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Isolation of GSH from Baker Yeast by Homogenizer Method

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Abstract: The purpose of this study was to determine the effect of operating parameters such as the yeast concentration, processing temperature and isolation time on the isolation of GSH using Homogenizer Method. The disrupted cells solution was centrifuged at 12,000 rpm for 20 min and the GSH content in the supernatant was analyzed by taking the optical density (OD) value of the solution using Spectrophotometer at 412 nm. Yeast solution with the concentration of 3, 6, 9 and 12 wt.% was disrupted at their original temperature for 30 sec to study the effect of yeast concentration. The result obtained shows that the optimum value of yeast concentration is 9 wt.% where 10.98 μmol mL^{-1} GSH was isolated. To study the effect of processing temperature, isolation process of the 9 wt.% yeast solution was done at various values of temperature such as 19, 22, 25, 28, 30 and 40°C for 30 sec. The results indicated that, the optimum GSH isolation temperature is 22°C with the isolation of 17.93 μmol mL^{-1} GSH. Then, to study the effect of isolation time, the isolation process of sample with the concentration of 9 wt.% was done at 22°C for 15, 30, 45 and 60 sec. The result indicated that the optimum isolation time for the Ultrasonic process is 30 sec.

Key words: Reduced glutathione (GSH), homogenizer method, protein flavor, antitoxin agent, anticancer

INTRODUCTION

Glutathione is a type of simple peptide which exists in yeast cell, tomato, orange etc. There are two types of Glutathione which is in a reduced form called GSH and in the oxidized form called GSSG. The purpose of this study was to isolate the GSH, a very useful material in our life from baker yeast (to be stated as yeast later on). In order to do so, the yeast cells have to be disrupted.

GSH has multi usage, from its use as a protein flavoring, antibiotic and antioxidant[1] to its use as coenzyme and enzyme in various types of biochemical reaction such as oxidization, reduction and antitoxin processes. GSH can also be used as an antitoxin of oxidized substances that are produced by the oxidization process of selenium inside human body which can cause cancer.

Although there are many sources of GSH, but among them yeast is found to be a suitable raw material not only due to easy handling of the process but also due to its low operational cost compared to other sources[2]. Furthermore, in producing a type of flavoring, it is better to use yeast rather than other sources because the use of yeast as a baking agent in the bakery industries is already familiar to us and safe.

Because of its low production, GSH is still not being used at commercial level. Many studies had been done to increase the yield of GSH production[3-10]. Furthermore, by the development of biotechnology area especially in genetic engineering and bioreactor engineering, the production of GSH can be improved. This include the manipulation of yeast gene for better production and then the work on finding the suitable medium for the growth of the recombinant yeast in the lab-scale bioreactor[11-13].

GSH is a volatile substance which is sweet in taste. From this characteristic, it is assumed that it also exists in Malaysian local fruits such as Durian, Star fruit, Jackfruit, Sapodilla etc. The production method of these materials is significantly different from the existing methods dealing with yeast. Renovation of the bioreactor for the production of GSH from this local fruits has to be done prior to further investigations.

Finally, GSH of the recombinant yeast and fruits will be produced in the large-scale bioreactor. For this purpose, a specific bioreactor might have to be developed. In producing an optimum value of GSH, the work on optimizing the operating parameters of the GSH production such as sample concentration, temperature of the process, sample's pH and incubation time etc. need to be studied.

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For sure, the production of GSH from the recombinant yeast and from fruits will be higher than that of the yeast. The GSH is finally needed to be isolated. Previously, GSH was isolated by disrupting the yeast cell by using ethanol as the extraction solvent[14]. But, this will create another step of job where the ethanol is needed to be separated in order to get a pure GSH. In this study, to avoid this step, homogenizer method[15-17] was used to disrupt the cells where in this method also, similar to Autolysis method[18], distilled water is being used as a solvent which is easy to be separated.

Isolation of GSH is affected by the operating parameters of the method used. Thus, the objective of this study was to determine the effect of the operating parameters such as yeast concentration, processing temperature and isolation time on the isolation of GSH from yeast by homogenizer method. The data can be used as a standard/control when dealing with the recombinant yeast and fruits etc.

MATERIALS AND METHODS

Fresh yeast used in this experiment was purchased at mini market in Balakong, Selangor Darul Ehsan, Malaysia. This yeast was stored in the frozen box (4°C) and was taken out just before running the experiment to avoid contamination and the reduction of its enzyme activities which can reduce the GSH isolation.

In this study, the yeast cells were disrupted by homogenizer method which based on the dispersion of solid particles by a shaft to get a product. The process breaks apart particles and liquid globules, reducing their size and substantially improving a number of important product qualities[19]. The disruption process was done at various conditions of the parameters inside the beaker at homogenizer's rotation speed of 11,000 rpm.

After disruption, the disrupted cells solution then was centrifuged at 12,000 rpm for 20 min. This will separate the solid phase that contains cell wall etc. from the supernatant which contains GSH etc. Before analyzing the GSH, 5 mL of the supernatant was mixed with 5 mL cold perchloric acid and stirred by a small glass rod to deproteinize it.

GSH analysis: To evaluate the GSH content in the supernatant, the OD value of the solution was taken by a Spectrophotometer at 412 nm, which gave better results than the results obtained at 240 nm[5,14]. Then, the GSH concentration was calculated by Bergmeyer method. In this method, two cuvets were prepared, that is control cuvet (CC) and experimental cuvet (EC). CC was filled by 2.55 mL phosphate buffer solution, 0.5 mL deproteinized sample and 0.15 mL albumin solution and stirred by small glass rod.

EC was also filled by the same substance and 0.01 mL glyoxalase solution was added and then the solution was stirred to make it homogenous. Then, 0.02 mL of methylglyoxal was added to EC and finally, 0.02 mL of methylglyoxal was once again added to EC. The concentrations of GSH were calculated by using the equation of Lambert-Beer law.

Effect of yeast concentration: To study the effect of yeast concentration on the GSH isolation by this method, several values of concentrations such as 3, 6, 9 and 12 wt.% had been used. Yeast was dissolved in distilled water at the selected weight percent (% of wt./volume). The isolation was done at their original temperature for 30 sec.

Effect of processing temperature: After getting the value of the suitable or optimum concentration of yeast by the previous experiment, the optimum temperature was then determined. Several yeast solutions with the optimum concentration of 9 wt.% but at the different temperatures such as 19, 22, 25, 28, 30 and 40°C were studied by running the isolation processes for 30 sec.

Effect of isolation time: The effect of isolation time was studied by heating the yeast solution at its optimum concentration of 9 wt.% and at its optimum isolation temperature of 22°C for 15, 30, 60, 300 and 900 sec.

RESULTS AND DISCUSSION

Effect of yeast concentration: The GSH isolation was increased gradually from 3 to 9 wt.% yeast concentration with the yield of 8.88 to 10.98 μmol mL^{-1}. But the GSH isolation was decreased to 10.43 μmol mL^{-1} at 12 wt.% (Fig. 1).

At low concentration (3 wt.%), their low densities enable the cells to be easily homogenized resulting in quite good a yield of GSH isolation. The higher the yeast concentration the higher the yield of GSH, but in contrast at the highest concentration (12 wt.%) the homogenization of the solution become slower than that of at 9 wt.% concentration. This happen probably due to its high density had inhibited the GSH isolation.

Effect of processing temperature: The GSH isolation was increased from 19 to 22°C with the yield of 13.13 to 17.93 μmol mL^{-1} but then decreased till the temperature of 40°C. The isolation of GSH was found low at low processing temperature of 19°C probably due to it was not

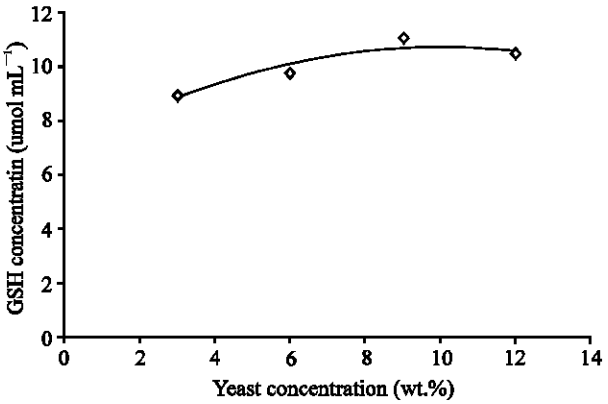

Fig. 1: Effect of yeast concentration on the isolation of GSH by Homogenizer Method run at the original temperature of samples for 30 sec

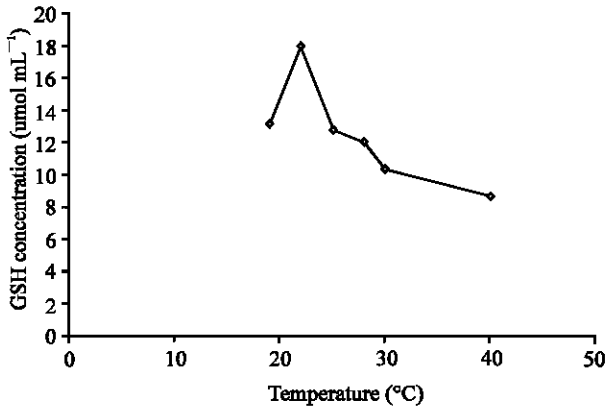

Fig. 2: Effect of temperature on the isolation of GSH by Homogenizer Method done at 9 wt.% yeast concentration for 30 sec

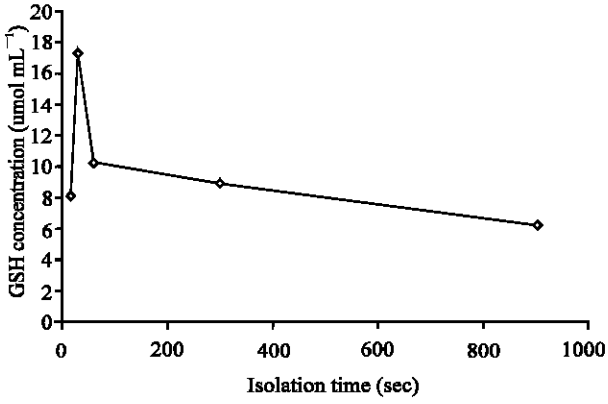

Fig. 3: Effect of isolation time on the isolation of GSH by Homogenizer Method done at the sample concentration of 9 wt.% and processing temperature of 22°C

fully active at this temperature that leads to less GSH isolation. Then, when the temperature was further increased the GSH isolation was decreased due to lost of its activity. This might be occurred because of the combination between temperature and mechanical effects (shaft) caused the cells become easily destroyed. Thus, the rate of denaturation became higher (Fig. 2).

Effect of isolation time: The optimum isolation time for this method was 30 sec and it is too short compared to Autolysis method (Fig. 3). The GSH isolation decreased when the isolation time used were longer than 30 sec probably due to the breakage of GSH structure while it was exposed to the shaft of the Homogenizer for a long duration of time. This evidence was also supported by the findings of Hopkins *et al.*[20] where they only applied 30 sec for each burst when using an Ystral homogenizer (shaft homogenizer).

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