Phytochemical screening and proximate analysis of soybeans 
(*Glycine max*) variety Gamasugen 1 and Gamasugen 2 derived from gamma rays irradiation

**E Lisanti** 1,* and **A Arwin** 2

1 Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Kampus A, Gedung HasjimAs'arie Lt. 5. Jl. RawamangunMuka Jakarta Timur, 13220, Indonesia
2 Center for Isotopes Radiation Application, National Nuclear Energy Agency of Indonesia, Jl. Raya Pasar Jum’ at No. 49 Jakarta Selatan, 12440, Indonesia

*elsalisanti@unj.ac.id*

**Abstract.** Soybean is very widely used in Indonesia, especially as a raw material for processed foods such as tofu and tempeh. This study aims to analyze the phytochemical content and proximate of variety irradiation soybean seeds Gamasugen 1 and Gamasugen 2. Phytochemical and proximate analysis of the seeds of soybean were carried out with quantitative and qualitative analysis. The proximate study analyzed included water content (oven method), ash content (furnace method), fat content (Soxhlet method), and crude fiber content (acid alkali digestion method). The results of phytochemical testing show that both soybeans (Gamasugen 1 and Gamasugen 2) contain flavonoid as quercetin, alkaloid, saponins, phenolic, triterpenoid, and glycoside. The proximate analysis results showed that Gamasugen 1 soybeans had a moisture content of 8.22%, lower than Gamasugen 2, which was 11.67%. Gamasugen 1 soybean content is 4.61%, lower than Gamasugen 2, which is 4.86%. Gamasugen 1 soybeans have a fat content of 14.50%, more economical than Gamasugen 2, which is 15.51%. Gamasugen 1 soybeans have a fiber content of 14.56%, higher than Gamasugen 2 soybeans, which is 9.96%. The conclusion shows the most top proximate analysis of soybean is found in fat content (Gamasugen 2) which is 15.51% and the lowest level is found in ash content (Gamasugen 1) which is 4.61%.

1. Introduction

The soybean plant belongs to the family of the papilionaceous. Soy (*Glycine max* (L.) Merry.) is one of the soybeans which is a source of plant foods that contain high protein but low cholesterol. One of the processed soybeans is tempeh. Soy is one of the most widely grown leguminous crops in the world grow in tropical, subtropical, and temperate climates and providing abundant protein and oil for human diet and animal feeding [1].

The Gamasugen 1 and 2 are irradiated soybeans from National Nuclear Energy Agency of Indonesia, research which has several advantages. Based on this, it is necessary to know the content of secondary metabolites (SM) and the proximate analysis of Gamasugen 1 and two soybeans. Because of SM exhibit a wide array of biological and pharmacological properties. They often interact with the main targets in cells, such as proteins, biomembranes, or nucleic acids. Whereas some SM appears to have been optimized on a few molecular targets, such as alkaloids on receptors of neurotransmitters, others (such...
as phenolics and terpenoids) are less specific and attack a multitude of proteins by building hydrogen, hydrophobic and ionic bonds, thus modulating their 3D structures and in consequence their bioactivities [2].

The proximate analyses of soybean samples for crude proteins, moisture, total ash, fat, and carbohydrate were carried out in triplicate using the methods described in AOAC [3]. This study aimed to estimate the proximate, and phytochemicals present in seeds of two soybeans irradiation (*Glycine max* (L.) Merry) genotypes Gamasugen 1 and 2 to explore their nutritional and medicinal values.

2. Methods

Gamasugen is the product of early maturing soybean mutants from Batan, Indonesia. Gamasugen is produced by radiation mutation using $^{60}$Co gamma rays. Soybeans were irradiated with 200 gray gamma rays.

Mutant lines that have more mature harvesting age than their parents are caused by genetic changes due to the influence of gamma ray radiation. This genetic change is permanent and inherited from the next generation, so that it is a new individual that has a different nature from its parent. The gene that controls early maturity has been reported by [3], that the age of early maturity in soybeans is controlled by the OT98-17 gene which causes faster harvesting (early maturity).

Proximate analysis and phytochemical tests were carried out at the Laboratory of Medicine and Medicine Plants Units (BALLITRO) in Bogor, Indonesia state.

2.1. Proximate Analysis

The proximate analysis aims to determine the physicochemical characteristics of Gamasugen 1 and 2. varieties of soybean. Observation parameters include a proximate analysis of soybeans (moisture content, ash content, fat, and crude fiber) [4]. The collected data were tabulated and analyzed descriptively.

2.1.1 Water concentration: Water level measurement is carried out by using the oven method. The cup to be used is dried in the oven at a temperature of 100-105°C for 30 minutes or until it gets massive permanent. After it's cooled in a desiccator for 30 minutes and later weighed. The sample is considered as much as 5 g (B1) in the cup, then dried in the oven at a temperature of 100-105°C until it is reached fixed weight (8-12 hours). The sample is cooled in the desiccator for (30 minutes) and then weighed (B2). Water content calculation is done as follows:

$$\text{Water content} = \frac{B1 - B2}{\text{sample weight}} \times 100\%$$

2.1.2. Ash concentration: Prepare a cup that has been previously heated in the kiln 400-600 ° C, cool it in the excavator, then weigh the cup (X). Weigh the sample ± 5 g, put it in the bowl then consider it (Y). Burn the example on the hot plate until it's not smoky. Enter into the furnace. Lift it, cool it in the desiccator. Weigh and record the weight (Z). Calculation:

$$\text{Ash concentration} = \frac{Z - X}{Y} \times 100\%$$

2.1.3. Crude fat levels: Pumpkin scatters with boiling stones in it was prepared which had been previously heated at a temperature of 105-110 ° C and cooled in the excavator. Weigh the pumpkin dancer (a). Weigh the sample ± 1 g (X). Put it in the seal sleeve, then cover it with non-fat cotton. Put the sealing sleeve into the soxhlet tool, then run using petroleum benzine. Then the extractor is connected to the condenser. This process is done using FATEX-S. Remove the pumpkin from the FATEX-S
device, then dry it in the oven 105-110°C until the weight remains (± 4-6 hours). Lift, chill in eczema. Final weighting (b). Calculation:

\[
\text{% Crude Fat Levels} = \frac{b - a}{X} \times 100\%
\]

2.1.4. **Crude fiber levels:** The sample is weighed as much as ± 1 g (x), put it in a cup glass. Then put it into the heater extract tool. Add 50 ml of 0.3 N H2SO4, cook for 30 minutes, then add NaOH 1.5 N and cook for 30 minutes. Prepare filter paper which has been heated in an oven 105 °C for 1 hour then weighed (a). Strain the liquid using filter paper above into the Buchner funnel. The filtering is carried out with a suction flask which is connected to a vacuum pump or water emitted. Wash in a row using 50 ml of hot water, 50 ml of 0.3 N H2SO4, 50 ml of warm water, and 25 ml of acetone. Insert filter paper and its contents into a porcelain dish. Then dry with oven 105 °C for 1 hour. Lift, chill in the executor then weigh (Y). Reinsert the cup into the furnace. Lift, relax, then weigh (Z). Calculation:

\[
\text{% Crude Fiber Levels} = \frac{Y - Z - a}{X} \times 100\%
\]

2.2. **Phytochemical analysis**

Phytochemical analysis can be carried out qualitatively and quantitatively. This analysis is used to determine the content of several compounds in plants such as alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids. The phytochemical analysis is carried out qualitatively and quantitatively. Qualitative tests for flavonoids, tannins, saponins glycosides, alkaloids were carried out using the Association of Official Analytical Chemist (AOAC) method. Quantitative testing of flavonoids using the aluminum chloride and tannin method using the Schanderl method [5].

2.3. **Phytochemical screening**

Qualitative screening of the soybeans with two varieties that were Gamasugen1 and two was carried out using the methods of Harborne for alkaloids, flavonoids, saponins, tannins, triterpenoid, and phenolic.

2.3.1. **Alkaloid test:** The sample extract added 0.1 g to 10 mL chloroform and a few drops of ammonia. The chloroform fraction was separated and acidified with ten drops of 2M H2SO4. The acid fraction was taken then added reagents Dragendorf, Meyer, and Wagner. The presence of alkaloids is characterized by the formation of white deposits by Meyer reagents, red deposits by Dragendorf reagents, and brown deposits by Wegner reagents.

2.3.2. **Saponin test:** The sample extract is 0.1 g plus enough water and heat for five minutes. The solution is cooled and then shaken. The occurrence of foam for ± 10 minutes shows the presence of saponins.

2.3.3. **Flavonoids and phenolic tests:** Extract the sample as much as 0.1 g plus 30% methanol until submerged and then heated. The filtrate is added by NaOH 10% (b / v) or H2SO4. The formation of red color due to the addition of NaOH shows the presence of hydroquinone phenolic compounds while the red color formed by the addition of concentrated H2SO4 indicates the presence of flavonoids.

2.3.4. **Triterpenoid and steroid tests:** Extract the sample as much as 0.1 g plus 25 mL ethanol 30% then heated and filtered. The filtrate is evaporated then ether is added. The ether layer was added by Lieberman Buchard reagent (3 drops of acetic anhydride acid and one drop of concentrated H2SO4). Red or purple indicates a triterpenoid, and green indicates the presence of steroids.
2.3.5. **Tanin test:** Extract the sample as much as 0.1 g of added water then boil for several minutes. Then filtered and the filtrate added FeCl₃ 1% (b / v). Dark blue or greenish black indicates tannins.

2.3.6. **Glycoside test:** The extracts were hydrolyzed with dilution HCl, and then the extracted test for glycosides [6].

3. **Results and discussion**

3.1. **Results**

Table 1 presents the proximate analysis of soybean varieties of Gamasugen 1 and Gamasugen 2. The proximate analysis is a study that will be used in the future that Gamasugen 1 and Gamasugen 2 soybeans can be used and developed for human food, because of their nutritional content. The results of the proximate analysis of Gamasugen 1 and Gamasugen 2 were given in Table 1.

| Analysis Test          | Varieties                  | Test Method | Analysis Test (%) | Source |
|------------------------|----------------------------|-------------|--------------------|--------|
|                        | Gamasugen 1 | Gamasugen 2 |                   |        |
| Protein content        | 37.65        | 37.34       | Gravimetri         | 37.69, 21.53 | [7,8] |
| Water content          | 8.22         | 11.67       | Gravimetric        |        |
| Ash content            | 4.61         | 4.86        | Gravimetric        | 4.29   |
| Fat content            | 14.50        | 15.51       | Shoxlet            | 28.20, 10.86 | [7,8] |
| Fiber content          | 14.56        | 9.96        | Gravimetric        | 5.44   |
| Dry matter             | 91.78        | 88.33       | Gravimetric        |        |
| NFE                    | 20.46        | 20.66       | Gravimetric        |        |

*NFE = Nitrogen Free Extract.*

The results as shown in Table 1, soy beans have a relatively low content of fat and a relatively high content of protein. Proximate analysis was used to compare the chemical characteristics of soybeans, Gamasugen 1 and Gamasugen 2.

According to Kishida et al. soy-based foods are known to have good nutritional and functional qualities, not only due to their high protein and oil content but also because of phytochemicals, notably the isoflavones [9]. Soybean consists of approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ [10].

| Constituents | Gamasugen 1 | Varieties | Gamasugen 2 | Varieties |
|--------------|-------------|-----------|-------------|-----------|
|              | Initial     | Final     | Initial     | Final     |
| Alkaloid     | Yellow      | Reddish   | Yellow      | Reddish   |
|              | faded       | brown     | white       | white     |
| Saponin      | Yellow      | Reddish   | Yellow      | Reddish   |
|              | faded       | brown     | white       | white     |
| Flavonoid    | Yellow      | Reddish   | Yellow      | Reddish   |
|              | faded       | brown     | white       | white     |
| Phenolic     | +           | +         | +           | +         |
| Triterpenoid | +           | +         | +           | +         |
| Glycosides   | +           | +         | +           | +         |
| Tanin        | -           |           | -           |           |
| Steroid      | -           |           | -           |           |

"+" Represents Present; "-" = Represent Absent.
3.2. Discussion

Today soybeans are a significant source of plant protein (70%) and oil (30%) and have become a globally important crop [11]. Soy with ethanolic extract is a versatile plant that has proven to have various health benefits [12]. Based on the phytochemistry test showed that both of the soybeans extracts from Gamasugen 1 and two were contained alkaloids, saponins, flavonoids, phenolic, triterpenoid and glycosides, and but were negative for tannin and steroid compounds. Tannins inhibit the activity of many different enzymes. Tannins are simply enzyme inhibitors [13].

According to Uwem et al. the results from qualitative analysis of phytochemical constituents of soy flour with the solvent of methanol and aqueous extract, respectively showed the tannins - - , phenolic + +, glycosides + +, steroids + +, saponins + +, and flavonoids + + [14]. The preliminary phytochemical screening indicated the absence of steroids, if the compared with Gamasugen 1 and Gamasugen 2.

The high protein content was 37.65, and 37.34% vs. 37.69% of the sample suggests that it could be used in the management of protein deficiency cases such as Kwashiorkor. This means the example could be used in improving the palatability of foods in which they are incorporated. The protein is important for tissue replacement, disposition of lean body mass and growth [8].

The ash content was 4.61, and 4.86% (The Gamasugen 1 and 2, respectively) vs. 4.29% is indicative that the flour sample could be significant sources of minerals. According to Patience et al. ash is an indication of availability of mineral [8]. The fat content of 14.5 and 15.51 v. 28.2% suggests that soya bean may be a viable source of oil, going by their raw fatty materials. Most legumes contain 1.5% crude fat. Soya bean crude fat is very high compared to most plants because it is an oilseed. Fat is important in the diet of infant and young children because it provides essential fatty acid, facilitates absorption of fat soluble vitamin, enhance dietary energy, density and sensory quality [15]. The sample fiber carried relatively high when compared to [7] that were 14.56, and 9.96% (Gamasugen 1 and 2) vs. 5.44% fiber, the presence of tissue in foods is known to be beneficial. Fibre has some physiological effects in the gastrointestinal, tract. These effects include variation in fecal water, fecal bulk and transit time and elimination of bile acids and neutral steroids, which lower the body cholesterol pool. Fibre plays a role in the increased the utilization of nitrogen and absorption of some other micronutrients. The low fibre content is in agreement with the report that food used for complementary feeding should contain low fibre as high fibre can lead to high water absorption and displacement of nutrient and energy needed for the growth of children less than two years [16]. Dry matter overhauled will be converted into energy and other molecules (CO$_2$ and H$_2$O) and then gets evaporated [17].

4. Conclusion

National soybean varieties Gamasugen 1 and 2 have superior chemical properties compared to imported soybeans. The advantages of Gamasugen 1 and two types compared to imported soybeans include ash content and low-fat content. The phytochemical test results of Gamasugen 1 and two qualitatively showed no difference in the content of secondary metabolites. The study has established the proximate nutrients concentrations of soybean indicate that the two samples were rich in proteins, fats, and fibers.

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