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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ISOFLAVONES AGLYCONES IN INDONESIAN SOYBEAN

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ABSTRACT: Soybean 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). Analysis of samples by HPLC using C18 reverse phased column. Genistein standard with a concentration 8 ppm, 10 ppm, 12 ppm respectively diluted with methanol: water (8:2) as much as 5 ml. Soybean extract, weighed as much as 3 mg and dissolved in 10 ml of methanol: water (8: 2). Standard and sample then analyzed with 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. From the results, show that average of genistein levels contained in the extract of soybean hydrolyzed for *Glycine max* is 0.5% and *Glycine soja* is 1.1%.

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}.

Actually soybeans are cultivated in two species, namely *Glycinemax* (yellow, white or green soybean) and *Glycinesoja* (black soybean). *Glycinemax* is native to subtropical regions of Asia such as China and Southern Japan, while *Glycinesoja* is native to tropical Asia in Southeast Asia.

This plant has spread to Japan, Korea, Southeast Asia and Indonesia. According to Indonesia National Standard (SNI)-01-3922-1995, soybeans can be divided into four, namely yellow soybeans, black soybeans, green soybeans and soy mix, while according Sniyeder and Kwon (1987), all originally green soybeans because of their chlorophyll

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content, but after maturity the chlorophyll becomes invisible⁵.

Soybean 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 health⁶. Soybean has a good potential as antimutagenic with $IC_{50} = 3.79 \times 10^{-4}$ mg/mL⁷.

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 its glycosides⁸.

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%⁹, and genistein levels previously obtained through the fermentation of soybean by *Lactobacillus bulgaricus* by 4.99%¹⁰. Hydrolysis can also be done chemically by the addition of hydrochloric acid¹¹. 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).

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 °C¹¹.

Preparation of Genistein:

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 Genistein:

Analysis of samples was conducted using High Performance Liquid Chromatography (HPLC), used C_{18} 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 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 $y = a + bx$ ^{8, 9}. 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 equation⁹.

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 of *Glycine max* 2.67 g and *Glycine soja* 1.87 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 $y = 108918x - 418941$, and $R^2 = 0.996$.

TABLE 1: CONCENTRATION AND AREA OF GENISTEIN STANDARD

| Sample | Concentration | Area |
|--------------------|---------------|--------|
| Genistein Standard | 8 ppm | 460371 |
| | 10 ppm | 654297 |
| | 12 ppm | 896042 |

TABLE 2: WEIGHT OF EXTRACT AND GENISTEIN LEVEL OF *GLYCINE MAX*

| Weight of Extract (g) | Vol (ml) | Area (Y) | Level of Genistein (µg/mg) | Average (µg/mg) | % w/w |
|-----------------------|----------|----------|----------------------------|-----------------|-------|
| 0.0031 | 10 ml | 134545 | 5.081 | 5.023 | 0.5% |
| 0.0032 | | 157111 | 5.288 | | |
| 0.0032 | | 93087 | 4.701 | | |

TABLE 3: WEIGHT OF EXTRACT AND GENISTEIN LEVEL OF *GLYCINE SOJA*

| Weight of Extract (g) | Vol (ml) | Area (Y) | Level of Genistein (µg/mg) | Average (µg/mg) | % w/w |
|-----------------------|----------|----------|----------------------------|-----------------|-------|
| 0.0031 | 10 ml | 995958 | 12.990 | 11.095 | 1.1% |
| 0.0030 | | 691372 | 10.194 | | |

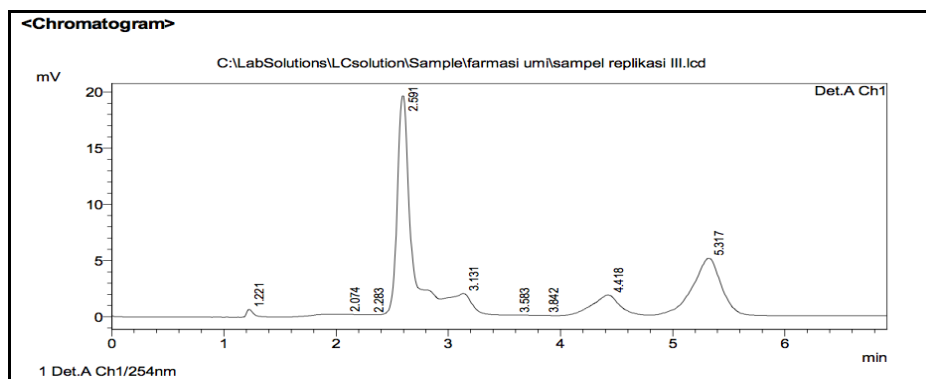


FIG.1: CHROMATOGRAM OF HYDROLYZED EXTRACT OF *GLYCINE MAX*

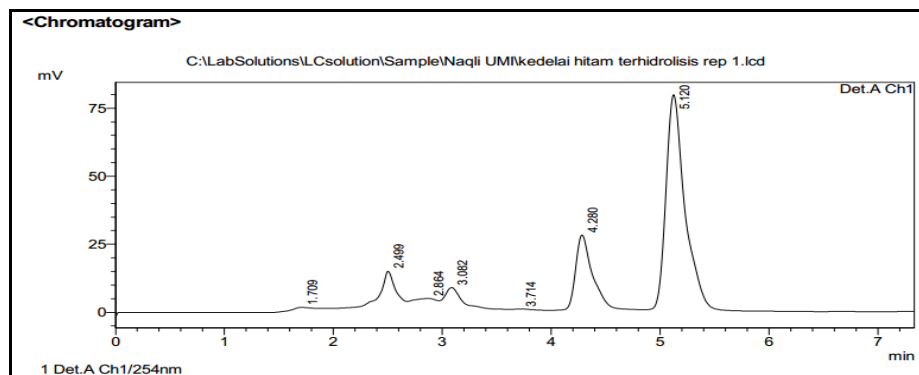


FIG.2: CHROMATOGRAM OF HYDROLYZED EXTRACT OF *GLYCINE SOJA*

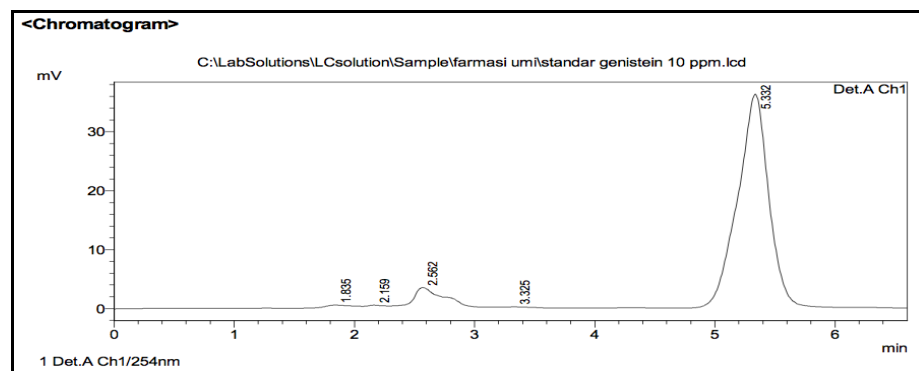


FIG.3: CHROMATOGRAM OF GENISTEIN STANDARD

CONCLUSION: This research show that the level of genistein as isoflavone aglycone of hydrolyzed extract of *Glycine max* 0.5% (w/w) and *Glycine sojais* 1.1%. The amount of genistein 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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