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Chemical Hydrolysis of Soybean (*Glycinemax* (L) Merrill) to Get Genistein Compound

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Abstract: Soybean (*Glycinemax* (L) Merrill) is one of the highest isoflavones source. Isoflavones are one type of phytoestrogen that have a chemical structure similar to estradiol, that's why it can be used not only to inhibit but also to prevent many symptoms related deficiency of estrogen. However in the small intestine, isoflavones like genistin will be hydrolyzed by β -glucosidase and produce aglycone like genistein, this process can be optimized by hydrolysis with enzyme or chemical. The aim of this study was to obtain the extract of soybean that hydrolysed by hydrochloric acid and to obtain scientific data about the content of genistein in the extract by High Performance Liquid Chromatography (HPLC). From the results, show that average of genistein levels contained in the extract of soybean hydrolyzed for three replication is 0.580%.

Keywords : Soybean, phytoestrogen, isoflavones, genistein, HPLC.

1. Introduction

Soybean (*Glycinemax* L. Merrill) is a source of complete food with a high nutrient content and good. Soybean seeds are a source of high quality protein, oligosaccharides, dietary fiber, minerals and phytochemicals particularly isoflavones. Levels of isoflavones in soybean seed is the highest among hypocotyledon group (> 20 mg / g)^{1,2,3,4}.

Soybean (*Glycine max* (L) Merrill) is known to contain very high isoflavone compounds, isoflavones are compounds that have a molecular similarity to estrogen that has been used to the clinical symptoms in women postmenopaus such as diabetes, cardiovascular disease, breast cancer and bone health⁵. Soybean has a good potential as antimitotic with $IC_{50} = 3.79 \times 10^{-4}$ mg/mL⁶.

Isoflavones in soy in the form of glycosides that genistein, daidzein and glisitin. Isoflavones in the form of glycosides are not absorbed by the body, to be absorbed, it is necessary isoflavones hydrolyzed by the enzyme β -glucosidase in the intestine to break the bonds of it's glycosides⁷.

Hydrolysis process can be done by adding a microbiology probiotic bacteria in soymilk. Results of research conducted by Fawwaz showed that the levels of genistein in soy fermented by *Lactobacillus acidophilus* by 3.46%⁸ and genistein levels previously obtained through the fermentation of soybean by *Lactobacillus bulgaricus* by 4.99%⁹. Hydrolysis can also be done chemically by the addition of hydrochloric acid¹⁰.

Based on previous research, this study was conducted to determine how many levels of genistein

contained in the extract of soybean that is hydrolyzed by chemical methods, determined by HPLC (High Performance Liquid Chromatography).

2. Materials and Method

Chemical Materials.

Standard genistein G6649 which contains 5 mg was purchased from Sigma Aldrich Chemie GmbH, with purity $\geq 98\%$.

Extraction Process.

Soybean seeds crushed (250 g) and added 500 ml ethanol 70% in the ratio 1:2 (g/ml), the mixture is then heated at a temperature of 90° C, stirring constantly for 2 hours. Solute mixture is separated using a vacuum filter (*Whatman*). The filtrate was added 37% hydrochloric acid until the mixture reaches a pH of 3. The mixture is then heated at 90° C, stirring constantly for 2 hours. The mixture is then added distilled water in the ratio 1:1 (ml/ml) and stirred constantly at room temperature. The precipitate formed is separated using a vacuum filter, the result is stored at 4° C¹⁰.

Standard Solution Preparation

Created a standard solution with a concentration of 200 ppm. Genistein weighed as much as 1 mg and dissolved in methanol: water (8: 2) for 5 ml. Pipette 1 ml of the stock solution and add 5 ml of methanol: water (8: 2) to obtain a concentration of 40 ppm.

Analysis of samples was conducted using High Performance Liquid Chromatography (HPLC), used C18 reverse phased column. Genistein standard with a concentration of 40 ppm and then diluted with methanol: water (8: 2) to obtain a concentration of 8 ppm, 10 ppm, 12 ppm and 14 ppm respectively as much as 5 ml. Series of concentration is then automatically injected into the tool as much as 10 mL. The mobile phase used was methanol: water (7: 3), a flow rate of 1 ml/min with a temperature of 28° C, at a wavelength of 254 nm. Data obtained in the form of an area, then determined the values of a, b and r by comparing the sample concentration (ppm) to the area. Created the linear regression equation $y = a + bx$ ^{7,8}.

Analysis of Genistein

Soybean extract, weighed as much as 3 mg and dissolved in 10 ml of methanol: water (8: 2). Inserted into the HPLC instrument and then analyzed. The results of the analysis will be obtained area (y) which is in turn embedded in the linear regression equation⁸.

3. Result and Discussion

Extract of Isoflavone Aglycone.

Isoflavone aglycone could be found if soybean was hydrolyzed, by hydrolyzing glycoside binding will be solved. Soybean hydrolyzed result was extracted with ethanol. We found extract in weight 2.67 g.

Isoflavone Aglycone Analysis by HPLC

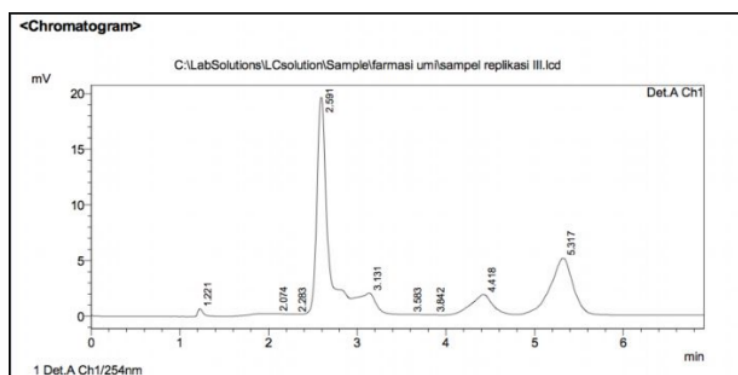
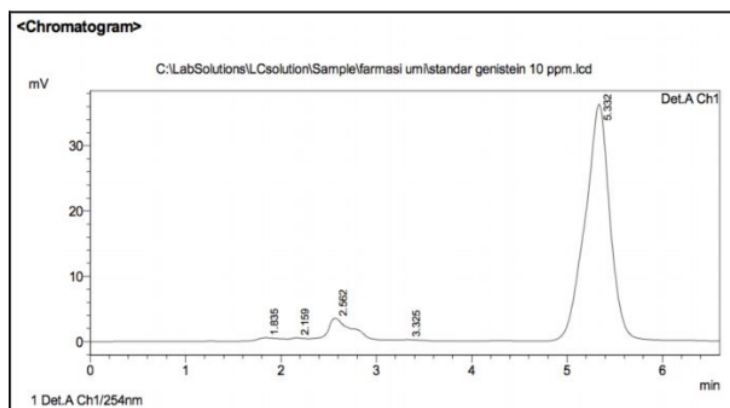
Pure standard genistein was used as isoflavone aglycone to identify and calculate the amount of genistein in extract soybean hydrolyzed. The result of linear regression, we found a, b and r value respectively: -194153, 84833 and 0.946.

Table 1. Genistein concentration and area

Sample	Concentration	Area
Genistein Standard	8 ppm	460371
	10 ppm	654297
	12 ppm	896042
	14 ppm	945345

Table 2. Weight extract and level of genistein

Weight of Extract (g)	Vol (ml)	Area (Y)	Level of Genistein (μ g/mg)	Average (μ g/mg)	% w/w
0.0031	10 ml	134545	6.046	5.807	0.580%
0.0032		157111	6.933		
0.0032		93087	4.442		

**Figure 1. Chromatogram of Extract Soybean Hydrolyzed****Figure 2. Chromatogram of Genistein Standard**

4. Conclusion

This research show that the level of genistein as isoflavoneaglycone in extract soybean hydrolyzed by hydrochloric acid average 0.58% (w/w).The amount lower than fermented result of soybean by probiotic bacteria. In conclusion enzymatic method more effective than chemical method, however enzymatic need higher cost than chemical method.

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