Preliminary Phytochemical Examination and some Analysis of Soybean Seeds Powder

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Abstract

It is concerned with preliminary phytochemical chemical examination and some analysis of soybean seeds powder. In the preliminary phytochemical tests, there was eleven chemical tests which carried out to investigate phytochemicals in soybean. Among them, nine phytochemicals such as glycosides, steroids, -amino acids, flavonoids, tannins, carbohydrates, alkaloids, saponins, and reducing sugar were presented. And, starch and terpenoids were not present or absent. In chemical analysis of soybean powder, it was found that moisture content (9.3 %), fat content (15.5 %), total ash content (4.36 %), acid insoluble ash content (0.46 %), water soluble ash content (2.92 %) and soluble matter contents in different solvents (ethanol, methanol, ethyl acetate, dichloroethane and pet-ether) were 12.67 %, 5.83 %, 15.22 %, 12.97 % and 11.8 % respectively.

Keywords: Soybean, Phytochemical, Chemical tests, Analysis, Solvents

INTRODUCTION

The soybean (*Glycine max*) is grown as a commercial crop in over 35 countries as the major oilseed (Smith & Huyser, 1987). The fruit of soybean is simple or in the shape of rescent pod. Its length is about 3-7cm, including 1 or 2seeds. The mass of 1000 seeds can be taken out 115-280g. On the fodder designed the seeds in mass about 180-200g.Unripen seeds are green , and mature have from light-yellow by green to brown colour. In practice are used seeds of different cultivars, what influence on colour and form of seeds. The soybean seeds of modern cultivars have spherical shape, and the yellow and green colour is the most desirable (Sikorski, 2007).The soybean products are used in food industry in the world. The soybean seeds contain high quantity of protein and its amino acid composition is approximate to the composition of animal proteins. Therefore, it is often used as replacement component of meat protein. Soybean seeds are used in oil industry. About 90% of soybean seeds make up cotyledons and 8% there are hulls. In the cotyledons are accumulated proteins and fats , the main components of seeds. In the cotyledons also are accumulated carbohydrates and anti-nutritional factors. As a result, the separation of this components or their extraction got different soybean products used in human and animals feeding.

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Materials and Methods

Collection and Preparation of Sample

The soybean seeds were collected from Nyaung Pin Lay Plaza, Yangon, Myanmar, in bulk in order to prevent the variation of the composition of the materials present in the raw materials. The seeds were kept air dry for one week. Dried seeds were ground to powder by using a grinding mill. The dried powder was stored in air-tight glass bottles.

Preliminary Phytochemical Examination of the Soybean Seeds

Test for Glycosides

Dried powdered seeds (ca. 1 g) was boiled with the distilled water for about 10 minutes, allowed to cool and filtered. The filtrate was treated with 10 % lead acetate solution. Observation was made to see if precipitation took place on addition of the reagent. (Steech, 1949)

Test for Steriods

Dried powdered seeds (ca. 5 g) was refluxed with benzene and the solvent was removed by distillation under reduced pressure. Acetic anhydride (3 drops) was added to the residue and the mixture was shaken. Then a few drops of concentrated sulphuric acid was carefully added and shaken. Observation was made to see if the solution turned to green colour. (Trease & Evans, 1954) ; (Charkravartic, 1982) ; (Rangaswami & Rav, 1955)

Test fo acid Amino Acid

Dried powdered seeds (ca. 5 g) was boiled with water for about 10 minutes and then filtered. An aliquot portion of the filtrate was transferred to a filter paper with the help of the micro pipette and allowed to dry. Then the filter paper was sprayed with ninhydrin reagent and allowed to dry at 110 ºC in an oven. Observation was made to see if a violet coloured spot appeared on the filter paper. (Linsted, 1955)

Test for Flavonoid

Dried powdered seeds (ca. 5 g) was refluxed with methanol (25 cm^3) and filtered. Alcoholic hydrochloric acid (95 % EtOH 1 vol + H_2O 1 vol + concentrated HCl 1 vol) (2 cm^3) was added to above filtrate (2 cm^3). A few pieces of Mg turning were added to the mixture. Observation was made to see if pink colour appeared within three minutes. (Harbone, 1984)

Test for Tannins

Dried powdered seeds (ca. 2 g) was refluxed with distilled water (10 cm^3) on water-bath for thirty minutes and filtered by using cotton wool. The filtrate was added 2 % sodium chloride solution (5 cm^3) and filtered through filter paper. The obtained clear solution was added 1 % gelatin solution (5 cm^3). Observation was made to see if white precipitate came down in the solution. (Marini Bettolo, et al., 1981)

Test for Carbohydrates

Dried poiwdered seeds (ca. 3 g) was boiled with water (10 cm^3) for about 20 minutes and filtered. The filtrate was placed into a test tube and a few drops of 10 % -naphthol in ethanol was added and shaken. This test tube was inclined at an angle of 45 ºC and concentrated sulphuric acid (ca. 1 cm^3) was slowly introduced along the side of the test tube. Observation was made to see if a red ring formed between two layers. (Vogel, 1966; Priestman & Edwards, 1993)

Test for Alkaloids

The dried soybean powder (ca. 2 g) was added 1 % hydrochloric acid (10 cm^3) and heated on water-bath for thirty minutes. The filtrate was added modified Dragendoff solution (5 drops). Observation was made to see if the reddish orange precipitate was observed in the solution. (Genus, 1978)

Test for Starch

Dried powdered seeds (ca. 1 g) was boiled with purified water (10 cm^3) for 30 minutes. It was then filtered and iodine solution (2 drops) were added to the filtrate. Observation was made to see if bluish-black precipitate were formed. (Harbone, 1984)

Test for Saponins

Dried powdered seeds (a little) were introduced into a test tube followed by the addition of distilled water and the mixture was vigorously shaken for a few minutes. Observation was made to see if frothing took place. (Trease & Evans, 1961)

Test for Terpenoids

Dried powdered seeds (ca. 1 g) were extracted with hot chloroform (20 cm^3) for 30 minutes and filtered. The filtrate was evaporated to dryness in vaccuo and the residue was dissolved in ethanol (2 cm^3). The solution was divided into two portions. One portion was transferred to watch-glass and the solvent was evaporated to dryness on a water-batch. The residue was dissolved in acetic anhydride, using a glass-rod. The solution was treated with a drop

of concentrated sulphuric acid and observation was made to see if greenish blue colour occurred. (cited in M T Y, 1993)

The second portion was transferred to a filter paper using a micro pipette and allowed to dry. The paper was then exposed to iodine vapour to see if a reddish-brown spot appeared.

Test for Reducing Sugar

The soybean powder (0.5 g) were boiled with dilute sulphuric acid (5 cm^3) for about 10 minutes and filtered. The filtrate was then neutralized with dilute sodium hydroxide solution. The resulting solution was mixed with 2 drops of Benefits solution and boiled for about 2 minutes. Then, the solution was allowed to cool. (Harbone, 1984)

Some Analysis of Soybean Seeds

Determination of Moisture Content

Toluene (150 cm^3) and water (1 cm^3) were placed in a dried round-bottomed flask (250 cm^3), some boiling chips were added and the apparatus was assembled. The mixture was then refluxed at 80 ºC for two hours. Both water and toluene were evaporated, condensed in the condenser and collected in the graduated side arm of Dean and Stark's apparatus. The condensed toluene which formed the upper layer, overflowed continuously back into the distillation flask, while the heavier condensed water remained at the bottom of the tube.

After all the added water (approximately 1 cm^3) collected at the bottom of the graduated side arm, the heating was stopped, allowed to cool for 30 minutes and the volume of water was read. Then, the dried powder of soybean seeds (ca. 10 g) was introduced into the distillation flask and some pieces of boiling chips were again added to the flask. The distillation was continued for about 5 hours until all the moisture was removed from the sample and the volume of water was read.

Determination of Ash Content

The ash content, the acid insoluble ash content and the water soluble ash content were determined by using the method described in "The Chemical Analysis of Foods".

Determination of Total Ash Content

Dried powder of soybean seeds sample (ca. 1 g) was accurately weighed and placed in a preheated, cooled and weighed porcelain crucible. The crucible was heated carefully on a hot plate until the organic matter dried and burned off without flaming. The partially decomposed sample was then incinerated in a Muffle furnace at 823 K for 6 hours until the resultant ash was

uniformed in colour (i.e., white or gray). The crucible containing the ash was then cooled to room temperature in a desiccators and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained. (Pearson, 1970); (Joslyn, 1970); (William, 1984). The ash content of soybean seeds powder was shown in Table (2).

Determination of Acid Insoluble Ash Content

The accurately weighed ash obtained from the determination of ash was put into a beaker and boiled with 25 cm^3 of dilute hydrochloric acid for about 5 minutes and then filtered, using an ashless filter paper. The residues was subsequently washed with hot water transferred to the Muffle furnace and heated at 823 K.

The crucible containing the insoluble ash was then cooled to room temperaturein a desiccators and weighed. Heating, cooling and weighing were repeated until the constant weight was obtained which correspond to the acid-insoluble matter in ash.

The acid-insoluble ash content of soybean seeds powder was shown in Table (2).

Determination of Water Soluble Ash Content

The accurately weighed ash obtained from the determination of ash was boiled with distilled water (25 cm^3) for about 5 minutes and then filtered, using an ashless filter paper. The residues was washed with hot water and kept in an oven for about 10 minutes.

It was subsequently transferred to the Muffle furnace and heated at 823 K for 5 hours. The crucible containing the water insoluble ash was then cooled to room temperature in a desiccators and weighed. Heating, cooling and weighing were repeated until the constant weight was obtained. When the weight of the insoluble matter was substracted from the weight of the ash,the difference in weight represented water soluble ash. The results were shown in Table (2).

Determination of Fat Content

Soybean seeds powder (ca. 50 g) was placed in a cloth bag and the bag was then placed in a Soxhlet extractor. Petroleum ether was poured into the extractor until some of it over flowed into the flask. The petroleum ether was heated by means of a water bath.

The extraction was assumed to be completed when a small amount of extract placed on a water bath did not leave any residue on evaporation of solvent. A duration of about 24 hours was required for the complete extraction during which the petroleum ether was recycled for about 480 times. The petroleum ether was removed by simple distillation until the volume of petroleum ether solution was transferred quantitatively into a tarred 50 cm^3 round-bottomed flask and the

residual petroleum ether by vaccum distillation at 333.2 K until frothing totally ceased. The outside of the flask was rubbed with a clean wetted with methylated spirit and then weighed. The difference in weight of the round bottom flask before and after the distillation gave the weight of fat content in the sample of soybean seeds powder. (Joslyn, 1970)

The fat content of soybean seeds powder was shown in Table (2).

Determination of Soluble Matters in Different Solvents

Alcohol Soluble Matter Content

Dried powdered soybean seeds sample (ca. 3 g) was placed in a conical flask. 95 % ethanol (100 cm^3) was added and the flask was stoppered with a cork. The sample was allowed to macerate for 24 hours. The flask was then placed on a shaker. It was continuously shaken for 6 hours and the suspension was allowed to stand for 18 hours. The contents were rapidly filtered through a filter paper and wash with small portions of alcohol to ensure complete removal of alcohol soluble matter. The filtrate and wash liquors were combined and the volume made up to 100 cm^3. A portion of the filtrate (25 cm^3) was taken in a tared round-bottomed flask and evaporated to dryness on a water bath. It was dried at 378 K to constant weight. The difference in the weight of the round-bottomed flask before and after the experiment was taken as the alcohol soluble matter.

The alcohol soluble matter content of soybean seeds sample was shown in Table (3).

Ethyl Acetate Soluble Matter Content

Ethyl acetate soluble matter content of soybean seeds sample was determined by the method given in "The British Pharmacopoeia" as described in procedure of alcohol soluble matter content, by using ethyl acetate instead of alcohol. The ethyl acetate soluble matter content of soybean seeds sample was shown in Table (3). (Steyermark, 1961); (Nadkec, 1954)

Methanol Soluble Matter Content

Methanol soluble matter content of soybean seeds sample was determined by the method given in "The British Pharmacopoeia" as described in procedure of alcohol soluble matter content, by using methanol (100 cm^3) instead of alcohol. The methanol soluble matter content of soybean seeds sample was shown in Table (3). (Steyermark, 1961); (Nadkec, 1954)

Dichloroethane Soluble Matter Content

Dichloroethane soluble matter content of soybean seeds sample was determined by the method given in "The British Pharmacopoeia" as described in procedure of alcohol soluble

matter content, by using dichloroethane (100 cm^3) instead of alcohol. The dichloroethane soluble matter content of soybean seeds sample was shown in Table (3). (Steyermark, 1961); (Nadkec, 1954)

Petroleum Ether Soluble Matter Content

Petroleum ether soluble matter content of soybean seeds sample was determined by the method given in "The British Pharmacopoeia" as described in procedure of alcohol soluble matter content, by using petroleum ether instead of alcohol. The petroleum ether soluble matter content of soybean seeds sample was shown in Table (3). (Steyermark, 1961); (Nadkec, 1954)

RESULTS AND DISCUSSION

Collection and Preparation of Sample

Soybean seeds were collected from Nyaung Pin Lay Plaza in Yangon. The seed were dried in air for one week. This drying reduced the moisture content to 10 % or less, the condition necessary to prevent growth of mould during the storage of the sample. The dried seeda were powdered in a grinding machine. The powdered sample was then stored in an air-tight container.

Preliminary Phytochemical Tests of the Soybean Powder

A literature survey showed that a very little work has been carried out on the chemical studies of locally soybean seeds. Therefore, the following preliminary phytochemical investigation carried out on the seeds powder with a view to determine the presence or absence of glycoside, steroids, -amino acids, flavonoids, tannins, carbohydrates, alkaloid, starch, saponins, terpenoids and reducing sugars in Table (1).

Table 1. Results of Preliminary Phytochemical Tests of Soybean Seed Powder

No.	Tests	Extract	Test reagent	Observation	Result
1.	Glycosides	H_2O extract	10 % lead acetate	White ppt.	+
2.	Steroids	Benzene extract	acetic anhydride and H_2SO_4	Green	+
3.	α-Amino cids	H_2O extract	ninhydrin reagent	Violet spot	+
4.	Flavonoids	MeOH extract	alcoholic HCland Mg turning	Pink solution	+
5.	Tannins	H_2O extract	2 % NaCl solution, 1 % gelatin solution 10 % α-naphthol	White ppt.	+
6.	Carbohydrates	H_2O extract	Dragendoff's reagent	Red ring	+
7.	Alkaloids	1 % HCl extract	Iodine solution	Orange-red ppt.	+
8.	Starch	H_2O extract	Distilled water	No bluish-black ppt.	-
9.	Saponins	H_2O extract	Acetic anhydride and conc.	Frothing	+
10.	Terpenoids	$CHCl_3$ extract	H_2SO_4, iodine vapour	No greenish blue colour, no reddish brown spot	-
11.	Reducing sugar	H_2SO_4 extract	NaOH solution and Benedict's solution	Brick-red ppt.	+

(+) = Present, (-) = Absent

Some Analysis of Soybean Powder

Dried powdered sample was submitted to analysis for the determination of moisture content, fat content, ash content, acid insoluble ash content, water soluble ash content and soluble matter contents in different solvents (ethanol, methanol, ethyl acetate, dichloroethane and pet-ether).

Determination of Moisture Content

Moisture content was determined by the Dean and Stark method and oven drying method.

Dean and Stark distillation method involves the reflux distillation of the sample with an immiscible solvent having a higher boiling point and lower specific gravity than water, e.g., toluene, heptanes, xylene. This method has the advantages that (a) it needs little attention once the apparatus has been set up and (b) any volatile oils which distilled over mixed with the solvent are not measured.

Dean and Stark distillation method directly measures the water content. The moisture content of the sample was determined to be 9.3 % as shown in Table (2).

Determination of Fat Content

Fat was determined by Soxhlet Extraction Method.

Fat was extracted with petroleum ether by heating on water bath using Soxhlet Extractor. And then petroleum ether was removed by vaccum distillation method. The difference of round-bottomed flask before and after the distillation gave the weight of fat content in the sample of soybean powder.

The average fat content of the sample was found to be 15.5 % as shown in Table (2).

Determination of Total Ash Content

Ash is inorganic residue remained after the organic matter has been burnt away. Ash was determined according to Jacobs. The sample was incinerated in a porcelain crucibile until all the carbonaceous material had been removed. Burning of the dried powdered sample was avoided by beginning the combustion at a low temperature. The combustion was completed at a temperature of approximately 550 ºC. the period of ashing is usually not specified, the ashing being continued until a uniformly light gray or white ash of constant weight is obtained. (Jacobs, 1958)

The average ash content of dried powder of soybean was 4.36 % as shown in Table (2).

Determination of Acid Insoluble Ash Content

The total ash is the residue remaining after incinerations. The determination of acid insoluble ash consists of boiling the total ash with dilute hydrochloric acid, filtering, igniting and weighing the acid insoluble ash. The result was shown in Table (2) and the average acid insoluble was calculated to be 0.46 %.

Determination of Water Soluble Ash Content

The determination of water soluble ash consists of boiling the total ash with distilled water, filtering, igniting and weighing. When the weight of the insoluble matter was substracted from the weight of the ash, the difference in weight represented the water soluble ash. The result was shown in Table (2) and the average ash content was found to be 2.92 %.

Table (2) Results of some Analysis of Soybean Seeds Powder

No.	Experiments	Results (%)
1.	Determination of moisture content	9.3
2.	Determination of fat content	15.5
3.	Determination of total ash content	4.36
4.	Determination of acid insoluble ash content	0.46
5.	Determination of water soluble ash content	2.92

Determination of Soluble Matters in Different Solvents

Ethanol, methanol, petroleum ether, dichloroethane, ethyl acetate soluble matter contents in dried powdered sample was determined by the method given in "The British Pharmacopoeia".

Ethanol, methanol, PE, dichloroethane, ethyl acetate soluble matter contents of the sample were listed in Table (3).

Table (3) Results of Determination of Soluble Matter Contents of Soybean

Sr. no.	Solvent	Soluble Matter content (%)
1	Ethanol	12.67
2	Methanol	5.83
3	Prtroleum ether	15.22
4	Dichloroethane	12.97
5	Ethyl acetate	11.8

CONCLUSION

In the preliminary phytochemical examination of soybean, it can be concluded as follows. There were eleven chemical tests carried out to investigate phytochemicals in soybean. Among

them, nine phytochemicals such as glycosides, steroids, -amino acids, flavonoids, tannins, carbohydrates, alkaloids, saponins, and reducing sugar were presented. And, starch and terpenoids were not present or absent. In chemical analysis of soybean powder, it was found that moisture content (9.3 %), fat content (15.5 %), total ash content (4.36 %), acid insoluble ash content (0.46 %), water soluble ash content (2.92 %) and soluble matter contents in different solvents (ethanol, methanol, ethyl acetate, dichloroethane and pet-ether) were 12.67 %, 5.83 %, 15.22 %, 12.97 % and 11.8 % respectively.

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