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Preparation of Organic Selenocystine Using Locally Isolated Yeast

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Abstract
Selenium is a toxic inorganic form at very low concentration in life, while the organic-selenium compounds are appreciable interest and various of them have essential roles in nutritional science and cell biochemistry. Selenium-enriched yeast (Se-yeast) is a public form of selenium used to additional dietary intake of this essential trace mineral. In this study, an organic selenocystine by using locally isolated bakery yeast (*Saccharomyces cerevisiae*)is prepared. A novel locally prepared date extract media enriched by 0.2% potassium phosphate (KH_2PO_4), 0.6% ammonium sulfate is adopted as alternative culture media. Selenium salt is used in different concentrations (30, 60, 120 and 240 µg/mL), which are added to the yeast culture media. While the best concentration of selenium added is 30µg/mL, it achieves optimal conditions for the growth of red color yeast identical to the standard. The organic selenocystine is analyzed by High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectrophotometer (AAS) as compared with standard product obtained from Sigma. Results confirmed the formation of similar selenocystine products.

Introduction

Selenium is a trace element, with atomic weight of 79 and is within the group in the periodic table of elements [1]. The selenium is essential trace elements in the human body as it is the most important nutrients necessary sources of aquatic organisms as it is present in animals milk, including cows, concentration level in the milk is 110.9 µg/mL [2, 3] due to the importance of this element, selenium currently added to breast-milk factory of children to protect them from diseases, anemia [4]. Selenium exists in all cells and tissues of the human and the biological fluid with different concentrations, depending on the type of tissue and depending on the level of selenium. The optimal concentration of selenium content in the tissue is about 15 mg of normal adults [5]. The crust of kidney was the richest of the body tissues selenium (4µg/kg dry weight), followed by liver (2.6-0.5 µg/kg), while for the heart muscle they contain a higher proportion of selenium from skeletal muscle as the heart muscle was found to contain 1.8-0.3 µg/kg. In the case of biological fluids which are the most changing factors in accordance with the activity need, therefore, selenium is of essential nutrients [6]. There are many factors affecting the balance of selenium in
the body of the organism such as the nature and quality of food [7] and the physiological status and health of the body [7-10] in addition to alcohol and smoking [9, 11]. The use of high selenium levels (2.5-3 g/day) is a case of poison, at the time the rate of human intake (50-200 µg/day) was needed to ensure safety for human health. Selenium was incorporated into molecules of an enzyme called GPX (glutathione peroxidase). This important enzyme protects cell membranes, red blood cells and sub-cellular components against disagreeable reactions with soluble peroxides. The invention of GPX helped to outlook how
Selenium is protective against cancer, heart disease, arthritis and accelerated aging [12, 13]. recently more scientific excitation is being generated with the recent research result of the study. Also selenium has e a bad effect on the central nervous system, liver and spleen [14]. Selenium was found within the protein content of the tissues, therefore called

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Selenoproteins [15]. Selenium composition present in the active sites of the enzyme Glutathione peroxidase, thioredoxin reductase and mammalian Iodothyronine 5-deiodinase [16].

Studies have confirmed the viability of selenium as cancer inhibition e.g. colon, cervical, breast, and liver cancer [17]. It was therefore highly recommended the use of this organic component for the purpose of cancer drugs, accompanied by the enzyme important role in the transformation of selenium salts.

Acorrding to this background and with new research evidence regularly appearing for the role of selenium in the reduction of viral virulence, many people are interested in ensuring they have sufficient selenium status by additional their diets with selenium. Since the selenium linked to the amino acid Cystine that confirms the importance of nutritional yeast since 1910 [23], and yeast also contains 70 trace elements with organic forms [24].

The study aimed to produce organic selenocystine using locally bread yeast isolation and locally prepared liquid media from date's juice as alternative culture media.

Materials and Methods

Micro-organism and the Material Basis

The yeast strain used by the manufacturers to produce selenium-enriched yeast in all cases was *Saccharomyces cerevisiae*. The selected strain of bakery yeast was isolated locally and kept as a reference source in 4 ^{o}C with a sequential activation.

Preparation of Culture Media

Locally prepared liquid media contain dates juice (8%) instead of sugar (5.6%) was prepared. The media were enriched by addition of 0.2% potassium phosphate (KH_2PO_4), 0.6% ammonium sulfate. The media were inoculated by yeast and incubated at 30 ^{o}C for 24 h with shaking at speeds of 150 cycles/ minute.

Addition of Inorganic Selenium Salts

Selenium salts were added to culture media in a concentration extremely high (30, 60, 120 and 240 µg/mL) during the exponential growth phase, in the form of six doses with periods of one hour for each dose continuously. The final product prepared after incubation for a period of 24 h. Later the yeast growth culture had been filtered and washed with distilled water several times and then dried using acetone.

Examination of the Outputs

The concentration of the yeast output selenocystine product was determined with the final estimation of organic transformation using HPLC, compared with authentic organic standard. It was dismissed on the type of reverse-phase column (C-8) dimensions (250 × 4.6 mm Id), using mobile phase consisting of THF: phosphate buffer (95:5v/v) [25].

Atomic analysis: In AAS, the sample is atomized into the vapor phase. A beam of electromagnetic radiation passes through the vaporized sample. The metals of interest have absorbed some of the radiation. The instrument measures the change in intensity, which is then converted into an absorbance reading. Finally doing calibration, the concentrations of various metals through the use of Beer-Lambert's law could be determined, depending the amount of absorption [26].

Results and Discussion

In the preliminary production study of organic seleno cystine, a local media of date juice has been prepared. The new novel culture media also contained source of nitrogen and carbon as fundamental to the growth of the organism in the media [27]. A novel locally date extract media was adopted as an alternative used locally appropriate culture media by researchers [15]. Bread yeast (*Saccharomyces cerevisiae*) was adopted in locally date extract media and then developed.

Different concentration of inorganic selenium (sodium selenite) (30, 60, 120, 240 µg/mL) were prepared in water and added to the growth culture. It is necessary to take consideration of the situation to avoid discouraging the growth of yeast, which can occur because of inorganic selenium salt concentration [28, 29], in addition to the quality and quantity of organic selenocystine as an output. So the inorganic

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selenium was added with each concentration used in the experiment in form of six doses and interval with one hour to avoid the affection of the yeast growth. The inorganic selenium salt were added during the process of stable growth (exponential), where they are converted inorganic selenium salts to the organic compounds within the yeast cell, as a result of the replacement of the sulfur atom of the amino acid (Cystine) by selenium, so the composition of organic (Selenocystine) was produced. Control of temperature, pH, selenium addition profile and aeration allows optimal growth of the yeast strain and maximum biomass production. Selenocystine product could be investigated by altering the color of yeast cells to the red indicating the formation of the organic Selenium, while the density of the color depended on concentration of organic selenocystine.

The ability of the yeast under appropriate conditions to accumulate quantities of trace elements such as selenium, where the selenium salts dissolved in water when added to the culture media, which is required for the development of yeasts. Absolutely, according to these stringent criteria not all material solid as Se-yeast is produced. At sometimes the amount of sodium selenite is excess such that most of the selenium is not bound to the yeast: at the worst, there may only be a mixture of sodium selenite and yeast [25]. Therefore, filtering and washing of yeast several times finally with distilled water necessary is to separate free Se salt mixture media contains, then drying using acetone.

It was known that selenium can be included in the other biological molecules such as DNA carrier (t-RNA) producing (Seleno-tRNAs) [30] and this in turn leads to a change in the production of vital proteins.

Fig. 1 confirms the results of the successful work using HPLC. The formed selenocystine concentrations were measured qualitatively and quantitatively by comparison with the peak area of the standard (Sigma). It shows various concentrations of the selenium salts noted in chromatograph salts added to the culture media. While the best inorganic selenium concentration added was 30 µg/mL, which performed optimal conditions for the best growth of yeast, and the obtaining of red yeast grew identical to the standard.

AAS by flameless has confirmed existence of produced from of yeast at various additions of selenium in the organic product as compared with the standard. The data also confirmed the presence of selenium in the required specifications.

AAS was used to determine concentrations of Se compounds in plant extracts by Zhang and Frankenberger [31]. They were able to separate Se into non-amino acid organic Se, Se-amino acids, selenite and selenate by an anion-exchange method. The atomization efficiency is greatly increased after hydride generation process. For selenium, HG-AAS (hydridegeneration atomic absorption spectrophotometer) requires the Se (IV) oxidation state (selenite), since Se (VI) is not reproducible [23]. Therefore, all selenium must be in the Se (IV) oxidation state for analysis [26]. As final, the results of both technical (HPLC and AAS) and morphological specifications of the product, confirmed a successful process in the preparation of organic selenocystine product using locally bread yeast isolation, in addition to the efficiency of the local media as alternative to the imported culture media. It is an integral selenocystine substitute which can be used to address the deficiency of Selenium in cancer patients.

Conclusion:

The evidence presented here has shown that selenocystine is similar to food-form selenium, safe and acceptable for use in food additive that can play a role as a precursor for selenoproteins synthesis and as a human anti-cancer agent. The product could be manufactured using bread yeast with alternative locally media. Control of pH, temperature,

Selenium feeding profile and aeration allow optimal growth of yeast strain and maximum biomass production. As a result of the fermentation the selenium organically bound to the yeast in the selenium-enriched medium.

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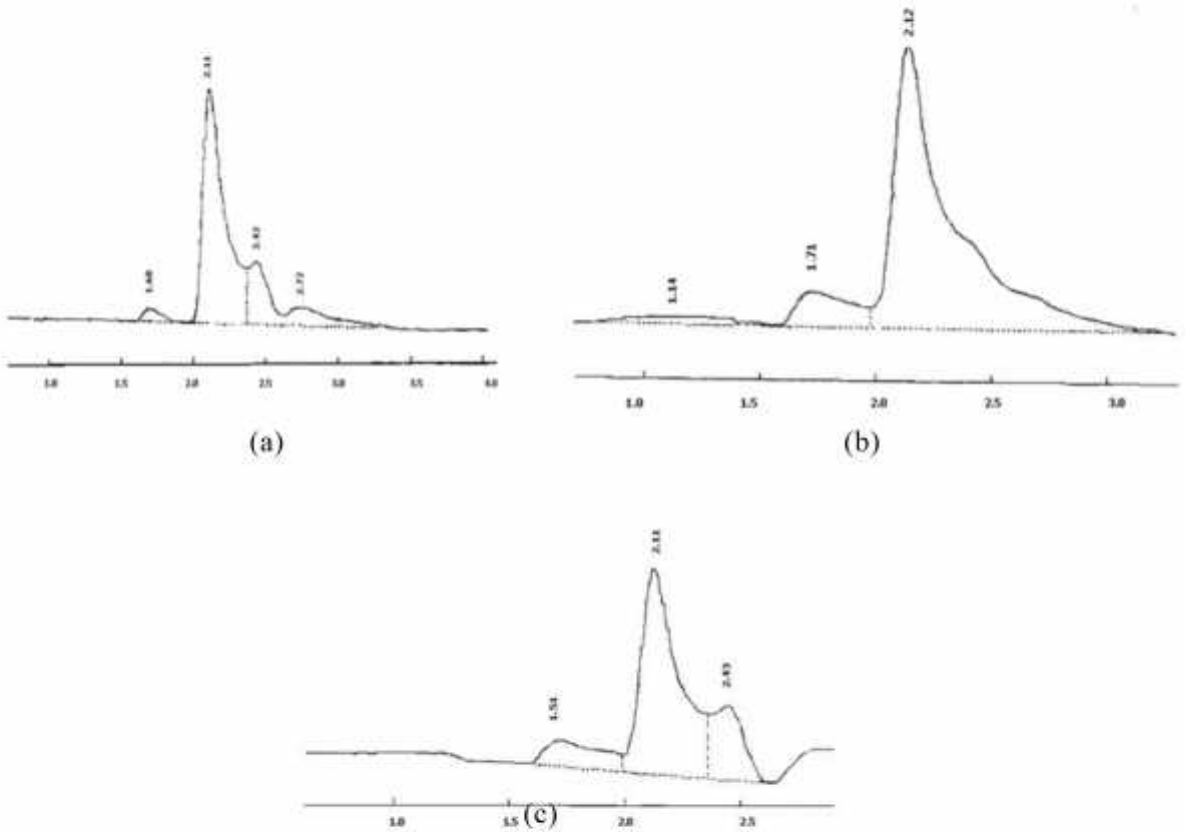

Fig. 1 Separation of Selenocystine salt result from different conc. of selenium added to the media, on reversed phaseisocratic HPLC methods on the following separation condition for standard and prepared complex on Column: reversed phase (50 × 4.6 mm under the same condition ID) 3 m particle size column, Mobile phase: 70:30 v/v 0.01 M potassium phosphate buffer: THF (Tetrahydrofurn) HPLC grade Merck Detection: 254 nm , Flow rate: 1 mL/min, Temp.: 30 °C. (A: Standard, B: 30, C: 60 µg/mL).

References

T.M.T. Sheehan, D.J. Halls, Measurement of selenium in clinical specimens, Am. Clin. Biochem. 36 (1999)301-315.

S.O. Knowles, N.D. Grace, K. Wurms, J. Lee,Significance amount and from of dietary selenium on blood, milk, and casein selenium concentration in grazing cows, J. Dairy. Sci. 82 (2000) 429-437.

P. Vandad, G. Vlaemynk, R. Van, H. Dedstra, Selenium content of cow's milk and its distribution in protein fraction, Z. Lebensm. Unters. Frsch 192 (1991) 422-426.

P. Bratter, L.N. Blasco, V.E. Negretti de Bratler, A. Raab, Speciation as an analytical aid in trace element research in infant nutrition, Analyst 123 (5) (1998) 821-826.

K. Ashton, L. Hooper, L.J. Harvey, R. Hurst, A. Casgrain,S.J. Fairweather-Tait, Methods of assessment of selenium status in humans, American Journal of Clinical Nutrition. 89 (6) (2009) 2025-2039.

H.J.Robboreet, R.E. Van Grieken, P.A. Van Den Bosch,H. Deelstra, D. Vanden Berghe, Losses metabolically in corporate selenium in common digestion procedure for biological material, Talanta 29 (1982) 1025-1028.

D.Beker, Z. Romic, H. Krsnjavi, Z. Zima, A contribution to the world selenium, Map. Biol. Trace Elem. Res. 33 (1992) 43-49.

J. Virtamo, E. Vakcila, G. Alfthan, S. Punar, J. Huttuncn, M.J. Kasrvoncn, Serum, selenium and risk of coronary heart disease and stork, Am. J. Epidemoil. 122 (1985) 276-282.

Al-Qadisiyah Journal of Agriculture Sciences
(QJAS)
| 2077-5822 |

P.J. Thuluvath, D.R. Triger, Selenium in chronic liver disease, J. Hepatol. 14 (1992) 176-182.

P.Anttila, I. Salmda, J. Lehto, O. Simell, Serum zinic, copper and selenium concentrations in health mothers during pregnancy and lactation, New York, 1988, pp. 262-272.

H.Robbereeht, H. Deolstin, Method inhaltion to blood selenium concentration values: Literature Review, J. Trace Lem. Lytes Health Dis. 8 (1991) 129-143.

H.J.Robbereeht, H.A. Deelstra, Selenium in human urine determination speculation and concentration levels, Talanta 31 (1984) 407-808.

L.C. Clark, G.F.Jr. Combs, Effect of selenium supplementation for cancer prevention in-patients with carcinoma of the skin, J. Am. Med. Ass. 276 (1996)1957-1963.

G.F.Jr. Combs, Essentiality and toxicity of selenium a critique of the recommended dietary allowance and reference dose, Risk Assessment of Essential Element, Washington, DC: ILSI Press, 1994, pp. 167-183.

J.R. Cronin, Dietary selenium: Elemental nutrition for muscles, immunity, well-being and cancer prevention, Alt. Complement. Therap. 6 (2000) 342-346.

J.R. Arthur, G.J. Beckett, New metabolic roles for elenium, Proc Nutr Soc. 53 (1994) 615-624.

M.P. Rayman, Selenium in cancer prevention: A review of the evidence and mechanism of action, Proceedings of the Nutrition Society 64 (2005) 527-542.

M.W.Yu, I.S. Horng, Y.C. Chiang, Y.F. Liaw, C.J. Chen, Plasma selenium levels and the risk of hepatocellular carcinoma among men with chronic hepatitis virus infection, Am. J. Epidemiol. 150 (1999) 367-374.

K.J. Helzlsouer, H.Y. Huang, A.J. Alberg, S. Hoffman, A. Burke, E.P. Norkus, et al., Association between alpha-tocopherol, gamma-tocopherol, selenium, and subsequent prostate cancer, J. Natl. Cancer Inst. 92 (2000) 2018-2023.

S.P.Hussain, A.R. Rao, Chemopreventive action of selenium on methylcholanthrene-induced carcinogenesis in the uterine cervix of mouse, Oncology 49 (1992) 237-240.

A.T.Nasseri, S. Rasoul-Amini, M.H. Morowvat, Y. Ghasemi, Single cell protein: Oroduction and process, American Journal of food technology 6 (2011) 103-116.

R.Lobinski, J.S. Edmonds, K.T. Suzuki, P.C. Uden, Species-selective determination of selenium compounds in biological materials, Pure. Appl. Chem. 72 (2000)447-461.

S.P.Hussain, A.R. Rao, Chemopreventive action of selenium on methylcholanthrene-induced carcinogenesis in the uterine cervix of mouse, Oncology 49 (1992)237-240.

A.T.Nasseri, S. Rasoul-Amini, M.H. Morowvat, Y. Ghasemi, Single cell protein: Oroduction and process, American Journal of food technology 6 (2011) 103-116.

R.Lobinski, J.S. Edmonds, K.T. Suzuki, P.C. Uden,Species-selective determination of selenium compounds in biological materials, Pure. Appl. Chem. 72 (2000) 447-461.

B.Welz, M. Sperling, Atomic Absorption Spectrometry,Wiley-VCH, Weinheim, Germany, 1999.

L.Diniz-Mendes E. Bernardes, P.S. de Araujo, A.D. Panek, V.M.F. Paschoalin, Preservation of frozen yeast cells by trehalose, Journal of Biotechnology and Bioengineering 65 (5) (1999) 572-578.

A.Suhajda, B. Janzso, J. Hegoczki, I. Pais, Accumulation of microelements in yeast, in: Proceedings of 6th international trace element symposium, 1994, pp.123-134.

I.Pais, B. Janzso, A. Suhajda, In production and application of selenium yeast in Hungary, 3rd Internat Symposium: Selenium in geochemistry, biological functions of selenium, Trend Biochem. Sci. 5 (1980)203-206.

S.I. Park , J.M. Park , H.S. Chittum , E.S. Yang , B.A. Carlson , B.J. Lee, et al., Selenocysteine tRNAs as central components of selenoprotein

Al-Qadisiyah Journal of Agriculture Sciences
(QJAS)
| 2077-5822 |

biosynthesis in eukaryotes, Biomed Environ Sci. 10 (2-3) (1997) 116-124.

Y.Zhang, W.T.Jr. Frankenberger, Speciation of selenium in plant water extracts by ion-exchange Chromatography- hydride generation atomic absorption spectrometry, Sci Total Environ. 269 (1-3) (2001) 39-47.

تحضير سلينوسستين العضوي باستخدام خميرة المعزولة محليا

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الخلاصة

تعد السلينيوم اللاعضوي من العناصر السامة بتراكيز الواطئة جدا للحياة، في حين ان المركب سلينوسيستين العضوي تعد موقع الاهتمام ولها الدور الاساسي في علوم التغذية والكيمياء الحياتية للخلية. ان الخمير الغنية بالسلينيوم **(Se-yeast) من المركبات الشائعة والمعتمدة** لتغذية الجسم بهذا العنصر الاساسي.

وفي هذه الدراسة تم تحضير سلينوسستين العضوي باستخدام خميرة الخبز (***Saccharomyces cerevisiae***) المعزولة محليا. **اعتمدت وسط زرعي محلي من** وسط خلاصة التمر الغنية بفوسفات البوتاسيوم **(KH_2PO_4) بنسبة 0.2%** و سلفات الامونيوم بنسبة **0.6%** كوسط زرعي بديل . كما استخدم ملح السلينيوم بتراكيز مختلفة **(30, 60, 120 and 240 µg /ml) مضافة الى الوسط** الزرعي للخميرة. وقد اتضح بان افضل تركيز مضافة هو (**30µg/ml**) للحصول على البيئة النموذجية للنمو والحصول على خمير حمراء اللون تضاهي نموذج الخميرة القياسية. تم مطابقة المنتج سلينوسيستين العضوي المحضر باستخدام جهـــاز كروماتوغرافي **ذو الأداء العالي (HPLC)و** جهاز الامتصاص الذري **(AAS)** مع المــادة القياسية المستوردة من شركــة (**Sigma**) ، **اكدت النتائج مطابقة المنتوج** المحضر من سلينوسيستين .

الكلمات المفتاحية : سلينوسيستين العضوي، سلينيوم، خميرة الخبز و سلينوسيستين