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## Isolation of GSH from Baker Yeast by Ultrasonic 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 Ultrasonic 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 OD value of the solution using Spectrophotometer at 412 nm. To study the effect of yeast concentration, yeast solution with the concentration of 3, 6, 9 and 12 wt.% was disrupted at their original temperature for 15 sec. The result shows that the optimum value of yeast concentration is 6 wt.% where  $12.93 \mu\text{mol mL}^{-1}$  GSH was isolated. To study the effect of processing temperature, Ultrasonic process of the 6 wt.% yeast solution was done at various values of temperature such as 19, 22, 25, 28, 30 and  $40^\circ\text{C}$  for 15 sec. The results indicated that, the optimum GSH isolation temperature is  $22^\circ\text{C}$  with the isolation of  $13.53 \mu\text{mol mL}^{-1}$  GSH. Then, to study the effect of isolation time, the Ultrasonic process of sample with the concentration of 6 wt.% was done at  $22^\circ\text{C}$  for 15, 30, 45 and 60 sec. The result indicated that the optimum isolation time for the Ultrasonic process is 15 sec.

**Key words:** Reduced glutathione (GSH), ultrasonic 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<sup>[1]</sup> 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<sup>[2]</sup>. 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.

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<sup>[3-10]</sup>. 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<sup>[11-13]</sup>.

GSH is a volatile substance which is sweet in taste. From this characteristic, it is assumed that it also exists in our 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<sup>[14]</sup>. 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, Ultrasonic method<sup>[15-18]</sup> was used to disrupt the cells where similar to the Autolysis and Homogenizer methods<sup>[19,20]</sup> 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 Ultrasonic 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 Ultrasonic method which based on high frequency sound as a way to disrupt the cells. The disruption process was done at various conditions of the parameters inside the beaker under the Ultrasonic conditions of 1.0 cycle and 100% amplitude. 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<sup>[5,14]</sup>. Then, the GSH concentration was calculated by Bergmeyer method. In this method, two cuvetts 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, 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. The isolation was done by the method described above at their original temperature for 15 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 6 wt.% but at the different temperatures such as 19, 22, 25, 28, 30 and 40°C were studied by running the isolation processes for 15 sec.

**Effect of isolation time:** The effect of isolation time was studied by heating the yeast solution at its optimum concentration of 6 wt.% and at its optimum isolation temperature of 22°C for 15, 30, 45 and 60 sec.

## RESULTS AND DISCUSSION

**Effect of yeast concentration:** Figure 1 shows the GSH concentration isolated from different concentrations of yeast at their original temperature for 15 sec. From these data, it can be observed that the concentration of GSH isolated was affected by the concentration of the yeast. GSH concentration was increased up to 6 wt.% and then remained constant by the increase of yeast concentration.

By analyzing the data in Fig. 1 it can be concluded that at a low concentration of 3 wt.%, the density of the solution is low enough to let the yeast cells to be penetrated by the Ultrasonic wave effectively due to the random motion of the cells. As a result, only some of the yeast cells were disrupted resulting in low concentration of isolated GSH.

The bigger value of yeast concentration is supposed to have stimulated higher concentration of isolated GSH. But from the results obtained, it can be viewed that the concentration of GSH isolated was increased only up to the yeast concentration of 6 wt.% and then it was almost constant at the yeast concentration of 9 and 12 wt.%. This phenomenon might have happen because at the higher value of yeast concentration, the density of the solution becomes higher and this make its cells too crowded to have chances to be penetrated by the Ultrasonic wave, so

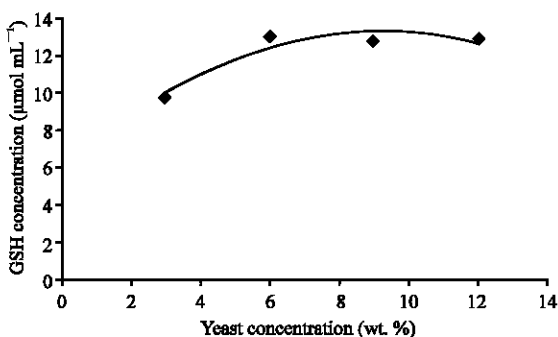


Fig. 1: Effect of yeast concentration on the isolation of GSH by Ultrasonic method run at the original temperature of samples for 15 sec

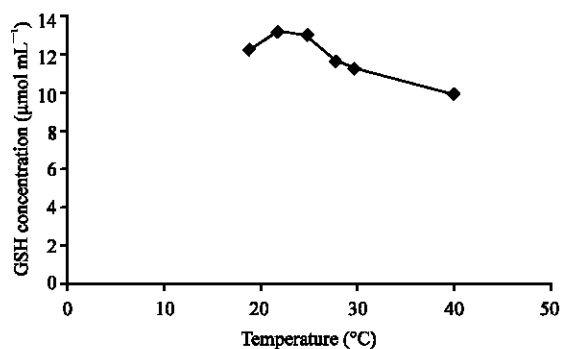


Fig. 2: Effect of temperature on the isolation of GSH by Ultrasonic method run at 6 wt.% yeast concentration for 15 sec

that the disruption process of the yeast cells becomes slow.

**Effect of processing temperature:** Figure 2 shows the relation between GSH concentration and temperature for the Ultrasonic process running at 6 wt.% concentration of yeast for 15 sec. It can be viewed that the concentration of GSH was increased up to the optimum Ultrasonic temperature of 22°C with the yield of 13.13 µmol mL<sup>-1</sup> GSH but then decreased till to the higher temperature of 40°C.

From Fig. 2 it can be inferred that the higher the heating temperature the more the yeast cells were disrupted. Beyond the optimum Ultrasonic temperature (22°C), even though the yeast cells are supposed to become more effectively disrupted but the concentration of the isolated GSH is decreased most probably because at higher temperature the cells motion become so random that their chances to be penetrated by the Ultrasonic wave were lower than those of at lower temperature.

**Effect of isolation time:** From the results of the study of the effect of isolation time on the isolation of GSH, the

optimum isolation time for the Ultrasonic process was 15 sec (data not shown).

It was found out that the optimum isolation time is 15 sec because at other times studied, the optimum processing temperature (22°C) was difficult to be maintained constant. It was experienced that processing the Ultrasonic for more than 15 sec increased the temperature of the sample higher than the optimum processing temperature. Of course, the temperature can be maintained constant by putting ice around the beaker but at the moment it can not be applied to the system used.

From the results of the study, the conclusion that can be made is that the concentration of yeast, processing temperature and the isolation time of the Ultrasonic affect the isolation of GSH. The optimum value of GSH concentration (18.130 µmol mL<sup>-1</sup>) was isolated from the Ultrasonic process with the following operating parameters; yeast concentration of 6 wt.%, processing temperature of 22°C and the isolation time of 15 sec.

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