINA MARINESCU, ANTONETA GABRIELA STOICESCU
"Dunarea de Jos" University of Galați, Faculty of Food Science and Engineering, 111 Domneasca St., 800201 Galati, Romani
marinescugina62@yahoo.com, antoneta.stoicescu@ugal.ro

LILIANA TEODOROF
Danube Delta National Institute of Research and Development,165 Babadag St., Tulcea, Romania

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The paper presents the preparation of selenium yeast from spent brewer's yeast by using industrial nutrient medium (malt wort and sparge water). The chemical treatment of the yeast biomass was done with sodium-selenite solution. The preparation of selenium – enriched *Saccharomyces uvarum* in different conditions of temperature, time, yeast biomass proportion and sodium-selenite concentration of the medium was analysed. Using a culture medium supplemented with 30–180 µg/ ml sodium-selenite results in total selenium accumulation in the range of 0.6–2.2 mg/g for malt wort and 0.4–1.6 mg/g for sparge water and in organic selenium accumulation in the range of 0.6–1.5 mg/g for malt wort and 0.3–0.9 mg/g for sparge water measured by ICP- MS method.

Keywords: organic selenium, sparge water, malt wort, sodium-selenite

Introduction

The spent brewer's yeast reuse is necessary and very important in Romania today as an environmental protection measure. A reuse possibility can be the yeast enrichment with minerals such as Selenium.

Selenium (Se) is an essential trace element for human and animal health. The physiological role of selenium was firstly appreciated following the evidence that the element is an essential component of glutathione peroxidase which has important antioxidant and detoxification functions (Suhajda *et al*., 2000; Zeng and Combs, 2008; Zhou *et al*., 2009). Selenium has structural and enzymatic roles as antioxidant and catalyst for the production of the active thyroid hormone; selenium is needed for the proper functioning of the immune system and appears to be a key nutrient in inhibiting HIV progression to AIDS (Rayman, 2000).

An elevated selenium intake may be associated with reduced cancer risk. Selenium has cancer protective effect, inhibiting the tumour cell invasion (Hsia *et al*., 2003; Rayman, 2000; Yin *et al*., 2009; Zeng and Combs, 2008).

Organic selenium complexes and selenium-containing amino acids are considered to be the most bio-available for human and animal consumption (Suhajda *et al*., 2000; Zhou *et al*., 2009). Under appropriate conditions, yeasts are capable of accumulating large amounts of trace elements such as selenium and incorporating them into organic compounds (Suhajda *et al*., 2000). This mineral is absorbed during the yeast growth process.

Previous works have shown that the used yeast was a pure culture of baker's yeast *Saccharomyces cerevisiae* and the Se enrichment of yeast was carried out at laboratory level (Kaur and Bansal, 2006; Nagodawithana and Gutmanis, 1985; Suhajda *et al*., 2000) or laboratory prepared natural fermentative medium: germinated brown rice juice, wort, soybean sprout juice (Yin *et al*., 2009).
The numerous studies concerning the bioproducts based on selenium obtaining were made in Romania. The Romanian researches have used the selected baker's yeast *S. cerevisiae* strains and molasses – yeast extract as culture medium (Ioniță *et al.,* 2008).

Industrial nutrient medium (malt wort and sparge water) and spent brewer's yeast *Saccharomyces uvarum* 5 generations (after five fermentation cycles) have been used in this work.

Materials and methods

The yeast used was a spent brewer's yeast slurry (a strain of *S. uvarum*), a by-product from a brewery, with a content of solids of ~ 20% provided by a Romanian brewery.

The malt wort (M) was taken from the wort kettle and the sparge water (W) was collected after spent grains washing in a lauter tun. The culture medium *p*H was between 5 and 5.7. The extract value of the industrial media was 11 Plato degrees for the malt wort and 6 Plato degrees for the sparge water. The nutrient media were sterilized at 121°C for 15 minutes by autoclaving.

The Sodium-selenite was obtained from Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

Spectrometer ICP-MS ELAN DRC-e, PerkinElmer/MDS SCIEX was used for the selenium content of yeast determination.

The spent brewer's yeast was cultivated in an experimental glass with 200 ml industrial nutrient medium for the yeast growth – malt wort or sparge water and selenium solution at different temperatures and different periods of time.

The selenium solution was obtained by Sodium-selenite (Na_2SeO_3 or Na-selenite) dissolution in distilled water or culture medium (Kaur and Bansal, 2006; Nagodawithana and Gutmanis, 1985; Suhajda *et al*., 2000). The selenium solution was added in the medium before the yeast cultivation.

The yeast cultivation was carried out at 30°C or 20°C and the agitation was carried out at 200 rpm. The amount of yeast inoculum was of 2%, 4% or 10%.
The medium selenium concentration was varied from about 13.67 ppm (30 ppm Na_2SeO_3) to about 81.75 ppm (180 pm Na_2SeO_3). At the end of selenium absorption process, the biomass separation from the culture medium was carried out by centrifugation for 20 min at 3500 rpm.
After washing the biomass with distilled water and separating the phases through centrifugation, the total Se found in the yeast biomass consisted of two different fractions: inorganic Se and organic Se with extra- and intracellular localization, respectively.

To determine the Se incorporation into the yeast cells, the biomass mixed with ultra-pure water was extracted in a boiling bath for 1 h and brought to a constant volume (Yin *et al*., 2009). Then the mixture was centrifuged at 4000 rpm for 15 min.
The supernatant liquor was filtered and the filtrate directly analysed. The organic Se content of the selenium yeast was calculated as the difference between the total Se and the inorganic Se (Yin *et al*., 2009).
The solids content of the samples was estimated by using a thermobalance for the moisture analysis. The digestion of the samples was performed with 10 mL HNO_3 or 9 mL HNO_3 + 3 mL HCl (US EPA 3015 / 1994). The Se content of the selenium yeast samples was determined in accordance with SR EN ISO 17294-2 / 2005, "The water quality. The application of the Inductively-Coupled Plasma Mass Spectrometry method. Two part – The 62 elements determination". The assays are performed in triplicate.

Results and discussion

In this experiment, the spent yeast selenium enrichment possibility was studied. The influence of many factors upon the amount of selenium in brewer's yeast was investigated. There was made a comparative study between the selenium accumulation processes when the yeast was cultivated in malt wort and in sparge water respectively.

The colour of the yeast products with selenium is drab for the lowest selenium content of the samples (maximum 1000 ppm total selenium), pink for the medium selenium content (1000 – 1700 ppm) and red for the greatest selenium content of the samples (minimum 1720 ppm).

Table 1. presents the total selenium content of the yeast and the organic selenium (selenium incorporated by absorption process) when the sodium – selenite concentration of the medium increases from 30 ppm to 180 ppm (μg/ml) Na_2SeO_3 at 30°C and 4% yeast biomass percentage, after 24 h, for two culture mediums. The total Se of the yeast was bigger in the case of the malt wort by 302.53 ppm (33%) and the organic Se was higher by 335.02 ppm (49%) for 30 ppm Na_2SeO_3 concentration which proves that the selenium absorption process is more intensive in the case of the malt wort. Whereas the Na_2SeO_3 concentration increases 6 times, the total Se content increases 1.7 times in the case of the malt wort and 1.8 times in

the case of the sparge water because the higher amounts of sodium-selenite in the culture medium have a strong inhibitory effect on the growth of the yeast (Suhajda *et al*., 2000). It is easily noticed that the enrichment efficiency is the highest up to 30 ppm sodium-selenite concentration of the medium.

Table 1. The influence of the Sodium – selenite concentration upon the Total Selenium and Organic Selenium content of the yeast

Na_2SeO_3 concentration of medium, ppm	Total Se of yeast from malt wort, ppm	Organic Se of yeast from malt wort, ppm	Total Se of yeast from sparge water, ppm	Organic Se of yeast from sparge water, ppm
30	1224.89	1020.33	922.36	685.31
60	1497.34	1233.80	1126.54	791.95
90	1701.45	1378.17	1373.46	921.59
120	1845.12	1448.41	1478.97	948.01
150	1965.25	1507.34	1553.26	966.12
180	2029.67	1520.22	1624.67	999.17

Figure 1. demonstrates that the total selenium content of the samples has a maximum value at 30°C for the malt wort with a 180 ppm sodium – selenite concentration of the medium and 10% yeast biomass percentage for 24 h.

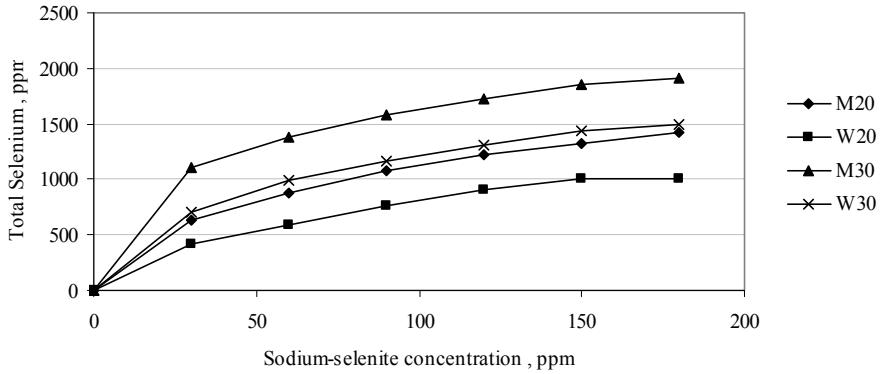

Figure 1. The influence of temperature upon the content of Total Selenium of the yeast for the malt wort at 20°C and 30°C (M20, M30) and the sparge water at 20°C and 30°C (W20, W30)

The total Se of the yeast cultivated at 20°C and 30 ppm Na_2SeO_3 concentration was 625.81 ppm (625.81 μg/g) and 412.88 ppm in the malt wort and the sparge water, respectively. At 30°C the total Se was 1101.32 ppm and 702.26 ppm in the malt wort and sparge water respectively. It was observed that a temperature higher by 10°C has determined an advance of the total Se with 76% in the first case and 70% in the second case. There is not much difference between the two behavioural patterns; however, the higher extract of the malt wort leads to higher total selenium

content of the yeast because the multiplication process was stimulated by the temperature. Using a medium at laboratory level supplemented with 30 µg/ml sodium-selenite, Suhajda *et al*. (2000) have obtained a selenium accumulation in the dried baker's yeast ranging from 1200 to 1400 µg/g.

Figure 2. shows the decrease of the total Se content while the yeast biomass percentage increases from 2% to 10%, at 30°C, after 24 h. The total Se value at a 30 ppm Na_2SeO_3 concentration was of 1261.22 ppm for 2% yeast, 1224.89 ppm for 4% yeast and 1101.32 ppm for 10% yeast in the malt wort case. In the case of the sparge water, at the same Na_2SeO_3 concentration, the total Se value was of 935.87 ppm for 2% yeast, 922.36 ppm for 4% yeast and 702.26 ppm for 10% biomass. Under the same temperatures and the same periods of time, the decrease of the total Se in the malt wort (12.7%) is less than the decrease of total Se in the sparge water (25%).

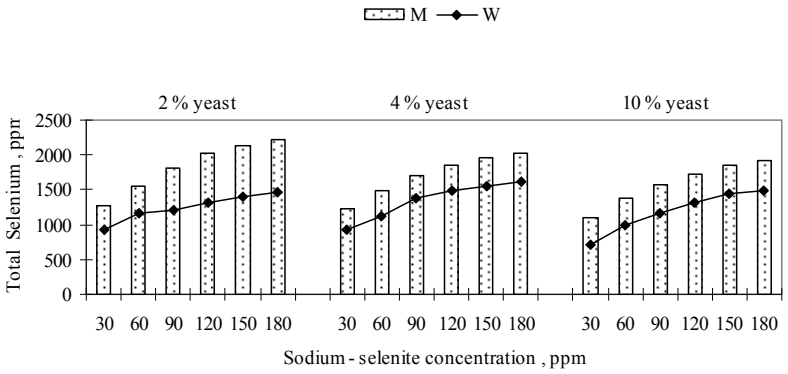

Figure 2. The influence of yeast biomass concentration upon the Total Selenium content of the yeast

In Figure 3. one may observe the results of the yeast biomass time of cultivation in two selenium enriched media for 12 h or 24 h at 30°C.

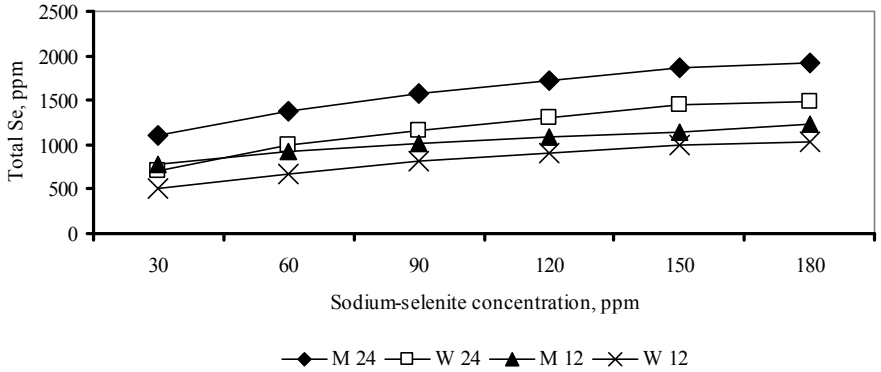

Figure 3. The influence of time upon the content of Total Selenium of the yeast

The total Se at 30 ppm Na_2SeO_3 concentration in the malt wort case was of 783.89 ppm for 12 h and 1101.32 ppm for 24 h (40.5% more). In the case of sparge water, at the same Na_2SeO_3 concentration, the total Se was of 506.93 ppm for 12 h and 702.26 ppm for 24 h (38.5% more). The results indicate that the selenium consumption rate has about the same value for both media. The total Se at 180 ppm Na_2SeO_3 maximum concentration in the malt wort case was of 1230.51 ppm for 12 h and 1916.44 ppm for 24 h. In the sparge water case, for the same Na_2SeO_3 concentration, the total Se was of 1039.61 ppm for 12 h and 1493.25 ppm for 24 h. It can be concluded that a shorter yeast cultivation period is more efficient than a longer yeast cultivation period if not intended the largest possible value for the incorporated selenium.

The *p*H values have a great influence upon the selenium content of the yeast under the same temperature (30°C), time (24 h) and biomass (10%) parameters using the sparge water as nutrient medium for the yeast growth (Figure 4). Selenium can be accumulated in yeast cells in organic bound and inorganic form (Suhajda *et al*., 2000) by the absorption and adsorption processes, respectively. The results obtained at various *p*H values show that increasing *p*H values lead to lower selenium consumption and a simultaneous lower inorganic selenium content according to Suhajda *et al*., 2000. The highest total Se, organic Se and inorganic Se contents were obtained for 3 *p*H units and the smallest total Se, organic Se and inorganic Se contents were obtained for 5 *p*H units. It was observed that at lower *p*H values both selenium accumulation processes are more intensive. Therefore, the selenium accumulation process can be optimized by a single *p*H adjustment.

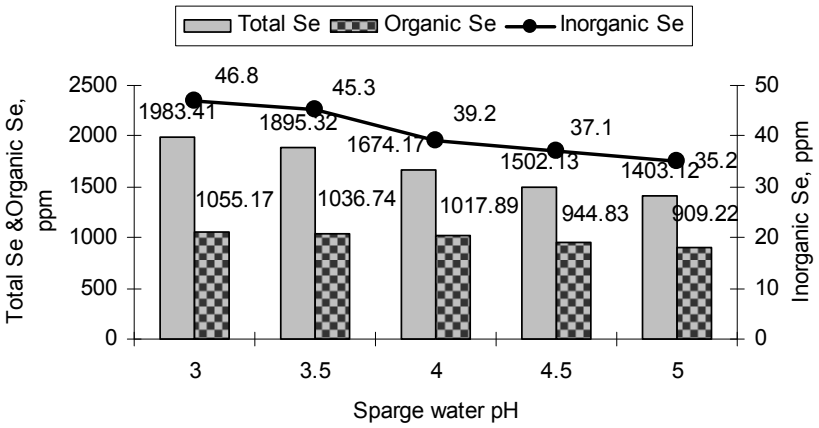

Figure 4. The influence of pH upon Total Se, Organic Se & Inorganic Se content of the yeast

A graphical comparison between the total Se or organic Se content of the *S. uvarum* cells accumulated after 12 h and the total or organic Se accumulated after 24 h using sparge water as nutrient medium for the yeast growth at 30°C was done in Figure 5. After 24 h of yeast cultivation time, the highest content of total Se and

organic Se for identical sodium-selenite concentrations was obtained. After 12 h, these chemical indicator values represent about 70% from the selenium quantity of the yeast after 24 h. Thereby, after 24 h, at 30 ppm Na_2SeO_3 concentration, were obtained: 702.26 ppm total Se (WT24) and 528.8 ppm organic Se content (WO24) of selenium yeast and after 12 h there were obtained: 506.93 ppm total Se (WT12) and 376.14 ppm organic Se content (WO12) of selenium yeast.

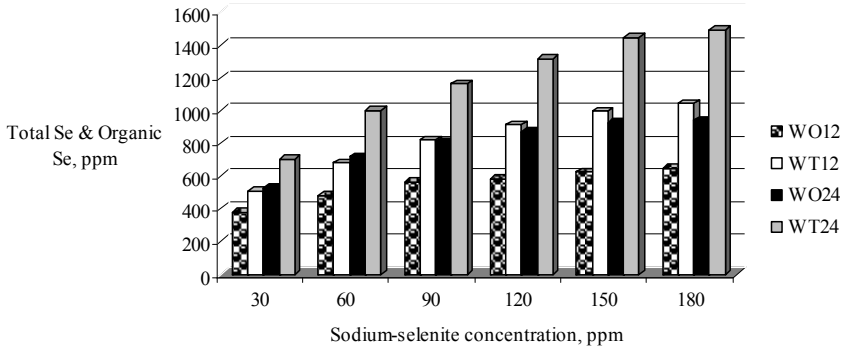

Figure 5. The influence of time and the Na_2SeO_3 concentration upon the content of Total Se and Organic Se of the yeast

Figure 6. shows the yeast adsorption capacity of the inorganic selenium content of the yeast samples in percentages. The nutrient medium was sparge water at 30°C, the yeast biomass proportion was 10% and sodium – selenite concentration varied from 30 to 180 ppm for 12 or 24 h. For each Na_2SeO_3 concentration of the medium, one may observe the bigger extra cellular Se proportion from the total Se after 12 h in comparison with the selenium values after 24 h. This may be thus explained: in the first 12 h, the yeast has a reduced multiplication activity that leads to a greater adsorption process than the absorption process of selenium from the medium.

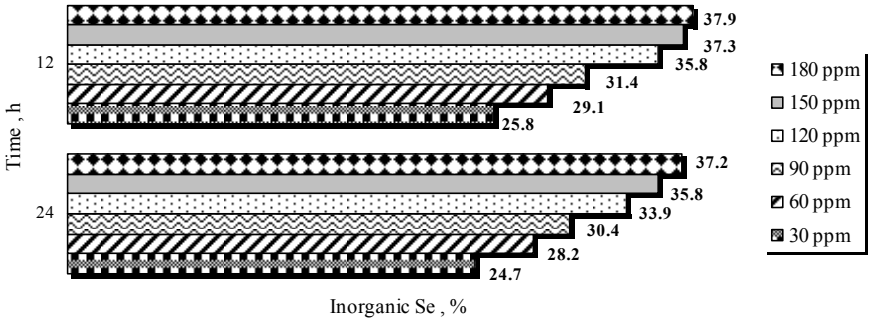

Figure 6. The influence of time and Sodium-selenite concentration upon the Inorganic Se content of the yeast

Figure 7. shows the total Se and organic Se values and inorganic Se percentage at 30°C, after 12 h and 10% inoculum in the sparge water, when the Na-selenite was added at the beginning of the cultivation period or gradually, in three identical doses: the first dose before the cultivation, the second dose after 2 hours and the last dose after 4 hours. Consequently, the organic Se content was increased by 3.6–4.0% and the inorganic Se was decreased by 1.6–9.6 % by a gradual Na-selenite addition comparatively with the Na – selenite addition at the beginning of cultivation.

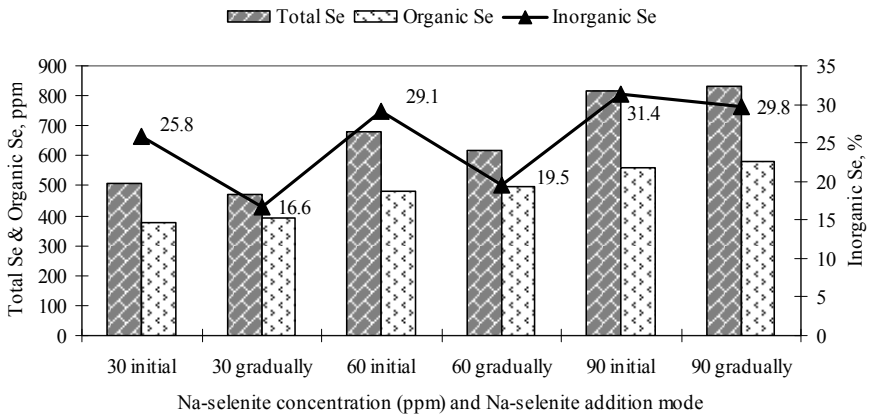

Figure 7. The influence of Na-selenite addition procedures upon the Total Se, Organic Se and Inorganic Se content of the yeast

Obviously the selenium absorption process is thus faster for small selenite amounts in the medium because the excessive selenite amounts in the medium have an inhibitory effect upon the growth of yeast cells. The presence of large selenite concentration of the medium causes a slowdown of the yeast absorption capacity but at the same time the inorganic Se has a smaller decrease. A higher Na–selenite concentration of the medium leads to the equalizing tendency of the effects of the Na–selenite addition procedure.

Conclusions

The Selenium yeast – a very important pharmaceutical product – can be obtained from the spent brewer's yeast *S. uvarum* species cultivation in the malt wort and sparge water industrial nutrient medium enriched with sodium-selenite.

The selenium absorption and selenium adsorption process as are influenced by the following factors: Na-selenite concentration of the medium, temperature, time, *p*H, yeast biomass proportion, Na-selenite addition mode and type of culture medium.

Yeasts are capable of accumulating large amounts of trace elements such as selenium during the cells growth phase.

Using a culture medium supplemented with 30–180 µg/ ml sodium–selenite results in total selenium – accumulation in the range of 625.81–2215.67 µg/g (0.6-2.2

mg/g) for the malt wort and of 412.88 – 1624.67 µg/g (0.4– 1.6 mg/g) for the sparge water and in organic selenium-accumulation in the range of 629.99 – 1520.22 µg/g (0.6-1.5 mg/g) for the malt wort and 376.14 – 999.17 µg/g (0.3– 0.9 mg/g) for the sparge water measured by the ICP-MS method but the optimal Na-selenite concentration for avoiding the inhibitory effects on yeast growth is 30 µg/ml.

The sparge water, by-product of breweries can be successfully reused as nutrient medium to obtain Selenium yeast.

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