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Selenium yeast from spent brewer's yeast

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Abstract

The work presents the preparation of selenium yeast from spent brewer's yeast by chemical treatment of yeast biomass with sodium-selenite solution. It was made a comparative study of preparation procedures in different conditions of temperature, time, yeast concentration and selenium solution concentration. The Wickerham culture medium and malt wort were used such as nutritive medium for selenium – enriched Saccharomyces uvarum. Using a culture medium supplemented with 30- 180 µg/ ml sodium–selenite results in total selenium – accumulation in the range of 575- 2400 µg/ ml and organic selenium-accumulation in the range of 530-2100 µg/ ml. The selenium content of yeast was measured by Inductively- Coupled Plasma Mass Spectrometry (ICPMS method).

Keywords: selenium brewer's yeast, trace mineral, malt wort, sodium selenite

1. Introduction

In Romania, in the past, a large spent yeast amount was used as animal feed, but a lot of farms are closed today and the biomass is overflowed in the city sewage and pollutes the wastewaters. Therefore, the spent brewer's yeast revaluation is necessary and very important as an environmental protection measure, too.

A revaluation possibility can be the yeast enrichment with minerals such as: selenium, chromium, iron, zinc. Is known the manufacture of selenium-enriched bakers'yeast Saccharomyces cerevisiae, especially.

The selenium yeast is used such as human dietary supplements or therapeutic product [1,2,3,5,6,7,]. Selenium (Se) is an essential trace element for human and animal health. The physiological role of selenium was first appreciated following evidence that the element is an essential component of glutathione peroxidase which have important antioxidant and detoxification functions.

Selenium has structural and enzymic roles as an antioxidant and catalyst for the production of active thyroid hormone [3, 6,7].

Selenium is needed for the proper functioning of the immune system and appears to be a key nutrient in inhibiting HIV progression to AIDS. An elevated selenium intake may be associated with reduced cancer risk .Selenium has a cancer protective effect, inhibe of tumor cell invasion [5,7].

It is generally believed that the ingestion of organic Se compounds is better and safer than that contained inorganic Se. In the present the best organic selenium sources are selenomethionine and selenium yeast.

Under appropriate conditions yeasts are capable of accumulating large amounts of trace elements such as selenium and incorporating them into organic compounds. Organic selenium complexes and selenium- containing amino acids are considered to be the most bioavailable for human and animal consumption [6,7].

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In this study it was used the spent brewer's yeast 5 Generations (after five fermentation cycles).

2. Materials and methods

2.1.Brewer's yeast. The yeast used was a spent brewer's yeast slurry (a strain of Saccharomyces uvarum), a by-product from a brewery, with a solids content of ~ 20% provided by a Brewery from Romania.

2.2. Chemicals. Reagents and chemicals were obtained from Sigma Chemical Co., St.Louis, USA, Merck KGAA, Darmstadt, Germany and other biochemical company of Romania.Sodium-selenite reagent was achieved from Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

2.3.Apparatus. Analytical balance Mettler Toledo xs 403 SM, Thermobalance MX50, Spectrometer ICP-MS ELAN DRC-e,PerkinElmer/MDS SCIEX, pH-meter Mettler Toledo S20 K, Centrifuge JANETSKI with refrigeration, Shaking incubator SI 300 R, Labo Autoclave Sanyo.

2.4. Selenium yeast from spent brewer's yeast preparation. Spent brewer's yeast was cultivated in a glass vessel with 200 ml culture medium for yeast growth and selenium solution in different conditions of temperatures and times.

The Wickerham medium (W) was selected such as culture medium in the first case and the industrial malt wort (M) was chosen in the second case [1,6]. Composition and characteristics of the culture mediums are given in Table 1 and Table 2. The Wickerham medium pH was between 5 and 6.The culture mediums were sterilized at 121^0C for 15 minutes by autoclaving.

Table 1. The Wickerham culture medium composition

Composition	**g/1000 cm^3**
Glucose	40
Yeast extract	2,5
Bactopeptone	5
$(NH_4)_2SO_4$	6
$CaCl_2.2H_2O$	0,25
KH_2PO_4	2
$MgSO_4.7H_2O$	0,25
Water	Until 1000 cm^3
Selenium content	0,000 µg/ ml (ppm)

The selenium solution was obtained by sodium-selenite (Na_2SeO_3) dissolution in distilled water or culture medium [3,4,6].The selenium solution was added in medium before yeast cultivation.

Cultivation was carried out at 30^0C or 20^0C and agitation was varied 200 rpm.The amount of yeast inoculum was 2% or 4%.

Table 2.The industrial malt wort characteristics

Characteristic	**Value**
Primitive extract	8,66 Balling degree
pH	5,08
Colour	6 EBC units
Acidity	1,1 ml 1N NaOH
Selenium content	0,000 ppm
Manufacturing recipe	75% malt + 25% maize

The medium selenium concentration was diversified from about 13,67 ppm to about 81,75 ppm.The definite values for analysed samples parameters are indicated in Table 3. The assays is performed in triplicate.

At the back end of the selenium absorption process the separation of the biomass from the culture medium was carried out by centrifugation for 20 min at 3500 rpm.

After washing by centrifugation with distilled water the yeast biomass contains total Se that is composed from extracellular inorganic Se and intracellular organic Se.

For determination of the Se incorporation into yeast cells the biomass mixed with ultra-pure water was extracted in boiling bath for 1 h and made a constant volume [1]. Then the mixture was centrifugated at 4000 rpm for 15 min. The supernatant liquor was filtrated and the filtrate could by analyzed directly. Organic Se yield was calculated from the difference between the total Se yield and inorganic Se yield [1].

*2.5. Chemical assays.*Solids content of samples was estimated by using a thermobalance for moisture analysis; The digestion of the samples was performed with 10 ml HNO_3 or 9 ml HNO_3 $^{+3}$ ml HCl [9]; The Se content of 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 "[8].

Table 3. The preparation conditions of selenium spent brewer's yeast and the definite values for analysed samples parameters

Sample	Culture medium	Temperature (^{0}C)	Time (h)	Yeast percentage (%)	Na_2SeO_3 concentration of medium (ppm)	Se concentration of medium (ppm)	Total Se (ppm) dry basis	Organic Se (ppm) dry basis	Colour selenium yeast
P1	W	20	24	4	30	13,67	875,34	802,15	Pink
P2	W	20	24	4	60	27,32	1012,35	902,31	Pink
P3	W	20	24	4	90	40,95	1123,54	995,82	Pink
P4	W	20	24	4	120	54,57	1294,38	1152,18	Pink
P5	W	20	24	4	150	68,17	1520,08	1350,10	Pink
P6	W	20	24	4	180	81,75	1775,64	1501,54	Red
P7	W	20	24	2	120	54,57	1506,54	1085,87	Pink
P8	W	30	24	4	120	54,57	2138,32	1603,91	Red
P9	W	20	12	4	120	54,57	620,76	576,12	Drab
P10	W	30	24	4	30	13,67	1967,94	1432,27	Red
P11	W	30	24	4	60	27,32	2012,11	1538,90	Red
P12	W	30	24	4	90	40,95	2067,39	1557,41	Red
P13	W	30	24	4	150	68,17	2415,34	1913,45	Red
P14	W	30	24	4	180	81,75	2611,52	2093,17	Red
P15	M	20	24	4	30	13,67	703,12	630,45	Drab
P16	M	20	24	4	60	27,32	721.78	665,13	Drab
P17	M	20	24	4	90	40,95	793,89	718,23	Drab
P18	M	20	24	4	120	54,57	892,42	792,34	Drab
P19	M	20	24	4	150	68,17	1319,73	1153,70	Pink
P20	M	20	24	4	180	81,75	1766,17	1084,35	Red
P21	M	20	24	2	120	54,57	940,89	813,29	Drab
P22	M	30	24	4	120	54,57	2085,12	1282,26	Red
P23	M	20	12	4	120	54,57	575,34	536,17	Drab
P24	M	30	24	4	30	13,67	1724,89	1133,45	Red
P25	M	30	24	4	60	27,32	1897,34	1311,56	Red
P26	M	30	24	4	90	40,95	2001,45	1467,42	Red
P27	M	30	24	4	150	68,17	2265,25	1598,67	Red
P28	M	30	24	4	180	81,75	2429,67	1814,89	Red

3. Results and Discussions

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 were determined. In Table 3 the principal preparation conditions are shown and the selenium content in samples was registered.

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

In Figure 1 on observe the difference between total selenium content of samples when the yeast biomasss proportion of the culture medium increase since 2% until 4% in the same conditions of temperature, time and sodium-selenite concentration of medium for two culture mediums.

The decrease of selenium content while with the increase of yeast percentage is more in the Wickerham medium use case.

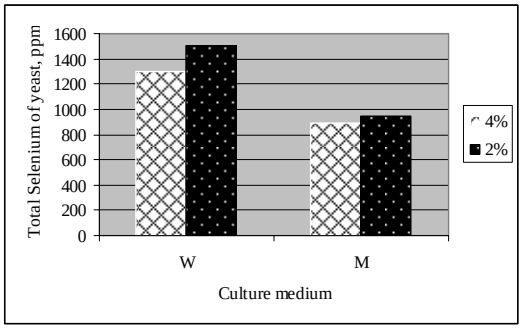

Figure 1. The influence of yeast biomass concentration upon total selenium content of yeast for two culture mediums after 24 h at 20^{0}C and 120 ppm Na_2SeO_3

Fig.2 shows that the total selenium content of samples is commensurate with sodium-selenite concentration of medium and the yeast selenium enrichment efficiency is highest until 30 ppm sodium-selenite concentration of culture medium. Furthermore, Fig. 2 shows that selenium absorption process is more intensive in the Wickerham medium use case.The complex malt wort chemical composition inconvenience the selenium absorption process, probably.

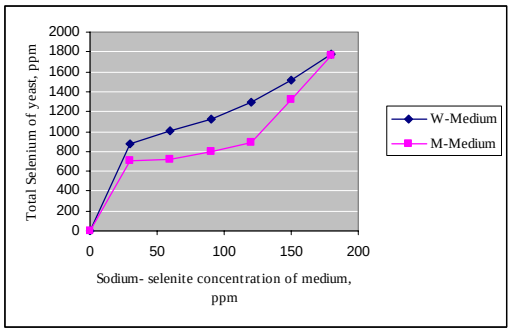

Figure 2. The influence of sodium-selenite concentration of medium upon total selenium content of yeast for two culture mediums after 24 h at 20^0C, for 4% yeast biomass concentration of medium

Fig. 3 presents the results of yeast biomass maintenance contact with two different selenium enriched mediums for 12 h and 24 h, respectively. In the first case, the difference between the total selenium content of sample from Wickerham medium and the total selenium content of sample from malt wort was 45,42 ppm (7,3%). In the second case, the total selenium difference was 401,96 ppm (31%). The yeast selenium absorption capacity was increased after 24 h.

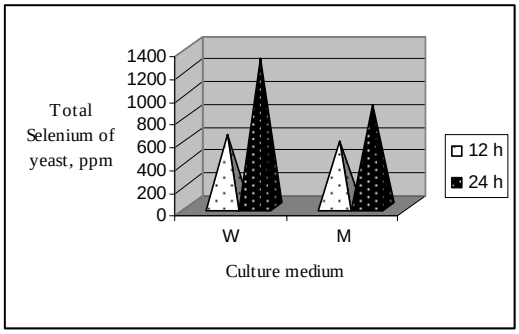

Figure 3. The influence of time upon total selenium content of yeast for two culture mediums at 20^0C, for 120 ppm Na_2SeO_3 , with 4% yeast biomass percentage

Figure 4 demonstrate that the total selenium content of samples have a maxim value at 300C for Wickerham culture medium with 180 ppm sodium – selenite concentration of medium and 4% yeast biomass percentage.

From 200C to 300C the selenium content increase approximately 1,8 times average in the Wickerham medium case. The curve allures are alike but at 300C is the lowest difference between total selenium content from Wickerham and malt wort medium, respectively.

Consequently, at a biggest temperature, the spent brewer's yeast absorbs about same selenium quantity from different culture mediums.

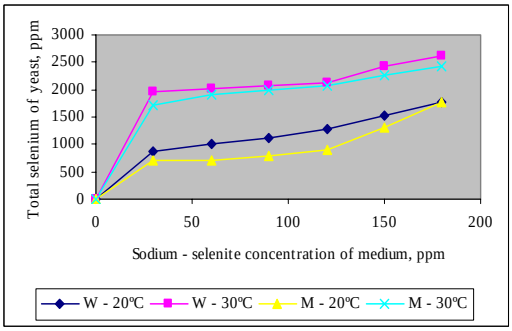

Figure 4. The influence of temperature upon total selenium content of yeast for two culture mediums with 120 ppm Na_2SeO_3 and 4% yeast biomass percentage, after 24 h

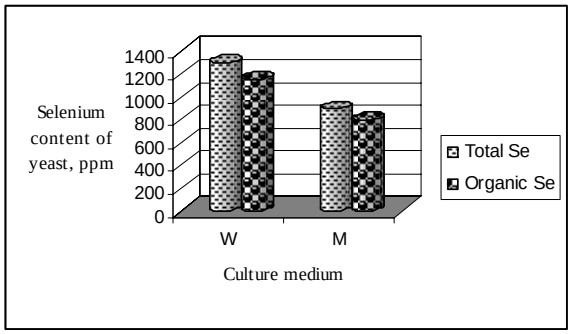

Figure 5. The difference between total selenium and organic selenium for two culture mediums with 120 ppm Na_2SeO_3 and 4% yeast biomass percentage, after 24 h at 20^0C

In Fig. 5 it was made a graphical comparison between the total selenium content and organic selenium content of samples for to dedicate the inorganic selenium adsorbed on the surface of the Saccharomyces uvarum cells.The inorganic selenium content represents 11,2% from total selenium content for malt wort and 11,0% for Wickerham medium. For all samples, the inorganic selenium percentage is maximum 40% from total selenium content.

4. Conclusion

The spent brewer's yeast Saccharomyces uvarum species, after five fermentation cycles can be enriched with trace mineral such as Selenium obtaining the Selenium Yeast, very important pharmaceutical product using sodium- selenite that selenium source.

In this study, the selenium amount absorbed by spent yeast is dependence by next factors: culture medium type, time, temperature, yeast biomass added percentage, sodium – selenite concentration of medium.

Using a culture medium supplemented with 30-180 µg/ ml (ppm) sodium–selenite results in total selenium – accumulation in the range of 575-2400 µg/ ml and organic selenium-accumulation in the range of 530-2100 µg/ ml.

The Selenium Yeast can be achieved from industrial malt wort and the optimum temperature used was 30^0C .

Subsequently, the parameters diversification is necessary for assess the optimum conditions selenium absorption and maximum selenium enrichment efficiency.

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