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Isolation of GSH from Baker Yeast by Autolysis 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 Autolysis method. To study the effect of yeast concentration, yeast solution with the concentration of 15, 30, 45 and 60 wt.% was disrupted at their original temperature for 1 h. The result obtained shows that the optimum value of the concentration for the Autolysis was 15 wt.% with the yield of 0.3942 μmol mL^{-1} GSH. To study the effect of processing temperature, Autolysis process of the 15 wt.% yeast solution was done at a various values of temperature such as 15, 25, 28, 31, 33 and 40°C. The results indicated that, the optimum GSH isolation temperature (suitable processing temperature) is 28°C with the yield of 1.367 μmol mL^{-1} GSH. Then, to study the effect of isolation time, the Autolysis process of sample with the concentration of 15 wt.% was done at 28°C for 1, 2 and 3 h. The results indicated that the optimum isolation time for the Autolysis process is 1 h.

Key words: Glutathione (GSH), autolysis, yeast, protein flavor, antitoxin agent

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 be used as an 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[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.

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[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 our Malaysian local fruits such as Durian, Starfruit, Jackfruit, Sapodilla etc. The production method of these materials is surely different from the method 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 influence parameters of the GSH production such as sample concentration, temperature of the process, sample's pH and incubation time etc need to be studied.

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

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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, Autolysis Method[15,16] was used to disrupt the cells where in this method distilled water is 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 Autolysis Method. The data can be used as a standard/control when dealing with the recombinant yeast and fruits etc.

MATERIALS AND METHODS

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.

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 at various conditions of the parameters inside the beaker. 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.

GSH analysis: To evaluate the GSH content in the supernatant, the OD value of the solution was taken by a Spectrophotometer at 240 nm. Then, the GSH concentration can be calculated by Bergmeyer method. Using this method, two civets have to be prepared, that is Control Civet (CC) and Experimental Civet (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 also filled by the same substance and 0.01 mL glyoxalase solution was added and then the solution was stirred to make it homogenous. The OD value of EC at this time is represented as E_1. Then, 0.02 mL of methylglyoxal was added to EC and the OD value were taken at 8, 10 and 12 min. Their average values are represented as E_2. Finally, 0.02 mL of methylglyoxal was added to EC and the OD values at 2, 4 and 6 min were taken and their average values are represented as E_3.

The concentrations of GSH were calculated by the equation of Lambert-Beer law as follow:

$$C_S = \Delta E_{GSH}/[(\varepsilon \times d)\times 10^6]$$

Where, C_S is the concentration of GSH; $\Delta E_{GSH} = 2\times$ (average of E_2) – E_1 - E_3 is the optical density change of the GSH solution; ε is a constant; d is the thickness of the civet. Table 1 described the samples of the GSH optical density data obtained from the Autolysis process conducted at yeast concentration of 15 wt.% and processing temperature of 28°C.

Effect of yeast concentration: To study the effect of yeast concentration on the GSH isolation, several values of concentrations such as 15, 30, 45 and 60 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 1 h.

Table 1: Sample data of the optical density (E) of the GSH. The GSH concentration (Cs) can be computed by $C_S = \Delta E_{GSH}/[(\varepsilon \times d)\times 10^6]$

	Optical density value (E)											
	E_1			E_2			E_3			Average		
I-th	-	E	-	E_8	E_{10}	E_{12}	E_2	E_4	E_6	E_1	E_2	E_3
1	-	2.71	-	5.14	5.15	5.17	3.03	3.08	3.05	2.71	5.15	3.06
2	-	2.70	-	5.18	5.11	5.21	3.02	3.09	3.04	2.70	5.17	3.05
3	-	2.67	-	5.16	5.15	5.20	3.04	3.09	3.06	2.67	5.18	3.07
4	-	2.81	-	4.32	4.34	4.38	2.97	3.06	3.04	2.81	4.35	3.01
5	-	2.83	-	5.12	5.11	5.13	2.95	2.97	2.96	2.83	5.12	2.96
6	-	2.81	-	5.12	5.10	5.15	3.05	3.10	3.09	2.81	5.13	3.08
7	-	2.79	-	5.08	5.12	5.11	2.99	3.12	2.99	2.79	5.10	3.10
8	-	2.82	-	5.04	5.09	5.10	2.95	2.98	2.96	2.82	5.08	2.97
9	-	2.81	-	4.35	4.39	4.34	2.96	3.00	2.97	2.81	4.35	2.99
10	-	2.76	-	5.04	5.14	5.11	2.98	3.14	2.97	2.76	5.09	3.04
Average	-	2.74	-	-	-	-	-	-	-	2.74	5.11	3.06

Table 2: Overall data obtained from various values of the operating parameters of the Autolysis process. The strikethrough (—), double underline (═) and double strikethrough (═) represent the data of GSH concentration obtained from the studies of effect of the yeast concentration (wt %), processing temperature (°C) and isolation time (h) on the isolation of GSH, respectively

	GSH concentration (μmol/mL)					
Isolation time (h)	15°C	25°C	28°C	31°C	33°C	40°C
Yeast concentration: 15 wt.%						
1	0	0.77	1.31	1.09	0.66	0
2	0	0.77	1.31	1.10	0.67	0
3	0	0.77	1.34	1.08	0.66	0
Yeast concentration: 30 wt.%						
1	0	0.80	1.35	1.10	0.77	0
2	0	0.76	1.35	1.12	0.77	0
3	0	0.80	1.25	1.12	0.80	0
Yeast concentration: 45 wt.%						
1	0	0.82	1.36	1.20	0.81	0
2	0	0.82	1.37	1.20	0.80	0
3	0	0.83	1.38	1.20	0.79	0
Yeast concentration: 60 wt.%						
1	0	0.79	1.31	1.18	0.77	0
2	0	0.80	1.32	1.18	0.77	0
3	0	0.79	1.32	1.18	0.78	0

Effect of processing temperature: After getting the value of the suitable or optimum concentration by the previous experiment, the suitable or the optimum temperature then have to be determined. Several yeast solutions with the optimum concentration of 15 wt.% but at the different temperatures such as 19, 22, 25, 28, 30 and 40°C were studied by running the Autolysis for 1 h.

Effect of isolation time: The effect of isolation time was study by heating the yeast solution at its optimum concentration of 15 wt.% and at its optimum isolation temperature of 28°C for 1, 2 and 3 h.

Overall data: To provide the overall data of the GSH isolation by Autolysis method, yeast solution with the concentration of 15, 30, 45 and 60 wt.% was disrupted at various value of temperature such as 15, 25, 28, 31, 33 and 40°C for 1, 2 and 3 h. By this data one can find the isolation condition of their interest.

RESULTS AND DISCUSSION

Effect of yeast concentration: From the data, it can be said that the concentration of GSH isolated were affected by the concentration of the yeast. GSH concentration was increased up to 45 wt.% and then decreased by the increase of yeast concentration (Table 2). From this observation, it can be concluded that at a low concentration of 15 wt.%, the density of the solution is low enough to let the yeast cells to make an intimate contact with the hot water to disrupt its cells wall. As a result, plenty of GSH were released from the yeast cells.

The bigger the value of the yeast concentration, the higher the concentration of GSH isolated. But, from the results obtained, it can be viewed that the concentration of GSH isolated from the 60 wt.% yeast solutions was lower than that of GSH at 30 and 45 wt.% yeast solution (Table 2). This phenomenon might be happen because of at the higher value of yeast concentration, the density of the solution becomes higher and this make its cells too crowded to make an intimate contact with the hot water. So, the disruption process of the yeast cells becomes slow.

Effect of processing temperature: From the previous experimental result the optimum yeast concentration in isolating the optimum value of GSH concentration was 45 wt.%. This value supposed to be used in this experiment, but since there is no such a difference in the concentration of GSH isolated between 15 and 45 wt.%, the concentration of 15 wt.% yeast solution was used because of it is easy to conduct the experiment using this concentration value. The data with double underline in Table 2 are the GSH concentration obtained from the study of effect of processing temperature on isolation of GSH done at 15 wt.% concentration of yeast for 1 h. From the data, it can be viewed that at low temperature (15°C), there was no GSH being isolated. The same phenomena occurred at high temperature (40°C), where no GSH were detected in the supernatant. GSH is an enzymatic protein that is not active at low temperature and lost its activity at high temperature.

At 15°C, the reason why there is no GSH isolated is probably due to the yeast cells were not disrupted or if disrupted, the GSH is not active or is not in the GSH form so that it cannot be detected. At the higher temperature of 40°C, the yeast cells were supposed to be well disrupted. The reason why the GSH was not detected is due to the fact that the GSH will lose its activity or it is not in the GSH form at this temperature.

For the temperature between these two temperatures i.e. 25, 28, 31 and 33°C, the concentration of GSH is increased up to the optimum Autolysis temperature of 28°C with the yield of 1.367 μmol mL^{-1} GSH but decreased till to the higher temperature of 40°C. The higher the heating temperature the more the yeast cells will be disrupted. Beyond the optimum Autolysis temperature (28°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 of losing its activity at such a high temperature.

Effect of isolation time: From the data, it is clearly understood that processing the Autolysis for 1 and 2 h did not increase the isolated GSH concentration (Table 2). Running the process for 3 h increased the isolation rate of the GSH. This is probably due to the cell wall of the yeast is become easily destroyed/disrupted after a longer contact with warm water.

Overall data: From the results of these studies, the conclusion that can be made is that the concentration of yeast, processing temperature and the isolation time of the Autolysis affects the production of GSH. The maximum value of GSH concentration (1.38 μmol mL^{-1}) was isolated from the Autolysis process with the following operating parameters; yeast concentration of 45 wt.%, processing temperature of 28°C and the isolation time of 3 h (Table 2). But the close value of 1.31 μmol mL^{-1} GSH can be isolated at the optimum operating parameters; yeast concentration of 15 wt.%, processing temperature of 28°C and the isolation time of 1 h. Thus, it is more economic if one isolates the GSH at the optimum condition of the process.

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