Journal of Applied Sciences 5 (1): 151-154, 2005
ISSN 1607-8926

Comparison Studies among the Methods Used in Isolating the GSH from Baker Yeast

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Abstract: The purpose of this study was to make a comparison among the methods used in isolating the GSH from baker yeast. This was done by comparing the optimum values of operating parameters such as the yeast concentration, processing temperature and the isolation time of each of the isolation methods used (Autolysis, Ultrasonic and Homogenizer). Among the three methods, Autolysis method produced the highest GSH concentration of 49.263 μmol mL^{-1} (15.14 g L^{-1}) followed by Homogenizer method (18.33 μmol mL^{-1} or 5.63 g L^{-1}) which is slightly higher than that of the Ultrasonic method (18.13 μmol mL^{-1} or 5.57 g L^{-1}). The different occurred probably due to the different of the isolation time applied to the three methods. The same duration time couldn't be used because it will increase the temperature of the sample higher that the optimum temperature of some of the methods. Unlike Ultrasonic and Homogenizer methods, the temperature for Autolysis method could be maintained within 1 h while for Homogenizer and Ultrasonic methods the temperature could only be maintained within 30 and 15 sec, respectively. The step down arrangement from best to worst for lab scale production was Autolysis>Homogenizer>Ultrasonic method. Autolysis was the best but the isolation time is longer compared to the other so that in large scale production, Homogenizer seems to be the best method due to the economical perspective. Ultrasonic is also can be considered as an excellent method but expensive compared to others.

Key words: GSH, autolysis, ultrasonic, homogenizer, 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.

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 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.

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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.

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, Autolysis, Ultrasonic and Homogenizer method[15-22] were used to disrupt the cells where in these methods, 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 and the optimum values of these parameters were already found by our previous studies[23-25]. In this study, the optimum values of each parameter for each method were compared in order to decide which method is the effective one in isolating the GSH.

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.

The yeast cells were disrupted by Autolysis, Ultrasonic and Homogenizer methods. 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 the using the equation of Lambert-Beer law.

Effect of yeast concentration: To study the effect of yeast concentration on the GSH isolation by each 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 1 h, 15 and 30 sec for Autolysis, Ultrasonic and Homogenizer methods, respectively.

Effect of processing temperature: After getting the value of the suitable or optimum concentrations of yeast by the previous experiments, the optimum temperatures were then determined. Several yeast solutions with the optimum concentrations but at the different temperatures such as 19, 22, 25, 28, 30 and 40°C were studied by running the isolation processes for 1 h, 15 and 30 sec for Autolysis, Ultrasonic and Homogenizer methods, respectively.

Effect of isolation time: The effect of isolation time for each method was studied by heating the yeast solution at its optimum concentration and at its optimum isolation temperature for 1, 2 and 3 h (Autolysis), 15, 30, 45 and 60 sec (Ultrasonic) and 15, 30, 60, 300 and 900 sec (Homogenizer).

RESULTS AND DISCUSSION

Effect of yeast concentration: Table 1 shows the difference among the values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum yeast concentration. From the Table 1, it is observed that Autolysis method was able to isolate the highest concentration of GSH (17.53 μmol mL^{-1}) followed by Ultrasonic (12.93 μmol mL^{-1}) and then Homogenizer method (10.98 μmol mL^{-1}). Considering only the yeast concentration (C) and isolation time (t), it seems that Ultrasonic (C = 6 wt.% and t = 15 sec) is the best isolation method followed by Homogenizer (C = 9 wt.% and t = 30 sec) and then Autolysis method. But if the cost of Ultrasonic and Homogenizer is taking into account, even the isolation time is longer autolysis

Table 1: The values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum yeast concentration

Methods	Yeast concentration (wt.%)	GSH concentration (μmol mL^{-1})
Autolysis	9	17.53
Ultrasonic	6	12.93
Homogenizer	9	10.98

Table 2: The values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum operating temperatures

Methods	Operating temperature (°C)	GSH concentration (μmol mL^{-1})
Autolysis	28	49.26
Ultrasonic	22	13.53
Homogenizer	22	17.93

Table 3: The values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum isolation time

Methods	Isolation time (sec)	GSH concentration (μmol mL^{-1})
Autolysis	3600	49.26
Ultrasonic	15	18.13
Homogenizer	30	18.33

Table 4: The values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum operating parameters. C, T and t represent the yeast concentration (wt.%), operating temperature (°C) and isolation time (sec), respectively

Methods	C (wt.%)	T (°C)	t (sec)	GSH concentration (μmol mL^{-1})
Autolysis	9	28	3600	49.26
Ultrasonic	6	22	15	18.13
Homogenizer	9	22	30	18.33

method can be considered as the best one followed by Homogenizer and then Ultrasonic method.

Effect of processing temperature: Table 2 shows the difference among the values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum yeast concentration (C) and operating temperatures (T). Autolysis (C = 6 wt.% and T = 22°C) is likely to be considered as the best isolation method followed by the Homogenizer (C = 9 wt.% and T = 22°C) and then Autolysis methods. But still Autolysis is ought to be considered as the best because the GSH isolated was almost triple higher than those of isolated by the Ultrasonic and Homogenizer methods.

Effect of isolation time: Table 3 shows the different among the values of GSH concentration isolated by Autolysis, Ultrasonic and Homogenizer methods at their optimum yeast concentration (C), operating temperatures (T) and isolation time (t). It seems that the best order of the methods is Ultrasonic>Homogenizer>Autolysis but if the isolation time of Autolysis is considered as a common value for this kind of operation, Autolysis should be considered as the best since its yield was triple higher than the other methods.

Table 4 summarized the overall consideration of the three methods. Among the three methods, Autolysis method produced the highest GSH concentration of 49.263 μmol mL^{-1} (15.14 g L^{-1}) followed by Homogenizer method (18.33 μmol mL^{-1} or 5.63 g L^{-1}) which is slightly higher than that of the Ultrasonic method (18.13 μmol mL^{-1} or 5.57 g L^{-1}). The different occurred probably due to the different of the isolation time applied to the three methods (Autolysis: 1 h, Ultrasonic: 15 sec and Homogenizer 30 sec). The same duration time couldn't be used because it will increase the temperature of the sample higher that the optimum temperature of the Ultrasonic and Homogenizer methods. Unlike Ultrasonic and Homogenizer methods, the temperature for Autolysis method could be maintained within 1 hour while for Homogenizer and Ultrasonic methods the temperature could only be maintained within 30 and 15 sec, respectively. The step down arrangement from best to worst for lab scale production was Autolysis>Homogenizer>Ultrasonic method. Autolysis was the best but the isolation time is longer compared to the other so that in large scale production, Homogenizer seems to be the best method due to the economical perspective. Ultrasonic is also can be considered as an excellent method but expensive compared to others.

ACKNOWLEDGMENT

This work was financially supported by Research Centre of the International Islamic University Malaysia, Kuala Lumpur, Malaysia under grant number of IIUM 1.11/RC/10/2-1 to Assistant Professor Dr. Mohamad Ramlan Bin Mohamed Salleh.

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