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## Effects of process parameters on the GSH isolation from baker yeast by autolysis

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The purpose of this study was to determine the optimum value of process parameters (yeast concentration, processing temperature, isolation time, rotation speed and pH) for the isolation of GSH from yeast by autolysis. For each experiment the unmentioned parameters were leaved unadjusted. To study the effect of yeast concentration, yeast with the concentration of 3, 6, 9, and 12 wt % (weight/volume) was disrupted for 1 h. Then, by using the optimum value of concentration the process was done at various values of processing temperature such as 19, 22, 25, 28, 30, and 40°C in order to find out the optimum processing temperature. To find the optimum isolation time, the process was done at the optimum value of concentration and processing temperature for 1, 2, and 3 h. To study the effect of rotation speed, the process was done at the optimum value of concentration, processing temperature and isolation time at various values of rotation speed of 250, 300, 350, and 400 rpm. The effect of sample pH was studied by running the isolation process of yeast solution with pH 1, 2, 3, 4, original pH of the solution, 6.5, and 7.5 at the optimum value of the other parameters. The result obtained shows that the optimum value of the process parameters was 9wt %, 28°C, 1 h, 350 rpm, and 3 for yeast concentration, processing temperature, isolation time, rotation speed of the process, and pH of the sample, respectively, where 24.863 mg/l GSH was isolated.

**Key words:** Reduced Glutathione (GSH); Yeast; Autolysis 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

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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 (Stephen and Jamieson, 1996) to its use as coenzyme and enzyme in various types of biochemical reaction such as oxidization, reduction and antitoxin processes. It has been proved that GSH can also be used as antitoxin of oxidized substance that 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 (Li *et al.*, 1997). 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 formed to increase the yield of GSH production (Ohwada and Sagisaka, 1990; Shimizu *et al.*, 1991; Sakato and Tanaka, 1992; Alfafara *et al.*, 1992a, b; Li *et al.*, 1998a, b; Liu *et al.*, 1999). 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 (Murata, 1982; Murata and Kimura, 1982; Murata *et al.*, 1983).

Previously, GSH was isolated by disrupting the yeast cell autolysis method which used ethanol as the extraction solvent (Riemersma, 1966; Breddam and Beenfeldt, 1991; Wei *et al.*, 2003). 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, distilled water was used as the extraction solvent because it is easy to be separated (Mohamad Ramlan *et al.*, 2004; <http://www.Isbu.ac.uk/biology/enztech.htm>; <http://www.emblheidelberg.de/ExternalInfo/geelof/draft frames/protocol database>).

Isolation of GSH is affected by the process parameters of the method used. The whole objective of this study is to study the effects of process parameters for the autolysis method such as yeast concentration, processing temperature, isolation time, operating parameter (rotation speed), and the sample pH on the isolation of GSH. Previously, the optimum yeast concentration, processing temperature, and the isolation time were found as 15 wt %, 28°C and 1 h, respectively (Mohamad Ramlan *et al.*, 2004). We found that it was difficult to handle the process with high concentration of yeast, so

that in this study we used the lower one. The optimum value of processing temperature and isolation time were once again determined.

## **Materials and Methods**

### ***Materials***

Fresh yeast was used in this experiment and 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.

### ***Disruption method***

In this study, the yeast cells were disrupted by autolysis method which based on heat as a way to disrupt the cells. The disruption process was done inside the beaker under the optimum parameters of the autolysis process. After disrupted, the disrupted cells solution then was centrifuged at 12,000 rpm for 20 min. This will separate the solid phase which contains cell wall etc from the supernatant which contains GSH etc. Before analyzing the GSH, 5 ml of the supernatant was taken and mixed with 5 ml cold perchloric acid and then stirred by a small glass rod to deproteinize it (<http://www.thermo.com>).

### ***GSH analysis***

To evaluate the GSH content in the supernatant, the OD value of the solution was taken by a Spectrophotometer (Anthelie Advanced, produced by SECOMAM, France) at 412 nm which gave better results than the results analyzed at 240 nm (Li *et al.*, 1998a, b; Riemersma, 1966). Then, the GSH concentration can be calculated by Bergmeyer method. Using this method, two cuvetts have to be 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 then stirred by small glass rod.

EC was 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 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 concentrations, the yeast solutions with the concentrations of 3, 6, 9, and 12 wt % (weight/volume) were used. Yeast was dissolved in distilled water for the selected weight percent. The experiment was done in the incubator shaker (250 rpm), at the room temperature for 1 h (Mohamad Ramlan *et al.*, 2004).

### ***Effect of processing temperature***

After getting the optimum concentration, the optimum processing temperature was determined. Several yeast solutions with the optimum concentration were prepared and the isolation process was done at different temperatures of 19, 22, 25, 28, 30, and 40°C for 1 h (Mohamad Ramlan *et al.*, 2004).

### ***Effect of isolation time***

In order to find the optimum isolation time, the isolation process was done for 1, 2, and 3 h by heating the yeast solution at its optimum concentration of 9 wt % and temperature of 28°C.

### ***Effect of rotation speed***

Instead of using the unadjusted speed which is considered not optimized, to find the optimum speed, several yeast solutions with the rotation speed of 250, 300, 350, and 400 rpm were studied by running the isolation processes at the original pH of the solution by the method described above at the optimum value of yeast concentration, processing temperature and isolation time of 9 wt %, 28°C and 1 h, respectively.

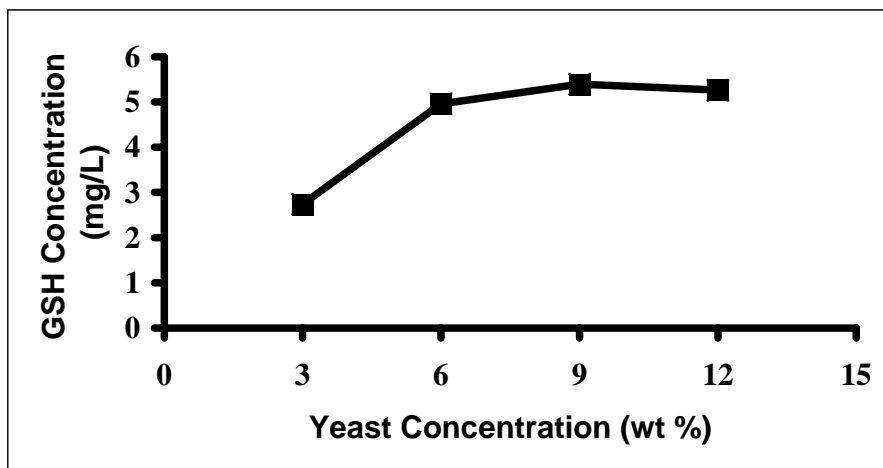
### ***Effect of sample pH***

After getting the value of the suitable or optimum rotation speed by the previous experiment, the suitable or optimum value of pH of yeast solution then was determined. To study this effect on the GSH isolation, several values of pH such as 1, 2, 3, 4, original pH of the solution, 6.5 and 7.5 had been used. The isolation process was done at the optimum value of yeast concentration, processing temperature, isolation time and rotation speed of 9 wt %, 28°C, 1 h and 350 rpm, respectively.

## Results and Discussion

### *Effect of yeast concentration*

From Fig. 1, it can be observed that the concentration of GSH, isolated by autolysis method, was affected by concentration of the yeast. By increase of the yeast concentration from 3 to 9 wt % the GSH concentration increased from 8.93 to 17.53  $\mu\text{mol/ml}$ . However, it decreased to 17.13  $\mu\text{mol/ml}$  when the yeast concentration used was 12 wt %. From this observation, it can be concluded that the concentration of GSH increases due to increase in yeast concentration up to certain extent. Beyond that, the concentration of GSH decreases probably due to slow disruption of the crowded yeast cells at high concentration of solution. The optimum concentration was found to be 9 wt % by using classical optimization method.



**Fig. 1.** Effect of yeast concentrations on the isolation of GSH by autolysis method. The process was done using yeast concentrations of 3, 6, 9, and 12 wt% (weight/volume) in the incubator shaker (250 rpm) at room temperature for 1 h.

### *Effect of processing temperature*

Fig. 2 shows that GSH isolation by autolysis method was lesser at low and high temperatures. This occurred, probably because at low temperature, GSH was not fully isolated due to incomplete disruption or it was not active, and at high temperature, it was not in the GSH form anymore. In general, the concentration of GSH increased from 19°C up to the maximum autolysis temperature of 28°C with GSH concentration of 22.330 to 49.263  $\mu\text{mol/ml}$  and then decreased to 29.497  $\mu\text{mol/ml}$ . These results indicated that GSH can be considered as an enzymatic protein that was not active at low temperature and

lost its activity at high temperature. The optimum temperature was found to be 28°C with a yield of 49.263  $\mu\text{mol/ml}$  GSH.

### ***Effect of isolation time***

Fig. 3 shows the effect of isolation time on the GSH isolation. It can be viewed that by increasing the isolation time, the isolated GSH concentration was decreased. This occurred might be due to the denaturizing of the GSH when it was exposed in longer time to the optimum processing temperature of 28°C. The optimum isolation time for this method was found to be one (1) hour.

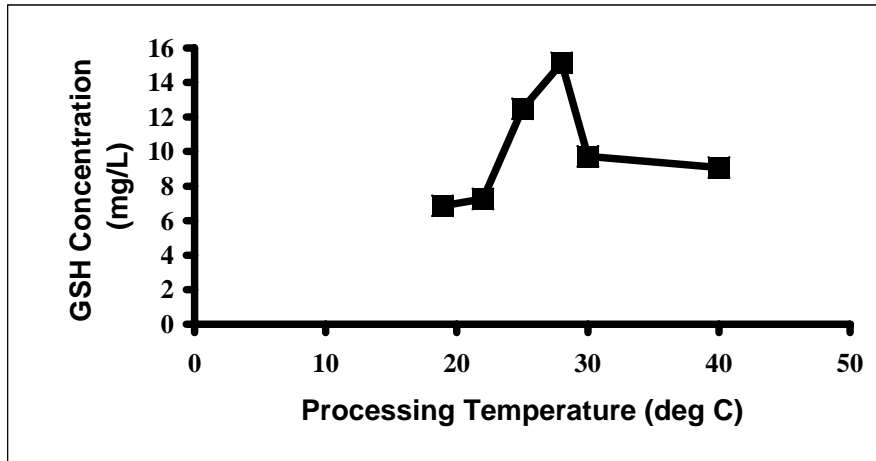
### ***Effect of rotation speed***

Fig. 4 shows the relation between GSH concentration and rotation speed for the autolysis process running at its optimum processing parameters. From the graph, it can be viewed that the concentration of GSH was increased up to the optimum rotation speed of 350 rpm where 7.797 mg/l GSH was isolated, then the GSH concentration reduced by the increase of rotation speed. It seems that the bigger value of rotation speed has stimulated lower concentration of isolated GSH. This phenomenon might be happen because of at the higher value of rotation speed, the shear stress worked to the GSH cell caused the destruction of its molecular structure.

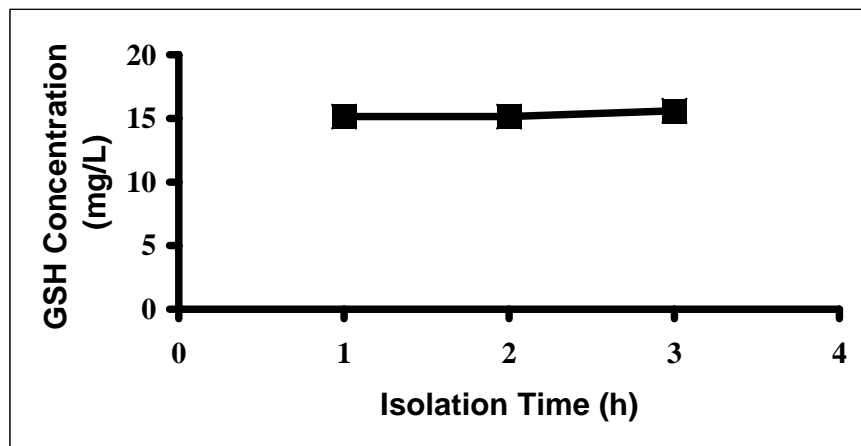
### ***Effect of sample pH***

Fig. 5 shows the GSH concentration isolated from different values of pH of yeast solution isolated by autolysis method at its optimum processing parameters. From these data, it can be observed that the concentration of GSH isolated was affected by the pH of the yeast solution. The result showed that the GSH concentration was the highest (24.863 mg/l) when the isolation was done at pH 3. Increase in pH reduced the concentration of GSH.

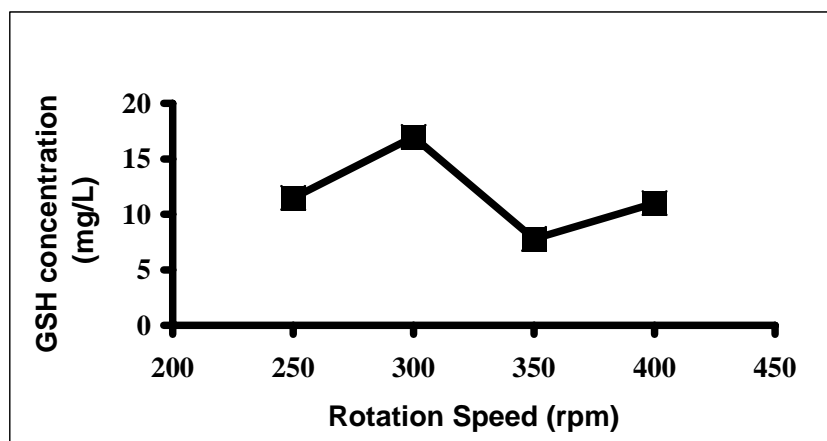
By scrutinizing the data in Fig. 5, probably it can be concluded that at a low pH value, the surface tension of the wall of the yeast cells was high that it was easily destroyed by the autolysis process. Another possible reason is that the GSH was polarized in the vicinity of the inner yeast cell wall so that it could be released easily to the outside of the cell even the wall cell is not break completely.



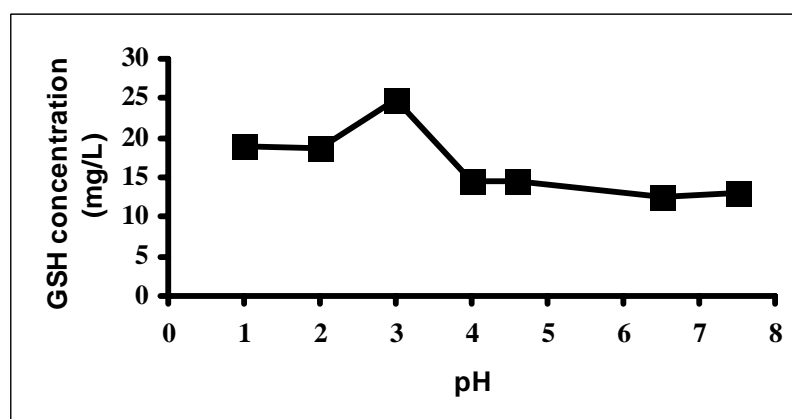
**Fig. 2.** Effect of processing temperature on the isolation of GSH by autolysis method. The process was done using yeast concentrations of 9 wt% (weight/volume) in the incubator shaker (250 rpm) at the processing temperature of 19, 22, 25, 28, 30, and 40°C or 1 h.



**Fig. 3.** Effect of isolation time on the isolation of GSH by autolysis method. The process was done using yeast concentrations of 9 wt% (weight/volume) in the incubator shaker (250 rpm) at the processing temperature of 28°C or 1, 2, and 3 h.



**Fig. 4.** Effect of rotation speed processing temperature on the isolation of GSH by autolysis method. The process was done using yeast concentrations of 9 wt% (weight/volume) in incubator shaker with the rotation speed of 250, 300, 350, and 400 rpm at the processing temperature of 28°C for 1 h.



**Fig. 5.** Effect of sample pH on the isolation of GSH by autolysis method. The process was done using yeast concentrations of 9 wt% (weight/volume) with pH value of 1, 2, 3, 4, original pH of the solution, 6.5, and 7.5 in incubator shaker with the rotation speed of 350 rpm at the processing temperature of 28°C for 1 h.

## Conclusion

From the results of these studies, the conclusion that can be made is that the process parameters of the autolysis process really affected the isolation of GSH. The optimum value of GSH concentration (24.863 mg/l) was isolated



from the autolysis process with the following process parameters; yeast concentration of 9 wt %, processing temperature of 28°C, isolation time of 1 h, rotation speed of 350 rpm and the sample pH of 3.

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