Comparison studies of GSH isolation from several Malaysian local fruits by mechanical methods

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Abstract

Glutathione (GSH) is a simple tripeptide produced by the liver. It is a volatile substance, which is sweet in taste, comprised of the amino acids cysteine, glutamic acid and glycine. In these modern days, the use of traditional medicine by the suffers of chronic disease is encouraged due to the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer more gentle means of managing such disease.

Therefore, the practice of traditionally used natural plants as medicines is one of the alternative ways to cure the illnesses. Besides, this study was done in order to investigate the production of GSH from fruits since most of the studies that had been conducted before were done on the isolation of GSH from other sources. In this study the reduced glutathione (GSH) was isolated from a potential local fruit of Malaysia. Three mechanical methods; homogenizer, ultrasonic and autolysis were screened for disrupting the fruits cells for maximum GSH content.

Different methods of cell disruption gave different effect on the GSH production. Watermelon (Citrullus lanatus) is the best potential local fruit that contain higher GSH followed by Jackfruit (Artocarpus heterophylus) and Sapodilla (Manilkara zapota), while ultrasonic is the best cell disruption method which can give the maximum amount of GSH, followed by homogenizer method and autolysis method.

Keywords: Autolysis, GSH, Homogenizer, Malaysian Fruits, Ultrasonic.

Introduction

The disruption of cell walls is a key step in the isolation and purification of many biological products which are present in the interior of cells. The complete recovery of these intracellular products is a delicate balance between efficient breakage of the cell walls, preservation of the cell contents, especially against denaturation of protein and effective separation from the cell debris. Breakage of the cell wall can be carried out mechanically or by non-mechanical technique[11]. One of the biological products that related to these techniques is Glutathione (GSH).

Glutathione is a simple tripeptide produced in all organs, especially in the liver[10]. It is present in virtually all mammalian tissues[10]. It is comprised of the amino acids cysteine, glutamic acid and glycine. Glutathione exists in yeast cell, tomato and orange etc[8]. There are two types of Glutathione which is in a reduced form called GSH and in the oxidized form called GSSG[9]. 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, Watermelon, Star fruit, Jackfruit, Sapodilla etc.[7]

Glutathione is the most important nonprotein thiol present in animal cells as well as in plants and bacteria. This active tripeptide possesses multifunctional properties, as an important biochemical drug for the treatment of liver diseases and also as an important cofactor widely used by biochemical scientists. Alongside the discovery of more functions and properties of GSH, this compound is of interest in the food additive industries, therapeutics and sport nutrition[14]. Glutathione is considered to be one of the most powerful antioxidants produced by the human body. It boosts the immune system by protecting cells from damage caused by free radicals produced during the normal process of cell oxidation.

Through this process, glutathione is able to detoxify the lungs, liver and intestinal tract and remove a wide range of toxins, including those produced by heavy metals, cigarette smoke, alcohol, radiation and cancer chemotherapy. Glutathione also facilitates carbohydrate metabolism and prevents oxidized fats from accumulating in arteries, where they can increase one's risk of developing cardiovascular disease.

Alongside the discovery of more functions and properties of GSH, this compound is of interest in the food additive industries, therapeutics and sport nutrition.[14] In addition to working at the cellular level, glutathione protects tissues in the brain, heart, kidneys, liver, lungs, eyes and skin from damage caused by oxidation. It also has anti-cancer properties due to its ability to target and remove carcinogens from the body, particularly from the liver.

Glutathione plays an important role in protecting the body from the effects of aging. Unfortunately, as the

body ages, levels of glutathione tend to decline. The lower glutathione levels become, the faster one becomes susceptible to the effects of aging. Glutathione is also depleted by illness and chronic exposure to heavy pollution.

In general, there were some studies that have been done on the topic related to GSH production. However, those studies focused more on the GSH isolation from yeast[3, 12]. They also used other sources like soybean[1], oat[15], rat[5] and rabbit kidney[2]. Few studies were done on the GSH isolation from fruits. Due to this reasons, this project is conducted to investigate the isolation of GSH from fruits. Besides, fruits were selected as the raw material in this project due to the ease to obtained and nutritious values. Furthermore, its process is easy to be handled as well as the operation cost is low compared to other sources.

There are several mechanical and non-mechanical cell disruption methods that can be used. However, since cell disruption is a sensitive process[6], some selected methods had been chosen. This project focuses on autolysis, homogenizer and ultrasonic methods.

Materials and Methods

Sample collection and preparation: The fresh fruits used in this experiment were purchased from the same supplier at the same fruits stall in Gombak, Selangor. In this study, three different fruits have been chosen as the raw materials which were Watermelon, Jackfruit and Sapodilla. They were chosen based on the sweet in taste characteristic[7]. Fruits were stored in chiller at 4°C and were then taken out just before running the experiment to avoid contamination and the reduction of its enzyme activities which may reduce the GSH isolation.

Each fruit cells (from Watermelon, Jackfruit and Sapodilla) were disrupted by Autolysis, Ultrasonic and Homogenizer methods during the extraction process. After disruption, the disrupted cells solutions then were centrifuged at 12,000 rpm for 20 min. This will separate the solid phase that contains cell walls from the supernatant which contains GSH and other compounds. Before analyzing the GSH, 5 mL of the supernatant was mixed with 5 mL of cold perchloric acid and stirred by a small glass rod to deproteinize it. Then, it was left at room temperature for 5 minutes to complete the reaction.

Cell disruption methods: Cell disruption involved several methods and the condition in each method are mentioned in Table 1 based on the previous study[8]:

- **Homogenizer** - This equipment will gently disrupt the fruits cells without damaging its subcellular structures. Several parameters were involved: Fruit concentration (wt %), processing temperature (ºC), rotation speed (rpm) and time of isolation (sec).

- **Autolysis** – (Auto-self; lysis splitting) which based on heat as a way to disrupt the cell. It is done in incubator shaker. It is a slow process that requires long processing periods in order to reach the desired result. Effects of the fruit concentration (wt %), processing temperature (ºC), isolation time (hr) and agitation speed (rpm) were studied.

- **Ultrasonic** – widely applied method in cell lysis with high frequency sound which is produced electronically and transported through a metallic tip to an appropriate concentrated cellular suspension. Effects of the fruits concentration (wt %), processing temperature (ºC), isolation time (sec), amplitude (%) and cycle were also studied.

Analysis of samples

The GSH content in the supernatant was analyzed by taking the OD value of the solution with spectrophotometer at 412 nm [4, 13], which gave better results than the results obtained at 240 nm, according to the specified protocols. 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.[6]

Table 1
Condition for each parameters for each cell disruption method*

	Conc. (wt %)	Temp. (^{0}C)	Time (sec)	Amplitude	Cycle	Rotation Speed (rpm)	Agitation (rpm)
Ultrasonic	6	22	15	100%	1.0	-	-
Homogenizer	9	22	30	-	-	13,000	-
Autolysis	9	28	3600	-	-	-	250

*conditions used for the three types of fruit used in the study

Results and Discussion

Table 2
GSH concentration isolated from Watermelon, Jackfruit and Sapodilla by autolysis method at its optimum values

Malaysian local fruits	GSH concentration (μmol mL^{-1})
Watermelon (Citrullus lanatus)	52.88 ± 0.5
Jackfruit (Artocarpus heterophylus)	51.05 ± 0.2
Sapodilla (Manilkara zapota)	50.91 ± 0.8

Screening of potential Malaysian fruits: Firstly, GSH is extracted from the selected three potential Malaysian local fruits (Watermelon, Jackfruit and Sapodilla) by using autolysis since from the previous study; it showed that autolysis can give the maximum amount of GSH extracts[8]. The condition set for the parameters was also the same (Table 1). The condition was the optimum condition for autolysis.

Table 2 shows the differences among the values of GSH concentration isolated from Watermelon, Jackfruit and Sapodilla by autolysis method at its optimum values. From Table 2, it is observed that Watermelon gave the best result. Watermelon gave the highest amount of GSH concentration which was 52.88 ± 0.5 μmol mL^{-1} followed by Jackfruit (51.05 ± 0.2 μmol mL^{-1}) and Sapodilla (50.91 ± 0.8 μmol mL^{-1}).

It can also be claimed that the GSH production is higher in fruits compared to yeast (from the previous study[8]) since they were extracted by the same method using the same condition. The highest concentration of GSH that has been extracted from baker yeast by autolysis was just 49.26μmol mL^{-1} compared to GSH in watermelon which was 52.88±0.5 μmol $mL^{-}1$.[8]

Screening of methods for cell disruption: Table 3 shows the differences among the values of GSH concentration isolated using ultrasonic, homogenizer and autolysis as cell disruption techniques. All the cell disruption methods were test on Watermelon since it gave the highest concentration of GSH compared to others. Then, to confirm whether the ultrasonic and homogenizer methods will give the same result to Jackfruit and Sapodilla, experiments were also done onto them. Table 3 summarizes the overall consideration and the process condition for each method. Figure 1 also shows the comparison between the three local fruits and the three cell disruption methods in diagram form.

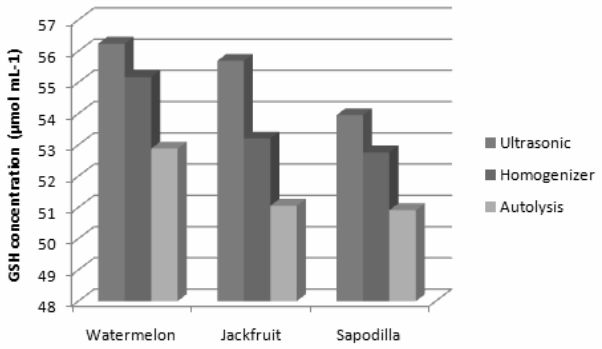

Fig. 1: The comparison between the three local fruits and the three cell disruption methods

Table 3
GSH concentration isolated using ultrasonic, homogenizer and autolysis for all three potential Malaysian local fruits at their optimum values

	Method	GSH concentration (μmol mL^{-1})
Watermelon (Citrullus lanatus)	Ultrasonic	56.24 ± 0.6
	Homogenizer	55.17 ± 0.1
	Autolysis	52.88 ± 0.5
Jackfruit (Artocarpus heterophylus)	Ultrasonic	55.69 ± 0.3
	Homogenizer	53.20 ± 0.4
	Autolysis	51.05 ± 0.2
Sapodilla (Manilkara zapota)	Ultrasonic	53.95 ± 0.1
	Homogenizer	52.76 ± 0.7
	Autolysis	50.91 ± 0.8

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 could not 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 highest to lowest for lab scale production was Ultrasonic>Homogenizer>Autolysis method. Ultrasonic is the excellent method for cell disruption but it is quite expensive compared to others. Homogenizer can also be considered as a good cell disruption method due to the economical perspective. Autolysis need longer isolation time compared to others and it will be efficient in large scale production.

Comparing to the GSH concentration that have been obtained from baker yeast[8], it was highest in autolysis method which was 49.26µmol mL^{-1}, followed by homogenizer method (18.33 µmol $mL^{-}1$) and ultrasonic method (18.13 µmol $mL^{-}1$).[8] The step down arrangement from highest to lowest for lab scale production for the yeast was autolysis>homogenizer>ultrasonic method.

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