Investigation on Antioxidant Activity and Protein Content of Glycine max (L.) Merr.

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Abstract

The sample soybean was collected from Meiktila Township, Mandalay Region, and Myanmar and carried out on analysis. The main aim of this research work is to determine the antioxidant activity, mineral and protein contents of soybean. The mineral content in soybean was measured by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrophotometer. The antioxidant activity of ethanol extract of sample was done by 1-1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. The moisture and ash contents of sample were done by AOAC method. The percentage of moisture and ash content of sample were found to be 7.89 and 0.8. The extraction and isolation of protein by using Trichloroacetic Acid (TCA) and acetate buffer method. The protein percentage of soybean was 37.94. This isolated protein was confirmed qualitative determination test, which gives rise to Biurest test, Millon's test, Formaldehyde test, Xanthoproteic test and Hopkin's-Cole test respectively.

Keywords: soybean, mineral content, antioxidant activity, protein

1. Introduction

Soybeans (*Glycine max* (L.) Merr) are widely distributed in East Asia, Australia, and Africa. Seeds contain high amount of components with health benefits, such as proteins, isoflavones (genistein, daidzein, and glycitein), coumestrol, phytate, saponins, lecithin, phytosterols, vitamin E, and dietary fibers. Soy protein and genistein (one of the main isoflavones in soybeans) supplements were reported to be beneficial for correcting hyperglycemia. [3]

The health benefits of soybeans come from the nutrients, vitamins, and organic compounds including a significant amount of dietary fiber and a very large amount of protein. According to the USDA Food Data Central, soybeans contain vitamin K, riboflavin, folate, vitamin B6, thiamin, and vitamin C. As for minerals, soybeans contain significant amounts of iron, manganese, phosphorus, copper, potassium, magnesium, zinc, selenium, and calcium. They are also a good source of organic compounds and antioxidants like isoflavones, which further help in boosting your health.[4]

1.1. Scientific classification

Family name : Leguminoceae
Scientific name : *Glycine max* (L.) Merr
English name : Soybean
Myanmar name : Pe-bok


Figure 1. The plant and seeds of soybean

2. Material and Methods

2.1. Sampling

The soybeans were collected from Meiktila Township, Mandalay Region. The clean air-dried sample was ground by grinding machine into finely powder. The powder was stored in stopper bottle and used throughout the experiment.

2.2. Elemental Analysis

Elemental compositions of soybean seed powder were measured at Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X-Ray Fluorescence spectroscopy) method.

2.3. Determination of Moisture and Ash Contents

The moisture and ash contents of sample by using (AOAC, 2000) method.[1]

2.4. Antioxidant Activity of Crude Extract

The antioxidant activity of ethanol extracts of the sample were done by using DPPH Radical Scavenging Assay.[5]

2.4.1. Preparation of Test Sample Solution

0.0031g of ethanol crude extract was dissolved in 10 mL of 95% ethanol under vigorous shaking. This solution was used as a stock solution. The stock solution was diluted with ethanol to obtain various concentrations of test sample solutions.

2.4.2. Preparation of 60 µM DPPH Solution

DPPH powder (2.4 mg) was weighed and it was thoroughly dissolved in 100 mL of 95% ethanol and stored in brown colored reagent bottle. It must be kept in refrigerator for no longer than 24 hours.

2.4.3. Measurement of antioxidant activity by spectrophotometric method

The control solution was prepared by mixing 2 mL of 60 µM DPPH solution and 2.0 mL of 95% under vigorous shaking. The blank solution was prepared by mixing 2.0 mL of test sample solution and 2.0 mL of 95% ethanol under vigorous shaking. Moreover, the prepared standard ascorbic acid solutions and the test sample solutions were also prepared by gently mixing each of 2.0 mL of 60 µM DPPH solution and 2.0 mL of test sample solution with various concentrations. After that, the solutions were allowed to stand for 30 minutes at room temperature. Then, the absorbance value of each solution at 517 nm was measured by UV spectrophotometer.

2.5. Extraction and Precipitation of Protein from Soybean

5g of the dried sample was weighed and grinded with a pestle and motor. The powder was extracted with an aliquot of ethanol: petroleum ether (2:1 v/v) mixture with stirring for a few minutes. The sediment was washed with ether. The solvent was then decanted, filtered and the residue was used for protein estimation. The residue was dried at room temperature to remove all traces of ether dissolved in 10% TCA (w/v) [prepare 10% TCA solution by dissolving 2.5g of TCA in 10 mL of distilled water]. The mixture was kept at 4℃ for about four hours. It was centrifuged at 6000 rpm for 15 minus. The precipitate was dissolved in 0.1M NaOH solution. Then, this suspension was used for protein estimation.

100 mL of sample solution was poured into a conical flask and warmed at 40℃ by using water bath. 100mL of sodium acetate buffer solution (pH 5.6) was also heated and slowly added into the sample while stirring constantly. The suspension was cooled to room temperature for 5 min and then it was filtered through a filter paper and the residue was washed into a beaker containing 30 mL of ethanol. Filtered paper was washed repeatedly to recover all the protein. Protein was left suspended in ethanol and then filtered on a funnel. The obtained protein was washed two times with a mixture of equal volumes (20mL) of ethanol and ether. Finally, the precipitate was washed with 50mL of ether and dried at room temperature. The weight of dried protein and the yield percent were calculated. [2]

2.6. Qualitative Tests of Protein

The qualitative determination of protein from soybean was tested by Biuret test, Millon's test, xanthoproteic test, Hopkin's-Cole test and Formaldehyde test.[1]

3. Results and Discussion

3.1. Elemental Composition of Soybean

The results of elemental compositions of soybean were shown in Table 1.

Table 1. Result of mineral content in soybean

No.	Element	Relative abundance (%)
1	Potassium	1.5621
2	Phosphorus	0.5234
3	Calcium	0.3500
4	Aluminum	0.1116
5	Sulfur	0.1062
6	Iron	0.0316
7	Silicon	0.0127

According to this table, the sample consists of potassium, phosphorus, calcium, aluminum, sulfur, iron and silicon respectively. Among them, potassium is the highest value containing in this sample. Potassium is one of the most important minerals in the body. It helps regulate fluid balance, muscle contractions and nerve signals.

3.2. Moisture and Ash Content

The results of moisture and ash contents of soybean were shown in Table 2 and 3.

Table 2. Result of moisture content

No.	Weight of sample (g)	Loss in weight of sample (g)	Moisture content (%)
1	1	0.0789	7.89
2	1	0.0788	7.88
3	1	0.0789	7.89

Table 3. Result of ash content

No.	Weight of sample (g)	Loss in weight of sample (g)	Ash content (%)
1	1	0.008	0.8
2	1	0.007	0.7
3	1	0.008	0.8

The moisture and ash content of soybean were found to be 7.89% and 0.8%.

3.3. Antioxidant Activity

The percent inhibition of the various concentrations of standard ascorbic acid was shown in Table 4.

Table 4.Result of % inhibition of various concentration of standard ascorbic acid

Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
50	89.936	11.98
25	64.103	
12.5	53.840	
6.25	45.350	
3.125	36.378	

The percent inhibition of the various concentration of the ethanol extract of the sample was shown in Table 5.

Table 5. Result of % inhibition of various concentration of sample

Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
100	91.634	35.24
50	79.767	
25	42.802	
12.5	32.102	
6.25	17.315	

IC_{50} value was calculated by using linear regressive equation.

In DPPH screening assays that, IC_{50} value of ethanol extract of soybean was found to be 35.24 µg/mL. IC_{50} value of standard ascorbic acid was 11.98µg/mL. So, the antioxidant activity of sample extract has low lower than standard ascorbic acid.

3.4. Protein Content of Soybean

The yield percentage of protein for soybean was shown in Table 6.

Table 6. Percent of protein from soybean

No.	Sample	Weight of dried sample (g)	Isolated protein (g)	Protein (%)
1	soybean	5	1.8972	37.94

The result of qualitative test of protein from soybean was shown in Table 7.

Table 7. Qualitative test of protein from soybean

No.	Experiment	Reagent	Observation	Inference
1	Biuret test	10% NaOH 1% $CuSO_4$	Bluish pink to violet	Presence of albumin
2	Millon's test	$HgSO_4$	White ppt	Presence of albumin
3	Xanthoproteic test	HNO_3 NH_4OH	Yellow color to orange color	Presence of tyrosine, tryptophan and phenylalanine
4	Hopkin's-Cole test	Glyloxylic acid, conc: H_2SO_4	Violet color	Presence of tryptophan
5	Formaldehyde test	HCHO, conc: H_2SO_4	Purple violet	Presence of tryptophan

4. Conclusion

In this research work, qualitative and quantitative determinations of protein from soybean were investigated. According to EDXRF analysis, the sample consists of potassium, phosphorus, calcium, aluminum, sulphur, iron and silicon respectively. The moisture and ash content of soybean were 7.89% and 0.8%.

According to DPPH radical scavenging assay IC_{50} value of sample was found to be 35.24 µg/mL.The sample extract has low antioxidant activity of standard ascorbic acid (IC_{50} = 11.98 µg/mL).

The yield percent of protein for soybean was 37.94. Moreover, protein was identified by five chemical tests. In Biuret test, sample was changed bluish pink to violet color. In Millon's test, the sample observed to white precipitate. In xanthoproteic test, sample developed in yellow color change to orange color. In Hopkins-Cole test, sample observed to reddish violet color. Finally, in formaldehyde test, sample appeared to purple violet color. According to this observation, soybean had the presence of protein.

The soybeans are very rich in nutritive components. The biggest benefits of soy come when they are eaten as a replacement for foods like red meat and other options high in saturated fat. Substituting soy over these foods may lower LDL cholesterol slightly, as well as reduce blood pressure. Therefore, soybeans are beneficial for human growth and development, help the body and repair cells and new ones.

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