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Original Article

Production of Organically Selenium Yeast by Fermentation and Analysis by Atomic Absorption Spectrophotometry

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ABSTRACT

Objectives: The objective of this study was to perform selenium yeast production and analyze the selenium and protein contained in selenium yeast obtained. **Methods:** Selenium yeast production was carried out by incubating the stationary phase culture of yeast with selenium solution for 24 hr, and yeast biomass was dried by Freeze-dryer to produce selenium yeast in powder form, then the selenium yeast was determined to the selenium content with atomic absorption spectrophotometer at a wavelength of 196 nm and protein content analyzing by the Bradford method at a wavelength of 595 nm using UV-Vis spectrophotometer. **Results:** Selenium yeast production reached 2.5 g of selenium yeast in powder form. The selenium and protein content of selenium yeast was 4258.0096 (±278.39) µg Se/g and 0.8505 (±0.0045) mg/mL, respectively. The method was easy, simple, and can prove that selenium yeast can still be produced and analyzed for the selenium and protein content. **Conclusion:** The present study demonstrated that production of selenium yeast in the form of dry powder could be carried out using the stationary phase culture of *S. cerevisiae* incubated with 30 µg/mL selenium solution, and the resulting selenium yeast can be analyzed for selenium content using atomic absorption spectrophotometer and protein content by Bradford method using UV-Vis spectrophotometer.

Key words: Atomic absorption spectrophotometry, Bradford, *Saccharomyces cerevisiae*, Selenium, Selenium-enriched yeast.

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INTRODUCTION

Yeast is a eukaryotic organism that belongs to a group of organisms called fungi. Yeasts are generally single-celled or unicellular organisms. Yeast is a chemoorganotroph because it uses organic compounds as an energy source and does not require sunlight for growth. Yeast can grow well at neutral or slightly acidic pH. Yeasts also need essential elements such as carbon, hydrogen, oxygen, phosphorus, iron, and magnesium. Carbon is obtained primarily from disaccharides such as sucrose and maltose or hexose sugars such as glucose and fructose.[1] The size of yeast cells can reach 2-3 µm to 20-50 µm with a diameter of 1-10 µm.[2] Yeast cells consist of various components such as cell wall, cytoplasmic membrane, nucleus, vacuoles, mitochondria, lipid globules, and cytoplasm. However, the overall cellular composition of yeasts may vary depending on the growing conditions.[3]

Saccharomyces cerevisiae or *S. cerevisiae* is one type of yeast that humans most often use, which has long been used to produce wine, bread, flavoring agent for foods industrial, and therapeutic. In pharmaceuticals, there has been increasing interest in using *S. cerevisiae* as a probiotic agent, not only for human health but also for promoting growth and health for animals.[2] *S. cerevisiae* is a species of the genus *Saccharomyces*, members of the family *Saccharomycetaceae*, subfamily *Saccharomycetoideae*, and characterized as unicellular fungi that reproduce asexually and sexually by ascospores.[4] To reproduce asexually, *S. cerevisiae* clones itself by creating a new daughter cell.[5]

Selenium (Se) is a non-metallic element with an atomic weight of 79. Selenium is one of the critical elements used in the body to carry out the normal functions of an organism.[6] Selenium can be obtained from food. The amount of selenium in food depends on several geological, geographical, and other factors.[7] The daily intake recommendation for selenium is 55 µg/day for adults, pregnant women should take an additional 5 µg/day and breastfeeding women an additional 4 µg/day.[8] Selenium is a trace element with a wide range of effects, such as anti-inflammatory, anti-cancer, and immunomodulatory.[9-11] Selenium also has antioxidant properties and protects the organism against the action of free radicals and carcinogenic factors.[6] Selenium deficiency can increase heart disease and cancer risk, and selenium is also associated with thyroid hormones. Animal studies have shown that selenium deficiency can lead to decreased immune function resulting in the inability of neutrophils and phagocytic macrophages to destroy antigens. Low selenium status in humans has been reported to cause a decreased immune response to poliovirus vaccination.[12]

S. cerevisiae is a type of yeast used to produce selenium yeast. It is known that selenium yeast or selenium-enriched yeast is one of the sources of selenium needed by the human body. Currently, selenium yeast is produced by fermenting *S. cerevisiae* in selenium-enriched media.[13] However, it is known that selenium can be toxic to yeast so that it will inhibit growth and will also affect the activity of *S. cerevisiae*.[14,15] Currently, supplements containing selenium yeast are widely available for human consumption, but in Indonesia, to obtain selenium yeast still relying on imports. Accordingly, this is an opportunity to develop methods for obtaining yeast selenium as a source of selenium that can be produced easily and simply. In this study, selenium yeast production was carried out in dry powder selenium yeast, which is made using stationary phase

culture of *S. cerevisiae*. The yeast suspension obtained was incubated with selenium solution to made selenium yeast. To determine the presence of selenium in the obtained dry powder selenium yeast was using an atomic absorption spectrophotometer, and for determination of the protein content was carried out using the Bradford method.

MATERIALS AND METHODS

Instruments

Atomic absorption spectrophotometer (AAS) (Shimadzu A-6300, Japan), Se Hollow Cathode Lamp (Hamamatsu Photonics KK, Japan), single beam UV-Vis spectrophotometer (Shimadzu, Japan), micropipette (Socorex, Switzerland), pH meter (Horiba pH 100, Japan), centrifuge, scale (Shimadzu AP 225 WD, Japan), shaker, oven (Memmert UM 200, Germany), water bath (Memmert, Germany), hot plate (Gerhardt, Germany), autoclave (Hirayama Hiclave HVE-50, Japan), freeze dryer (Heto FD4, Japan), magnetic stirrer (IKA Hs-7, Germany), centrifuge tubes, Erlenmeyer flasks, volumetric flasks, and glass tools.

Materials

Saccharomyces cerevisiae, sabouraud dextrose agar media (Himedia, India), dextrose, peptone (Himedia, India), yeast extract (Himedia, India), sodium selenite (Indukern S.A, Spanish), concentrated nitric acid (65%), concentrated hydrochloric acid (37%), 0.1 M sodium hydroxide, 3.5% sodium chloride, Bradford reagent, bovine serum albumin (Sigma-Aldrich, USA), deionized water, distilled water (Ikapharmindo, Indonesia) and all other chemicals used were of analytical grade from other Indonesian manufacturers.

Preparation of yeast inoculum

S. cerevisiae was pre-cultured to sabouraud dextrose agar media. Sabouraud dextrose agar media was made by dissolving 6.5 g of sabouraud dextrose agar media powder to 100 mL of distilled water in Erlenmeyer flask. After the media was dissolved, the Erlenmeyer flask was tightly closed with cotton and then sterilized media using an autoclave at 121°C for 15 min. The sterile sabouraud dextrose agar media was poured aseptically into a sterilized tube, and then the tube was tilted and stored at 4°C for 24 hr until a solid medium was obtained. After that, *S. cerevisiae* was inoculated into a solid sabouraud dextrose agar medium aseptically and incubated at room temperature for 84 hr.

Preparation of selenium solution

Selenium solution has been prepared by dissolving 0.1095 g sodium selenite in 50 mL deionized water to obtain a selenium standard stock solution 1000 µg/mL. The final concentration for Selenium solution was made by pipetting 0.75 mL of selenium standard solution 1000 µg/mL into a 25 mL volumetric flask until a selenium solution with a concentration of 30 µg/mL was obtained. The final pH of the selenium solution was adjusted to 5.0, and the selenium solution was sterilized using an autoclave at 121°C for 15 min.

Production of selenium yeast

Yeast extract peptone dextrose broth medium was used for the fermentation medium. Yeast extract peptone dextrose broth medium was made by dissolving 4 g of dextrose, 4 g of peptone, and 2 g of yeast extract in 200 ml of distilled water in Erlenmeyer flask, stir until all components were dissolved entirely and the final pH of the medium was 4.8-5.6. Then the Erlenmeyer flask was closed tightly with cotton and sterilized using an autoclave at 121°C for 15 min. The yeast extract peptone dextrose broth medium was inoculated with 84 hr pre-cultured *S. cerevisiae*, then incubated using a shaker (150 rpm) at room temperature for 84 hr. After 84 hr of incubation, 25 mL selenium solution (concentration 30 µg/mL) was added, then incubation again using a shaker (150 rpm) at at room temperature for 24 hr. The yeast biomass was obtained by centrifugation (3000 rpm, 10 min, 4°C) of the medium, and the pellet was rinsed twice using sterile deionized water. After that, the yeast biomass was dried by Freeze-dryer to produce selenium yeast in powder form.

Calibration curve of selenium

The calibration curve of selenium was made by pipetting 0.125 mL, 0.250 mL, 0.375 mL, 0.500 mL and 0.625 mL of selenium standard stock solution 1000 µg/mL into a 25 mL volumetric flask until selenium solutions with concentrations of 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, and 25 µg/mL was obtained, respectively. The final series solutions were measured at a wavelength of 196 nm with an atomic absorption spectrophotometer. The measured absorbance's of the final series solutions were used to make a calibration curve of selenium.

Analysis of Selenium

Analysis of selenium was carried out using atomic absorption spectrophotometry. 0.1 g of dry powder selenium yeast was digested at 120°C for 20 min with 10 ml of concentrated nitric acid (65%). Then added 2 ml of concentrated hydrochloric acid (37%). After the solution was cooled, heated again at 80°C for 10 min. After cooling, the solution was filtered, then 25 mL of deionized water was added using a volumetric flask. The absorption of the final solution was measured at a wavelength of 196 nm with an atomic absorption spectrophotometer.

Calibration curve of bovine serum albumin

A standard protein solution was prepared by dissolved 0.05 g of bovine serum albumin in 50 mL of sterile distilled water to obtained bovine serum albumin standard solution 1 mg/mL (Protein stock solution). The protein stock solution was diluted to obtain a concentration of 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, and 1 mg/mL. Then the protein standard solutions series measurement was carried out by adding 100 µL of the standard solution series with 4 mL of Bradford reagent. Then the solution was homogenized and incubated at room temperature (25°C) for 10 min. The final series solutions were measured at a wavelength of 595 nm with a single beam UV-Vis spectrophotometer. The measured absorbance's of the final series solutions were used to make a calibration curve of bovine serum albumin.

Analysis of protein

Analysis of protein was carried out using a single beam UV-Vis spectrophotometer. 0.25 g of dry powder selenium yeast was heated at 60°C for 90 min with 15 ml of 0.1 M sodium hydroxide in 3.5% sodium chloride. Then the solution was centrifuged at 4000 rpm for 30 min at 4°C. 100 µL of supernatant was pipetted and added 4 ml of Bradford reagent, homogenized, and incubated at room temperature (25°C) for 10 min. The absorption of the final solution was measured at 595 nm with a single beam UV-Vis spectrophotometer.

RESULTS

In this present study, pre-cultured *S, cerevisiae* was inoculated in yeast extract peptone dextrose broth medium and incubated at room temperature for 84 h. After 84 hr incubation, the selenium solution (concentration 30 µg/mL) was added before incubating again at room temperature for 24 hr. The yeast biomass was obtained by centrifugation of medium using a centrifuge (3000 rpm, 10 min, 4°C), and the pellet was rinsed twice using sterile deionized water. After that, the pellet was dried by Freeze-dryer to produce 2.5 g of selenium yeast in powder form. After the production stage and obtained selenium yeast

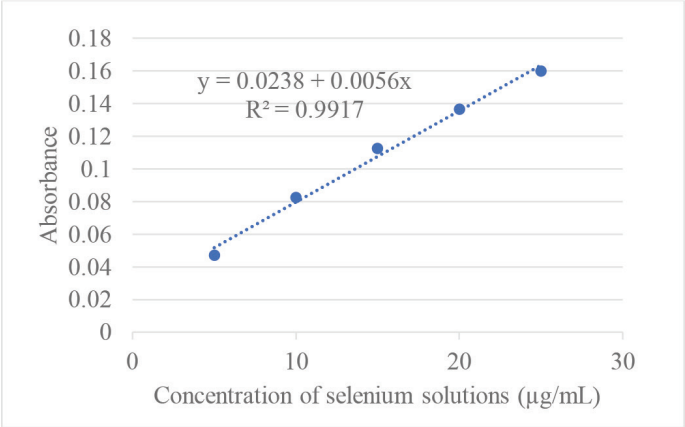

Figure 1: Calibration curve of selenium.

Table 1: Data results of the calibration curve of selenium.

No	Concentration of selenium solutions (µg/mL)	Absorbance
1	5	0.0471
2	10	0.0823
3	15	0.1123
4	20	0.1364
5	25	0.1597

Table 2: Data results of selenium content in selenium yeast.

No	Absorbance	Concentration (µg/mL)	Selenium content in selenium yeast (µg Se/g)	Average (±SD) (*n*=3) of selenium content in selenium yeast (µg Se/g)
1	0.1183	16.8750	4185.2679	
2	0.1141	16.1250	4023.2036	4258.0096 (±278.39)
3	0.1275	18.5179	4565.5572	

in powder form. Then the analysis of selenium and protein contents in selenium yeast powder was carried out.

Selenium content was analyzed by digesting selenium yeast powder at 120°C with concentrated nitric acid. Furthermore, concentrated hydrochloric acid was added and then heated again at 80°C. The absorption of the final solution was measured at a wavelength of 196 nm with an atomic absorption spectrophotometer. Then the measured absorption was calculated using the linear regression equation as shown in Figure 1 to obtain the concentration of Se content in the selenium yeast. The linear regression equation was obtained from the absorption measurements of the selenium solutions series, as shown in Table 1. The average of three repetitions of selenium content in selenium yeast was 4258.0096 (±278.39) µg Se/g, and it was used as the final result of selenium content in selenium yeast powder, complete data of 3 repetitions of measurements of selenium content in selenium yeast were presented in Table 2.

Analysis of protein content was carried out by the Bradford method. The Bradford method uses a Coomassie Blue G-250 (Bradford reagent), which binds to proteins to form a blue ionic solution. The absorbance of the final solution was measured using a UV-Vis spectrophotometer at a wavelength of 595 nm. Before analyzing the protein content of selenium yeast, selenium yeast was extracted to obtain the protein. Protein extraction was carried out by combining water, sodium hydroxide, sodium chloride at high temperatures. Then the solution was centrifuged using a centrifuge at 4000 rpm for 30 min at 4°C. A certain supernatant was added with Bradford reagent and incubated at room temperatures (25°C) for 10 min. The absorption of the final solution was measured at 595 nm with a single beam UV-Vis spectrophotometer. Then the measured absorption was calculated using the linear regression equation shown in Figure 2 to obtain the concentration of protein content in the selenium yeast. The linear regression equation was obtained from the results of the absorption measurements of the protein standard solutions series, as shown in Table 3. The average concentration of three repetitions of protein content in selenium yeast was 0.8505 (±0.0045) mg/mL and used as the final result of protein content in selenium yeast powder, complete data of 3 repetitions of measurements of protein content is presented in Table 4.

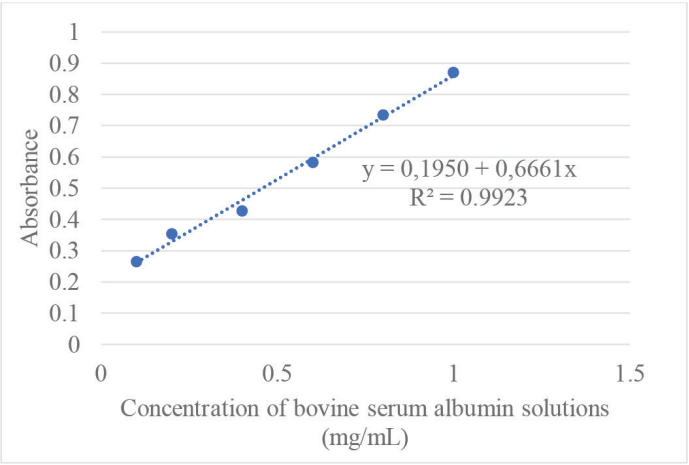

Figure 2: Calibration curve of bovine serum albumin.

Table 3: Data results of the calibration curve of bovine serum albumin.

No	Concentration of bovine serum albumin solutions (mg/mL)	Absorbance
1	0.1	0.2645
2	0.2	0.3544
3	0.4	0.4277
4	0.6	0.5834
5	0.8	0.7344
6	1	0.8707

Table 4: Data results of protein content in selenium yeast.

No	Absorbance	Concentration of protein content in selenium yeast (mg/mL)	Average (±SD) (*n*=3) concentration of protein content in selenium yeast (mg/mL)
1	0.7601	0.8484	
2	0.7595	0.8475	0.8505 (±0.0045)
3	0.7650	0.8557	

DISCUSSION

Selenium is a trace element with a wide range of effects, such as anti-inflammatory, anti-cancer, and immunomodulatory.[9-11] Selenium also has antioxidant properties and protects the organism against the action of free radicals and carcinogenic factors.[6] The use of selenium in the form of dietary supplements has been popular and widely used because it has excellent potential as a source of selenium. Seeing the immunomodulatory effect of selenium, it is necessary to explore the safest way to increase selenium levels in the human body, mainly in the form of the selenium yeast, but in Indonesia to obtain selenium yeast still relying on imports. Currently, selenium yeast is produced by fermenting *S. cerevisiae* in selenium-enriched media.[13] However, it is known that selenium can be toxic to yeast so that it will inhibit growth and will also affect the activity of *S. cerevisiae*.[14,15] The toxicity of selenium can be explained by the fact that this element is an analog of sulfur. Thus, when selenium is present in large quantities, it then enters the yeast, which will cause conformational changes and functional activity of the yeast.[16] In this case, the determination of simple and easy production methods needs to be well defined to improve biomass production of selenium yeast, and it is of great concern in the food and pharmaceutical industry.

In the present study, to obtain selenium yeast, stationary phase culture of *S. cerevisiae* was added with selenium solutions, then incubating again using a shaker at room temperature for 24 h. This was done to prevent the inhibition of yeast growth. It was known that the presence of selenium in the growth medium causes mutation and inactivation of various metabolic pathways of yeast, even selenium also affects the characteristics and morphology of yeast, such as changes in the structure and complexity of the membrane of the cell wall so that it will ultimately affect the growth of yeast.[17] After 24 hr of incubation, the yeast biomass was obtained by centrifugation using a centrifuge (3000 rpm, 10 min, 4°C). The centrifugation was used to separate unnecessary parts such as selenium which was not bound by yeast, and fermentation products such as alcohol. The pellet obtained was rinsed twice with sterile deionized water to remove selenium which may still not be bound by yeast and other materials that might remain in the pellets. Drying of the pellets was done using a Freeze-dryer to produce 2.5 g of selenium yeast in powder form.

Analysis of selenium content was carried out using atomic absorption spectrophotometry at a wavelength of 196 nm. Analysis of selenium content using atomic absorption spectrophotometry was chosen because it is a specific method for analyzing selenium in samples.[18] Before being analyzed, selenium yeast powder was digested at high temperature with concentrated nitric acid, which functions to digested and reduced the disturbing effect that can come from the sample. Nitric acid was chosen because it has the oxidizing capacity, accessibility, and availability in the market, then concentrated hydrochloric acid was added to complete the sample digestion process. The absorption of the final solution was measured at a wavelength of 196 nm with an atomic absorption spectrophotometer. From the results obtained, it is known that the addition of selenium solution with a concentration of 30 µg/mL, resulted in selenium content was 4258.0096 (±278.39) µg Se/g in selenium yeast. Several things influence the accumulation of selenium both intracellularly and extracellularly in yeast cells. Extracellular selenium accumulation is influenced by the ability of yeast to bind selenium, where this process involves ionic bonding and the complexation of Se ions by yeast cell wall components. The yeast cell wall structure consists of two layers: the inner layer in the form of carbohydrate polymers β-1,3-glucans, and the outer layer mannoproteins of the cell wall.[19] The cell wall also consists of β-1,6-glucans, and chitin makes up between 1 and 2% of the total cell wall composition. All cell wall components are interconnected through covalent bonds to form a homogeneous structure. Low molecular weight but highly branched and water-soluble as β-1,6-glucan covalently bonded to β-1,3-glucan. In addition, β-1,6-glucans are linked to chitin via β-1,4-glycosidic bonds.[20] In general, mannoproteins are highly glycosylated polypeptides and are rich in mannose. Decreased permeability of yeast cell walls can be caused by the presence of long branched carbohydrate chains linked to polypeptide chains via O-glycoside bonds with serine or threonine hydroxyl groups. The N-glycosidic bond can also be distinguished for the amide group of asparagine, leading to forming the rigid structure that make up the protective layer for the cell wall.[21] The mannoprotein layer forms an outer protective barrier and is decisive yeast cell wall permeability is quite influential in the process of binding selenium by yeast cells. Biosorption of selenium occurs due to the presence of a functional group that shows a negative charge on the cell wall surface, such as phosphodiester, sulfide bridges, mannose phosphate residues, negatively charged phosphate, and hydroxyl groups. The rate of biosorption is strongly influenced by the surface hydrophobicity of the yeast cell wall, which depends on the presence of polysaccharides, proteins, and lipids. The polysaccharide component that composes the yeast cell wall is a barrier that can reduce the penetration of selenium into the cell interior. The process of intracellular accumulation of selenium occurs through active transport inside the yeast cell interior.[17] The binding of selenium by yeast cells can depend on the culture conditions, selenium concentration in the experimental medium, selenium concentration in the fermentation medium, and the type of yeast used. It has an impact on the results biomass and selenium content in the yeast cell biomass produced. The transport and bioaccumulation mechanisms of selenium are related to the presence of different transport conveyors or the presence of nonspecific transport of complex ions with a sugar substrate contained in yeast cells.[17,22,23]

In this study, selenium yeast powder was analyzing for the protein content using the Bradford method. The Bradford method was chosen because this method is easy to practice, has relatively good accuracy, and rapid method for analyzing protein content in samples. The Bradford method is a test for measuring the colorimetric concentration of proteins in a colored solution. In the Bradford method, a Coomassie G-250 (Bradford reagent) reacts with an ionize able group on the protein so that it disrupts the tertiary structure of the protein and exposes a hydrophobic pocket followed by a dye that binds hydrophobic amino acids to form a stable complex that gives a color (blue) that can be measured for absorbance by using UV-Vis spectrophotometer at a wavelength of 595 nm.[24,25] From the results obtained, the protein content was 0.8505 (±0.0045) mg/mL in selenium yeast. The cell wall of *S. cerevisiae* consists of protein. The protein layer of the cell wall can consist of at least 20 different types of glycoproteins. The composition of the protein layer can vary depending on growing conditions. Proteins contained in yeast cell walls are helpful for making it easier for yeasts to reproduce and form biofilms. Proteins also help yeast cells to retain iron and facilitate the absorption of sterols needed for yeast growth under anaerobic conditions. The amount of protein in yeast cells is also influenced by the growth medium of yeast. Therefore, it is also possible to use selenium yeast as a source of protein and minerals to be used as a supplement to overcome selenium deficiency in the diet.

CONCLUSION

The present study demonstrated that production of selenium yeast in the form of dry powder could be carried out using the stationary phase culture of *S. cerevisiae* incubated with 30 µg/mL selenium solution, and the resulting selenium yeast can be analyzed for selenium content using atomic absorption spectrophotometer and protein content by Bradford method using UV-Vis spectrophotometer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Se: Selenium; **AAS:** Atomic absorption spectrophotometer; ***S. cerevisiae:*** *Saccharomyces cerevisiae*.

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