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Original Article

Production of Zinc-Yeast from *Saccharomyces cerevisiae* Fermentation and Determination of Zinc Content by Atomic Absorption Spectrophotometry

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ABSTRACT

Objectives: This study aimed to obtain an optimum method for zinc-yeast production. **Materials and Methods:** Production of zinc-yeast was prepared using Yeast Extract Peptone Dextrose (YEPD) as a culture medium. Yeast that had been inoculated into culture media was incubated with a shaker at room temperature for 84 hrs, then zinc sulfate was added with various concentrations of 200, 300, and 400 mg/L, and then incubation continued for 24 hrs. The obtain yeast biomass was dried with a Freeze-dryer and analyzed for total accumulated zinc and protein content. Determination of zinc was using atomic absorption spectrophotometry (AAS) at a wavelength of 196 nm, whereas protein content was determined with Bradford method at a wavelength of 595 nm using UV-Vis spectrophotometer. **Results:** Zinc-yeast with the addition of 200 mg/L zinc sulfate obtained 0.3111 g that contains 2228.60 µg/g of zinc and 0.8515 mg/L of protein. While zinc-yeast with the addition of 300 mg/L zinc sulfate obtained 0.3704 g that contains 2458.30 µg/g of zinc and 0.8682 mg/L of protein. And the last, zinc-yeast with the addition of 400 mg/L zinc sulfate obtained 0.2370 g that contains 2648.43 µg/g of zinc and 0.8935 mg/L of protein. **Conclusion:** Production of zinc-yeast with the highest zinc and protein content can be obtained by adding 400 mg/L zinc sulfate solution to the stationary phase culture of *S. cerevisiae*. The modified zinc-yeast production method is simple and can be used for the production of functional food products.

Key words: Yeast, *Saccharomyces cerevisiae*, Zinc, Fermentation, Bradford.

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INTRODUCTION

Recently, the world is being overwhelmed in managing the severe acute syndrome respiratory coronavirus 2 (SARS-CoV-2) as known as coronavirus disease 2019 (COVID-19). According to Yasui *et al.* (2020), most of the patients suffering from this disease showed zinc deficiency. Patients with zinc deficiency experience are more complicated, especially if they stay in the hospital sufficiently long, which can build mortality.[1] The aftereffect of the examination with rats showed that the immune response was significantly decreased (more than 50%) in zinc deficiency sufferers. Zinc has a role in immune response, including the development of immune cells and gene expression in immune cells. Additionally, zinc has an important role in cell multiplication and separation, signal transduction, energy-related supplement digestion, body development, and improvement. Zinc can be obtained from various sources, including from yeast fermentation which is abundant with protein, amino acids, and peptides.[2]

Yeast is a group of microorganisms that are a source of enzymes in various food, feed, and agricultural production. Also, the enzymes in yeast can be used as specific biocatalysts in chemical and pharmaceutical synthesis. The considerable use of yeast affects the need for the main ingredients of yeast extract.[3]

Zinc-yeast production is generally done by enriching yeast with zinc, as was done by Kamra Azad *et al.* (2014), namely adding zinc to yeast cultivation media. However, in this method, using a very low concentration of zinc to avoid inhibition of growth in yeast, thus causing the zinc content in yeast is quite small. While, in this research, zinc-yeast production was modified Kieliszek *et al.* (2019) method which is the addition of zinc sulfate to the stationary phase culture of *S. cerevisiae*, with YEPD medium as cultivation media. The obtained dry zinc-yeast will also determine the content of zinc using atomic absorption spectrophotometry (AAS) and also protein using Bradford method.[4,5]

MATERIALS AND METHODS

Materials

Atomic Absorption Spectrophotometer (Shimadzu®), UV-Vis Spectrophotometer single beam (Shimadzu®), micropipette (Socorex®), pH meter (Horiba pH 100), centrifuge (NF 400R), shaker (SHO-2D Daihan Scientific®), oven (Memmert UM 200), analytical scale, autoclave (HVE-50 Hiclave Hirayama), hot plate stirrer (IKA RH Basic 2), sonicator (Ultrasonic Branson 1800), freeze-dryer, oven, and glass tools.

Saccharomyces cerevisiae, Sabouraud Dextrose Agar (SDA), zinc sulfate, sulfuric acid, peptone, dextrose, sodium hydroxide, sodium chloride, distilled water, deionized water, nitric acid, hydrogen peroxide, Bradford reagent, and bovine standard serum albumin.

Preparation of aqueous solutions of Zinc

Zinc sulfate was prepared at a concentration of 10% in deionized water. Then diluted to obtain zinc sulfate with a concentration of 200, 300, and 400 mg/L. Each zinc sulfate was sterilized using an autoclave (1 atm, 121°C) for 15 min.

Preparation of yeast inoculum

S. cerevisiae was adapted to sabouraud dextrose agar (SDA) media. SDA media was made by dissolving 6.5 grams of SDA media in 100 mL of distilled water with heating. SDA media was sterilized using an autoclave at a temperature of 121°C and a pressure of 1 atm for 15 min. Then SDA media are poured aseptically 10 mL into a sterilized test tube. The test tube was tilted and kept at 4°C for 24 hrs. After that, *S. cerevisiae* was inoculated into SDA media aseptically and incubated with a shaker at room temperature for 84 hrs.

Culture media

Zinc-yeast was prepared using YEPD as a culture medium. YEPD media was made by dissolving 2 grams of dextrose, 2 grams of peptone, and 1 gram of yeast extract in 100 mL distilled water. Stir until dissolved and pH was checked (4,8-5,6). After that, YEPD media was sterilized using an autoclave (1 atm, 121°C) for 15 min.

Culture yeasts supplemented with Zinc

Yeast fermentation was carried out by aseptically inoculating *S. cerevisiae* in YEPD media. Then media was incubated using a shaker (150 rpm) at room temperature for 84 hrs. 12.5 mL of zinc sulfate with various concentrations of 200, 300, and 400 mg/L were added after 84 hrs of incubation, and then incubation continued for 24 hrs with shaker (150 rpm). Every cell suspension was centrifuged at 3000 rpm at 4°C for 10 min. Biomass were suspended twice with sterile water. The obtained biomass was dried using Freeze-dryer to obtain dry zinc-yeast.

Determination of total accumulated zinc in yeast cells

0.1 g of yeast biomass is purified using 15 ml of 65% (v/v) nitric acid and heating for 1 hr at 90°C. Then 2 mL of hydrogen peroxide (30%) are added and the temperature is increased to 140°C for 20 min. After cooling, the sample was diluted with 30 ml of deionized water. The solution was filtered and the filter results were used for zinc content analysis by atomic absorption spectrophotometry method with wavelength 213.9 nm and zinc lamp 8 mA.

Determination of protein content

0.5 g of zinc-yeast was added 30 mL of 0.1 M sodium hydroxide in 3.5% sodium chloride. Then incubated at 60°C for an hour and a half, and centrifuged at 4000 rpm at 4°C for 30 min. For estimation, 100 µL of the supernatant was added to 5 mL of Bradford reagent, homogenized and incubated for 10 min at room temperature. Standard curves were made from BSA with a concentration of 0.1; 0.2; 0.4; 0.6; 0.8; 1.0; and 1.2 mg/mL. The absorption was measured by UV-Vis spectrophotometry with a wavelength of 595 nm.

RESULTS

S. cerevisiae is characterized as a unicellular fungus that reproduces vegetatively by multilateral budding and sexually through ascospores. The vegetative cells are globose, ovoidal, or cylindrical in shape and appear granular also light cream-colored, as can be seen in Figure 1. These yeasts can be cultivated with various media, one of "rich" media that can be used for yeast is YEPD (yeast extract peptone dextrose). Yeast that had been inoculated into culture media was incubated with a shaker (150 rpm) at room temperature for 84 hrs (the stationary phase culture of yeast). The suspension of yeast was added zinc sulfate with various concentration of 200, 300, and 400 mg/L, and then incubation continued for 24 hrs. After that, the suspension of yeast was centrifuged to obtain yeast biomass and washed twice with deionized water. The biomass was dried with a Freeze-dryer to obtained 0.3111, 0.3704, and 0.2370 g of dry zinc-yeast with the

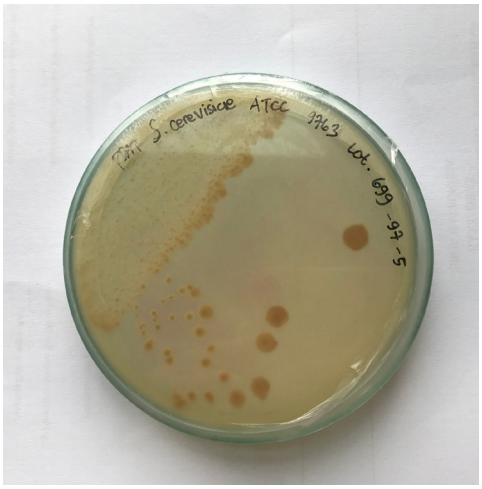

Figure 1: *Saccharomyces cerevisiae*

Table 1: Result of total accumulated zinc in dry zinc-yeast.

The Concentration of Zinc Sulfate in Yeast (mg/L)	Biomass of Zinc Yeast (g)	Absorbance	Concentration (mg/L)	Content (µg/g)	Average Content (µg/g)
200	0.3111	0.0528	0.0895	2226.44	2228.60 ± 3.06
		0.0529	0.0897	2230.76	
300	0.3704	0.0585	0.0994	2477.79	2458.30 ± 27.57
		0.0576	0.0978	2438.81	
400	0.2370	0.0628	0.1069	2661.41	2648.43 ± 18.36
		0.0622	0.1058	2635.44	

Values are means ± SD

added concentration of zinc sulfate being 200, 300, 400 mg/L, respectively. (Table 1).

Analysis of zinc content in yeast zinc carried out using an atomic absorption spectrophotometer. Zinc-yeast needs to be prepared by digestion with 65% nitric acid at 90°C for 1 hr. Then 30% hydrogen peroxide was added and heated again at 140°C for 20 min. The solution appears to have changed from a yellowish color to colorless. Because the concentration is high enough, the solution is diluted again with a 50-dilution factor. The results of the analysis of zinc content can be seen in Table 1 as calculated using calibration curve of zinc sulphate (Figure 2). From these data, it can be seen that the addition of zinc sulfate to yeast is equivalent to the total amount of zinc content, with the smallest value of zinc content is 2228.60 ± 3.06 µg/g and the largest is 2648.43 ± 18.36 µg/g (Table 1).

Determination of protein in zinc yeast was also carried out using the Bradford method. This method uses Coomassie Blue G250 reagent which binds to proteins to form blue ionic. The added zinc-yeast to the reagent was then measured using UV-Vis Spectrophotometry with a wavelength of 595 nm. The measurement of protein content was initiated by making a calibration curve using bovine serum albumin (BSA) (Figure 3). From the calibration curve, a linear regression equation was obtained which was used to measure protein content in zinc-yeast and the results

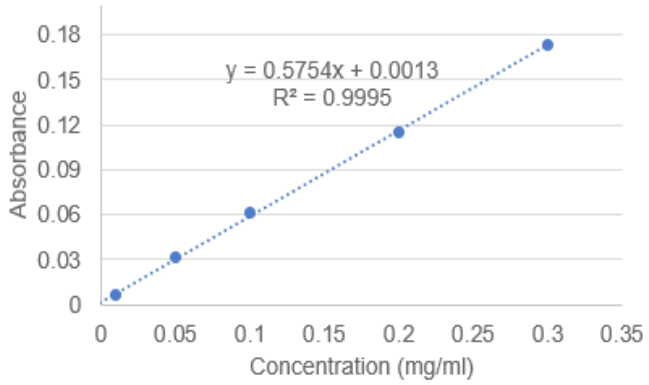

Figure 2: Calibration curve of zinc sulfate.

Table 2: Result of protein content in zinc-yeast.

Concentration of Zinc Sulfate in Yeast (mg/L)	Absorbance	Concentration (mg/L)	Average Concentration (mg/L)
200	0.7150	0.8512	0.8515
	0.7144	0.8502	
	0.7139	0.8493	
300	0.7247	0.8682	0.8682
	0.7244	0.8677	
	0.7250	0.8688	
400	0.7395	0.8942	0.8935
	0.7384	0.8923	
	0.7393	0.8939	

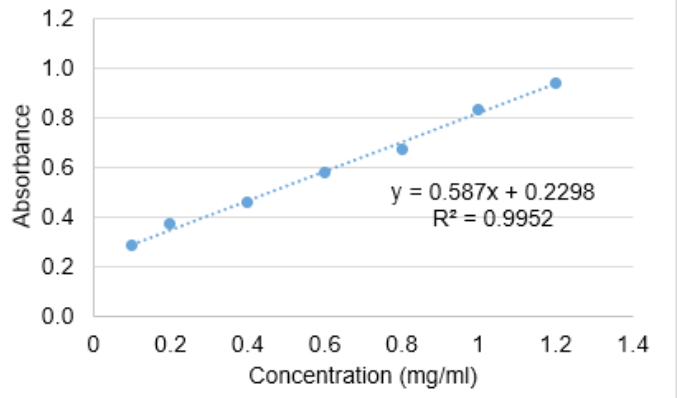

Figure 3: Calibration curve of bovine serum albumin.

can be seen in Table 2. From these data, it can be seen that the addition of zinc sulfate to yeast is equivalent to protein content. The results of analysis protein content were 0.8515, 0.8682, and 0.8935 mg/L in zinc-yeast with the addition of zinc sulfate concentrations of 200, 300, and 400 mg/L respectively (Table 2).

DISCUSSION

Zinc is an essential trace element for the organism which has a role in immune response, including the development of immune cells and gene expression in immune cells. While lack of zinc in the body can reduce the immune response and cause many diseases. As it has been observed that patients suffering from COVID-19 are known to have zinc deficiency, which will lead to complications if a patient in the hospital too long.[1] In addition, lack of zinc in the body can inhibit growth, interfere with nervous systems, and affect almost all other organ systems.[2]

Zinc can be obtained from various sources including zinc-enriched yeast. Generally, the produce of zinc-enriched yeast is by adding zinc to the yeast culture medium which may affect cell growth yeast because zinc can involve in the regulation of structure and metabolic activity of yeast.[4] But zinc levels must be adjusted properly, because if adding a high concentration of zinc salts on culture media will be toxic and inhibit yeast growth. However, if zinc concentration is smaller than 0.1 mg/L then the fermentation will slow down due to the decreased activity of metallo-enzyme alcohol dehydrogenase.[6]

Therefore, in this research the production of zinc enriched yeast was modified which is the addition of zinc will not be added to the culture media, but to the stationary phase culture of yeast. The various zinc concentration is 200, 300, and 400 mg/L was added after 84 hrs of incubation, and then incubation continued for 24 hrs. The purpose of modification is to avoid the toxicity of zinc in yeast and inhibition of yeast growth. After completion of the incubation period, the fermented yeast was centrifuged, and the obtained biomass was washed twice with deionized water to separate the yeast from other substances that were not bound by yeast. The yeast biomass was then dried in a Freeze-dryer to obtained 0.3704, 0.3111, and 0.2370 g of dry zinc-yeast with each addition of zinc sulfate concentration of 200, 300, 400 mg/L (Table 1).

The total zinc content in zinc-yeast was determined by using an atomic absorption spectrophotometer because it is a specific method for metal including zinc. Before being measured, zinc-yeast needs to be digested using concentrated nitric acid (65%) as strong acid and added hydrogen peroxide (30%) as a strong oxidizing agent which serves to increase the oxidation ability. From the results, it is known that the addition of zinc sulfate to yeast is equivalent to the total amount of zinc content, with the smallest value of zinc content is 2228.60 ± 3.06 µg/g which the addition of 200 mg/L zinc sulfate to yeast fermentation and the largest is 2648.43 ± 18.36 µg/g which the addition of 400 mg/L zinc sulfate to yeast fermentation. The absorption of zinc in yeast generally occurs at the exponential phase of culture when the availability of the highest energy source. Nevertheless, this method can be used because at the stationary phase, the yeast population has reached the maximum number and is already in the adaptation stage and generally cultured cells are resistant to physical and chemical stress. Thus, the addition of zinc in the stationary phase can avoid inhibition of culture growth and the possibility of zinc toxicity to yeast cultures.[7]

In addition to determining the total zinc accumulation, zinc-yeast was also determined protein content using the Bradford method because this method is practical and has fairly good accuracy. The principle of the Bradford method is protein binding to Coomassie Blue G250 dye. In this method, the determination of protein content can be estimated through the dye in the form of a blue ionic whose absorption can be measured using UV-Vis Spectrophotometry at a wavelength of 595 nm.[8] The results of analysis protein content were 0.8515, 0.8682, and 0.8935 mg/L in zinc-yeast with the addition of zinc sulfate concentrations of 200, 300, and 400 mg/L respectively.

The cell wall of S. *cerevisiae* consists of protein, mannan, and β-glucan. The amount of protein in yeast cells is also influenced by the growth medium of yeast. Zinc is an important catalytic and/or structural cofactor for many types of proteins. About 9% of genes in eukaryotic organisms and ~5% of prokaryotic genes code for proteins that bind zinc to function and are needed by living things, one of which is yeast. Organisms have

evolved many mechanisms of zinc homeostasis. In *Saccharomyces cerevisiae*, the transcription factor Zap1 is useful as a central regulator of zinc homeostasis in yeast. Zap1 is a transcriptional activator protein that is low in activity in zinc-containing cells and high in cells that lack it. Zap1 increases the expression of many genes, including those encoding the uptake transporter of zinc in the plasma membrane, which is involved when yeast attracts zinc present in the medium. Zap1 also increases the expression of organelle transporters that control zinc levels in intracellular compartments such as vacuoles and endoplasmic reticulum.[9]

CONCLUSION

Production of zinc-yeast with the highest zinc and protein content can be obtained by adding 400 mg/L zinc sulfate solution to the stationary phase culture of *S. cerevisiae*. The zinc content of zinc-yeast can be determined using the AAS method and the protein content can be determined by Bradford method using UV-Vis spectrophotometer. The modified zinc-yeast production method is simple and can be used for the production of functional food products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

YEPD: Yeast extract peptone dextrose; **AAS:** Atomic absorption spectrophotometry; ***S. cerevisiae:*** *Saccharomyces cerevisiae*; **Zn:** Zinc.

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