Cysteine content obtained from the variation of temperature and acidity on soybean extraction

D Mustikaningtyas¹,²*, S Widyarti², M Rifa'i² and N Widodo²

¹ Student of Biology Doctoral Program, Faculty of Mathematics and Natural Science, Brawijaya University, Malang, Indonesia
² Biology Department, Faculty of Mathematics and Natural Science, Brawijaya University, Malang, Indonesia
³ Biology Department, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang, Semarang, Indonesia

*Corresponding author: dewi_mustikaningtyas@mail.unnes.ac.id

Abstract. Modifying the Saccharomyces cerevisiae culture medium with cysteine supplementation is an effort to increase the production of glutathione. Soybeans contain high cysteine, where its extract has potential value as enrichment media for S. cerevisiae to glutathione synthesis. Soybeans that used in this study were local varieties of Grobogan Central Java. This study aimed to analyze the effects of temperature, pH, and the drying process of soybeans extraction, and to obtain high content of cysteine in soybean extract. Soybeans were extracted using acetic acid in pH 4.0; 4.5; and 5.0 at room temperature and 50°C. Pellets obtained were dried using freeze dryer or heat dryer. The result showed that the highest cysteine content at 7.4 mg/g was derived from soybean extraction at pH 5.0, room temperature, and heat dryer. Therefore, by combining the pH and the incubation temperature during the extraction process can be suggested to increase the cysteine levels in soybean extract, especially for local varieties of Grobogan Central Java.

1. Introduction

Glutathione (GSH) is a tripeptide that composed of cysteine, glutamic acid, and glycine. Glutathione can be found in most cells, especially in eukaryotic cells. Glutathione has a role as an antioxidant that can reduce oxidative stress. Therefore it has been used as an active raw material in food and cosmetic products because it has anti-aging effects. Moreover, it is also a therapy for cancer patients who are undergoing chemotherapy or radiotherapy, viral infections, and cystic fibrosis [1, 2]. The benefits of diverse glutathione provide opportunities for the development of glutathione production.

Saccharomyces cerevisiae is often used for glutathione production because it can accumulate glutathione more efficiently [3]. This is also supported by research conducted by Tahmasebi which isolated yeast from fruits to identify and measure their ability to produce glutathione, where all isolates obtained were S. cerevisiae [4]. This yeast does not produce pyrogens and is not pathogenic in humans [5]. S. cerevisiae has been used frequently in protein production and has been used commercially [6]. It has ATP regeneration ability through the glycolysis pathway, so no additional ATP is needed in
glutathione production [7]. According to these considerations, *S. cerevisiae* is the right choice to be used as a host cell to produce glutathione.

Glutathione synthesis in *S. cerevisiae* is divided into two stages. The first stage is the phosphorylation mechanism of γ-carboxylate on L-glutamate by ATP produces γ-glutamyl phosphate intermediate, then L-cysteine as nucleophile attaches to γ-glutamyl phosphate intermediate to form γ-glutamylcysteine. In the second stage, the dipeptide then binds to glycine to form glutathione [8, 9]. Supplementation of amino acids, especially cysteine, is needed in the production process of glutathione because the reduced antioxidant glutathione (GSH) is a strong electron donor, which is influenced by the presence of sulfhydryl group (SH), ie, cysteine [10]. The concentration of glutathione produced depends on the amount of cysteine availability in the cell. The positive effect of glutathione production when cysteine concentration is greater than 3 mmol / L [11]. The importance of cysteine material in glutathione production is the reasoning for cysteine supplementation in *S. cerevisiae* culture media [12]. By looking for natural ingredients rich in cysteine, it is expected that it can be as resources of cysteine supplementation in *S. cerevisiae* culture media. One natural ingredient that is easy to find in Indonesia and rich in cysteine is soybeans.

Soybean is one of the seed with high protein content, which is more than 40% dry weight of soybean extract [13]. One of the local varieties soybeans in Central Java is Grobogan varieties. There were fifteen amino acids present in this variety of soybeans, but there was no cysteine included [14]. To obtain soybean extract with cysteine content, it is necessary to take concern about the extraction technique from soybean. This study aimed to analyze the effects of temperature, pH, and the drying process of soybeans extraction. And to obtain high content of cysteine in soybean extract.

Triyono conducted the study about the effect of acid addition and pH variation in mung bean protein extraction process [15]. The results of the research showed that the mung bean protein flour product, which had the highest protein content was obtained from the addition of acetic acid with a pH value of 4.5. Protein solubilization is determined by several parameters, i.e. solvent pH, solvent ionic point (pI), solvent temperature, and solubilization time, which will affect the concentration of soybean protein produced [13]. Previous research has also been conducted by Rosenthal *et al.* that the protein yield from soybean extraction is influenced by pH and temperature in multistage extraction [16].

2. Methods
This is a preliminary study that was conducted in the Biochemistry Laboratory, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang. In this research, the following critical steps are included. The effect of four control parameters in the soybean extraction has been investigated. These control parameters included soybean/water ratio, acidity using acetic acid addition, the temperature of incubation, and the drying process. The soybean-to-water ratio used were 1:4, 1:5, 1:6. Extraction was carried out at the variation of pH (4.0; 4.5; and 5.0) with acetic acid addition, NaOH 0.1N were used for pH adjustment. The temperatures of incubation after acetic acid addition were 50 °C and room temperature for 30 min. The drying process was carried out by using heat dryer and freeze dryer. Protein content, especially cysteine content, was measured by using the high-performance liquid chromatography (HPLC).

2.1 Preparation of soybeans
Local soybeans varieties (Grobogan) used in this study were taken from the farmer in Grobogan Purwodadi. Firstly, 600 g soybean seeds that would be extracted were soaked in distilled water with a soybean/water ratio of 1:5 for five hours at 60 °C. Then it was drained for treatment forward.
2.2 Aqueous extraction of soybeans
Water extraction of soybean was developed based on the modified method from Sartini et al. and Sundarsih [17, 18]. Soybeans that were soaked for five hours then were weighed and soaked in distilled water with a variation of soybean/water ratio (1:4; 1:5; 1:6 respectively). After that, soybeans in the distilled water were blended then boiled in 90 °C for ten minutes.

The soybean suspension was filtrated, then the filtrate divided into three part and added acetic acid in some of pH (4; 4.5; and 5 respectively). The each of filtrate in some of pH was divided into two part, one part was boiled in 50 °C for 30 minutes, and another part without boiling. The filtrate in the variation of pH was precipitated overnight. The supernatant was removed, and the precipitate was drained by using heat dryer and freeze dryer. Then the cysteine content test was carried out to determine the level of soybeans that would be used in the research treatment.

2.3 Analysis of cysteine content from a soybean extract
The cysteine level in soybean extract with four control parameters (soybean-to-water ratio, pH, incubation temperature, and drying process) was measured using HPLC (Shimadzu, Japan). The total of 0.205 g of each soybean extract sample that was analyzed in this step. The analysis was performed on a Shimadzu high-performance liquid chromatography system, equipped with an SCL 10 AVP system controller, RF 20-A Fluorescence Detector λ 450 OPA detector, C 18.5 μm Shimadzu 120 x 4.6 mm column, and wavelength detector was 450 nm. In this research, the level of protein yields also was measured as the complementary data.

3. Results and Discussion
3.1 Soybean-to-water ratio
Each stage in soybean extraction can determine the protein content, especially cysteine obtained at the end of the process. Water extraction methods were carried out in this study, where the first stage begins with soaking in water for 5 hours. It can increase the size of protein bodies within the cotyledon cells of soybean to double their original size [13]. The protein body in the water medium is easily disturbed compared to the smaller protein body [19].

The next stage was the soybeans that were soaked for five hours then were weighed and soaked in distilled water with a variation of soybean/water ratio (1:4; 1:5; 1:6 respectively). After that, soybeans in the distilled water were ground then boiled in 90 °C for ten minutes. The addition of water also serves to remove carbohydrates that are contained in soybean seeds. The removal of insoluble carbohydrate and dietary fiber via an acidic precipitation step using acetic acid addition [13]. Soybean cotyledon cell walls consist of a series of polysaccharides, which often cross-links with phenolic compounds and proteins. Primary cell walls contain pectin, hemicellulose, and cellulose microfibrils, which are interrelated with proteins. There is a secondary cell wall inside the primary wall containing cellulose and hemicellulose, which is also capable of binding proteins. Therefore removing carbohydrates can release proteins in soybean cells.

3.2 Effect of acidity
The soybean suspension was filtered, then acetic acid addition for getting the variation of acidity (pH 4; 4.5; and 5). Some of the previous studies have been established that pH influence the product of amino acid. According to the study of acetic acid addition, The optimum production such as protein content 88.56 % was obtained from 4 % acetic acid and temperature 55 °C [20].

The Solubility of soybean proteins also depends on their isoelectric points (pI), which is on average at pH 4.5 [13]; therefore most of the soybean proteins are soluble at pH values below 3 and above 6. This is following the results of this study, which obtained optimum results at pH 5 (Table 1). And it is still possible to increase the pH value above 6.
3.3 Effect of incubation temperature after acetic acid addition

The next treatment is incubation with temperature variations (50 °C and room temperature). Increased temperature can result in enzyme inactivation. The occurrence of enzyme inactivation can affect the level of protein to be obtained. In this study, optimal cysteine levels were obtained from treatments at room temperature. Where in this case shows that an increase in temperature exceeding room temperature can reduce protein levels, especially cysteine.

| No | Sample Code | Protein Content (g/100g) | Amino acid level (mg/g) |
|----|-------------|--------------------------|-------------------------|
|    |             |                          | Cysteine    | Glutamic acid | glycine   |
| 1  | Control A (KA) | 29.4                     | 5.13        | 45.16        | 13.53     |
| 2  | Control B (KB) | 28.9                     | 5.36        | 46.97        | 14.11     |
| 3  | Control C (KC) | 29.6                     | 4.93        | 43.76        | 13.13     |
| 4  | A1bi        | 26.3                     | 5.95        | 52.70        | 15.87     |
| 5  | A2bi        | 24.0                     | 6.26        | 55.08        | 16.54     |
| 6  | A3bi        | 22.8                     | 6.37        | 56.16        | 16.88     |
| 7  | A1bii       | 28.1                     | 5.76        | 50.82        | 15.20     |
| 8  | A2bii       | 26.9                     | 5.71        | 51.01        | 15.30     |
| 9  | A3bii       | 25.4                     | 5.99        | 52.75        | 15.86     |
| 10 | B1bi        | 24.3                     | 6.53        | 58.22        | 17.51     |
| 11 | B2bi        | 22.0                     | 6.81        | 60.47        | 18.15     |
| 12 | B3bi        | 20.6                     | 6.95        | 61.22        | 18.33     |
| 13 | B1ai        | 25.7                     | 6.38        | 56.06        | 16.83     |
| 14 | B2ai        | 23.3                     | 6.41        | 57.02        | 17.10     |
| 15 | B3ai        | 22.1                     | 6.76        | 59.58        | 17.99     |
| 16 | B1bii       | 25.6                     | 6.26        | 55.00        | 16.56     |
| 17 | B3bii       | 23.6                     | 6.31        | 56.23        | 16.85     |
| 18 | C1ai        | 24.2                     | 6.81        | 60.14        | 18.08     |
| 19 | C2ai        | 22.0                     | 6.98        | 61.52        | 18.49     |
| 20 | C3ai        | 21.3                     | 7.24        | 64.27        | 19.31     |
| 21 | C1bi        | 23.1                     | 6.96        | 61.75        | 18.55     |
| 22 | C2bi        | 20.8                     | 7.28        | 63.38        | 19.11     |
| 23 | C3bi        | 19.5                     | 7.40        | 65.10        | 19.66     |
| 24 | C1aii       | 26.2                     | 6.57        | 57.59        | 17.31     |
| 25 | C2aii       | 23.9                     | 6.67        | 58.48        | 17.63     |
| 26 | C3aii       | 23.2                     | 6.61        | 58.83        | 17.71     |
| 27 | B1aii       | 27.0                     | 6.14        | 53.84        | 16.26     |
| 28 | B2aii       | 24.8                     | 6.23        | 54.86        | 16.47     |
| 29 | B3aii       | 24.4                     | 6.40        | 56.27        | 17.00     |
| 30 | B2bii       | 24.6                     | 6.38        | 56.07        | 16.87     |
| 31 | C1bii       | 24.7                     | 6.43        | 57.31        | 17.24     |
| 32 | C2bii       | 23.2                     | 6.52        | 58.12        | 17.45     |
| 33 | C3bii       | 22.7                     | 6.88        | 60.17        | 18.07     |

A = 1:4; B = 1:5; C = 1:6; (Soybean: water); 1 = 4; 2 = 4.5; 3 = 5 (Acidity/pH);
**a** = 50°C; **b** = room temperature (incubation temperature after acetic acid addition);

**i** = heat drying; **ii** = freeze drying (drying process)

The controls (KA, KB, KC) in this study are the soybean extract that was produced from variations of the soybean/water ratio without treatment of pH and temperature. Interestingly, the result of protein analysis showed that protein content from the controls is higher than the samples with pH and temperature treatment whereas the amino acid levels (included cysteine) are lower than the samples with pH and temperature treatment. The controls with variations of the soybean/water ratio (1:4; 1:5; 1:6) have a protein content of 29.4 g/100g, 28.9 g/100g, 29.6 g/100g respectively. And cysteine levels are 5.13 mg/g, 5.36 mg/g, 4.93 mg/g. While the samples with pH and temperature treatment (C3ai, C2bi, C3bi) are the soybean/water ratio of 1:6 with pH and temperature treatment (5 and 50°C; 4.5 and room temperature; 5 and room temperature, respectively). These have a protein content of 21.3 g/100g, 20.8 g/100g, 19.5 g/100g. And cysteine levels are 7.24 mg/g, 7.28 mg/g, 7.40 mg/g respectively (Table 1). This is in line with previous research, which stated that deactivating enzyme because of heating process slightly reduces protein yields, small reductions in protein extraction might be justified to produce soy protein products with different degrees of hydrolysis and functional properties [19].

In general, the extraction parameters that affect the production of protein from soybean are pH, solids-to-liquid ratio (S/L), agitation rate, particle size and/or type of cellular disruption, enzyme type, enzyme concentration, extraction time, and temperature [19, 21]. So, by varying the crucial processing parameter as pH and temperature, cysteine content that obtained was highly influenced.

### 4. Conclusion

This study shows that the cysteine content is influenced by key processes parameters such as acidity and temperature. The highest cysteine content is 7.4 mg/g was derived from soybean extraction at pH 5.0, room temperature, and using heat dryer. Accordingly, Adding acetic acid to regulate pH and determine the incubation temperature afterward can optimize the acquisition of amino acids, especially cysteine in Grobogan local varieties of soybeans extract.

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