Investigation of the Amino Acids Content Extracted from Sesame Seed Meal Cake, Defatted Powder (*Sesamum indicum*)

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Abstract

The meal cake, which is rich in protein may be used as food for humans and animals. The protein fraction of the meal cake is considered to be the most important one because of its nutritional value. In this paper, the chemical composition of meal cake extracted from sesame seeds collected from Kyauk–Pan–Taung Township, Mandalay Region was investigated. When oil from the sesame seeds was removed by solvent extraction with petroleum ether, meal cake for analysis was obtained. Micro Kjeldahl's method was used for the determination of protein content. Nineteen amino acids were used, but seventeen amino acids were isolated in meal cake, namely alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, phenylalanine, proline, serine, threonine, tyrosine, leucine, isoleucine, lysine, methionine and valine. The contents of eight selected amino acids, namely alanine, arginine, glycine, histidine, phenylalanine, serine, threonine and lysine were evaluated from the respective calibration curve by two dimensional paper chromatography.

Key words : Sesame seed, Meal cake, Petroleum ether, Micro Kjeldahl's method, Amino acids

Introduction

The human body needs a variety of nutrients. The types of nutrients in foods are proteins, carbohydrates, fats, vitamins, minerals and water. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago (Levit, B. 1951). Sesame seeds are a good source of healthy fats, protein, B vitamins, minerals, fibre, antioxidants, and other beneficial plant compounds. Regularly eating substantial portions of these seeds may aid blood sugar control, combat arthritis pain, diabetes, lower cholesterol and against heart disease. Sesamin, a compound in sesame seeds, may help reduce joint pain and support mobility in arthritis of the knee. Sesame seeds may aid blood sugar control because they are low in carbs and high in quality protein and healthy fats (M. Lanson, 1982).

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Aim and Objectives

Aim

The aim of this research is to investigate the amino acids content in the sesame seed meal cake after defatting using petroleum ether.

Objectives

- To prepare the sesame seed meal cake from sesame seed
- To determine the chemical composition of sesame seed meal cake
- To separate and identify the amino acid from meal cake

Botanical Description

Family – Pedaliaceae

Scientific name – *Sesamum indicum*

English name – Sesame

Mvanmar name – hnan

eeds of Sesame

Materials and Methods

The sesame seeds used for the extraction of oil were collected from Kyauk–Pan–Taung Township, Mandalay Region. The extraction was done by solvent extraction using petroleum ether (b–pt, 60–80°C) after removing the husk.

Removal of Oil by Solvent Extraction

Sample (10 g) was accurately weighed and introduced into a weighed thimble. The open end of the thimble was closed with a cotton wool and placed in a Soxhlet extractor. The Soxhlet extractor was connected with the round–bottomed flask. Petroleum–ether (bpt.60°–80°C) was poured into the extractor until some of it overflowed into the flask. The other end of the extractor was connected with water condenser. The flask was heated on a water–bath for 8 hours. After the extraction, the petroleum ether was removed by distillation until the volume of the pet–ether was remained to about 10 cm^3. The last trace of the solvent was then removed by placing the content in an oven at about 100°C until the constant weight was obtained. It was then stored in an air–tight desiccator for analysis throughout the work. The oil content of sample was calculated by the following equation.

$$\text{Oil (\%)} = \frac{\text{weight of oil (g)} \times 100}{\text{weight of sample (g)}}$$

Determination of Protein Content

Defatted powder (0.5 g) was accurately weighed and introduced in the dry Kjeldahl's digestion flask. Potassium sulphate (0.2 g) and copper II sulphate (0.05 g) were added to the flask. Then concentrated sulphuric acid (10 cm^3) was poured carefully into the flask and the contents were shaken until well mixed. The flask was placed on the digestion rack in an inclined position heated gently. Digestion was continued until the mixture became clear and almost pale green color. Then, the flask was cooled at room temperature. This solution was used as digested solution.

The digested solutions were carefully poured into the distillation apparatus which had previously been cleaned by passing steam through the apparatus for about 30 minutes. (50 cm^3) of 0.05 M sulphuric acid solution a drop of methyl orange indicator were introduced in a conical flask (receiver). This receiving conical flask was placed in a position so that the tip of the condenser lies just below the surface acid solution. The digested solution was poured into the distillation flask and then (100 cm^3) of 40 % NaOH solution was added to make the mixture strongly alkaline and heated. The distillation was assumed to be completed within 15 minutes after boiling the solution. The excess amount of acid remained, unreacted with ammonia was titrated with 0.1 M sodium hydroxide solution.

The percentage of protein content can be calculated by using the following calculation.

$$\text{Protein (\%)} = \frac{0.014 \times 100 \times (V_1 - V_2)M_B \times 6.25}{W}$$

Where,

V_1 = volume (cm^3) of NaOH solution used in blank

V_2 = volume (cm^3) of NaOH solution used in test

M_B = Molarity of NaOH solution

W = weight (gm) of the sample

Amino Acids

Hydrolysis of the Meal Cake Protein

The meal cake protein was directly hydrolysed into amino acids. The hydrolysis may be carried out by strong inorganic acids, strong bases or by enzymes. The acid hydrolysis (6NHCl) is the most convenient of the three methods.

Separation and Identification of Amino Acids by One Dimensional Paper Chromatography

Reagents

(1) Standard amino acid solutions

Standard solutions of nineteen different amino acids were prepared in 75 % ethanol (v/v).

(2) Solvent system 1 : n–Butanol : Acetic acid : Water (BuA) (120 : 30 : 50 v/v)

(3) Locating reagent (Ninhydrin reagents, 0.4 % in acetone, w/v)

Ninhydrin powder (0.4 g) was dissolved in acetone and made up to 100 mL with acetone.

Procedure

For one dimensional paper chromatography (descending technique), Whatman No. 1 (47 cm × 57 cm) chromatographic papers were chosen. The nineteen amino acids solution, the standard

amino acid mixture and the sample were applied individually on the different original points by means of capillary tube. The paper was then fold, back about 6 cm from the origin situated end of the paper for descending chromatography. The paper to be chromatographed was placed in the tank (Panglas Shandon 500 chromatographic tank) with one end of the paper being dipped well into the solvent system 1. (n–butanol, acetic acid, water), but taking care so that the surface of the solvent was well below the points of origin. The chromatogram was developed for 20 hours at room temperature. The paper was then removed from the tank and dried in the air for half an hour. The chromatogram was then sprayed with 0.4 % ninhydrin reagent and placed in an oven at 105°C for a few minutes, where purple spots were revealed. These chromatograms were recorded as photographs shown in Figure (2). R_f values of amino acid spots revealed were determined.

$$R_f = \frac{\text{Distance traveled by solvent}}{}$$

Separation and Identification of Amino Acids by Two Dimensional Paper Chromatography

Procedure

Twenty sheets of Whatman No. 1 chromatographic paper (47 cm × 47 cm) were taken and developed in the first dimension by the descending technique using as solvent system 1. About 20 hours, the paper chromatograms were taken out to dry. The dried papers were then turned at right angle and developed in the second dimension by the descending technique for 18 hours using phenol : water (160 : 40 v/v) solvent system 2 as solvent. The chromatograms were taken out and left over night in the air to dry. The amino acid spots were then located with the help of ninhydrin spray reagent (0.4 % ninhydrin in acetone w/v) and their R_f values were calculated. The two–dimensional paper chromatography was repeated with a standard amino acid mixture (10 μL) and the hydrolysate of meal cake samples (10 μL). By comparison of the standard mixture and protein hydrolysate chromatograms (Figure 4 and Figure 5) and with the help of the R_f values of standard amino acids, the presence of the amino acids in the hydrolysate sample were identified.

Separation and Identification of Amino Acids by One Dimensional Thin Layer Chromatography

Procedure

The tank used for TLC was first cleaned and dried. TLC sheet (20 cm × 20 cm) was applied with standard amino acids and sample hydrolysate for one dimension. To be spotted on the sheet, eighteen points were marked on the base line with a pencil. The techniques for TLC were same as those for PC, but the size of spots should not exceed 3 mm in diameter.

Quantitative Determination of Amino Acids

The amino acids (Arginine, Histidine, Alanine, Glycine, Lysine, Serine, Phenylalanine and Threonine) present in the hydrolysate were determined quantitatively by separating them on a paper chromatogram. The amino acids were eluted from the paper strips with spectroscopic methanol and quantitative determination carried out by measuring the colour intensities of their solutions.

Procedure

The aliquots of standard alanine solutions of different concentrations (1 cm^3 each) were taken in the 10 cm^3 graduated boiling tubes. An empty 10 cm^3 graduated boiling tube was also taken for blank. Ninhydrin reagent (4.0 cm^3), EDTA reagent (0.2 cm^3) and deionized water (0.1 cm^3) were added into the above tubes. With constant shaking, the tubes were heated in a water bath at 373 K for 20 minutes. The purple colour was gradually produced. The tubes were cooled to room temperature and the volume was made up to 10 cm^3 with deionized water. The absorbances of alanine solutions were determined with spectronic–21 spectrophotometer at wave length 570 nm. Then the concentration absorbancy curve of standard alanine was drawn in Figure (6). Standard calibration curves for arginine, histidine, glycine, lysine, serine, phenylalanine and threonine were also constructed in the same way as described for the concentration–absorbancy curve of standard alanine.

Results and Discussion

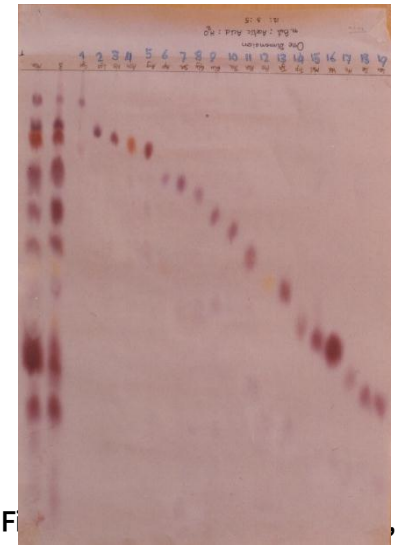
In this result, the yield percent of extracted oil and meal cake were 36.2 % and 63.7 % respectively. Therefore, the sesame seed oil can be applied for edible, medicine, cosmetic and antioxidant and the seed cake provides as a protein concentrate for humans. The protein content of meal cake was determined by Micro–Kjeldahl method. The yield of protein content was 27.8 %.

To determine the amino acids in the meal cake of sesame seed was an emphasis of this research.

In the paper chromatography, one and two dimensional techniques were used and the developing method was descending. In the thin–layer chromatography, one– dimensional method was applied and the developing technique was ascending.

By comparison of R_f values and colour intensities of the spots of standard amino acids with the sample spots, the amino acids obtained from the hydrolysate were identified. It was observed that seventeen amino acids, except asparagine and tryptophan, were present in the sample hydrolysate according to the chromatograms of paper and thin–layer.

One dimensional
paper chromatogram



F , Standard Amino Acid Mixture and Hydrolysate of Sesame Meal Cake

One dimensional
thin–layer aluminium chromatogram

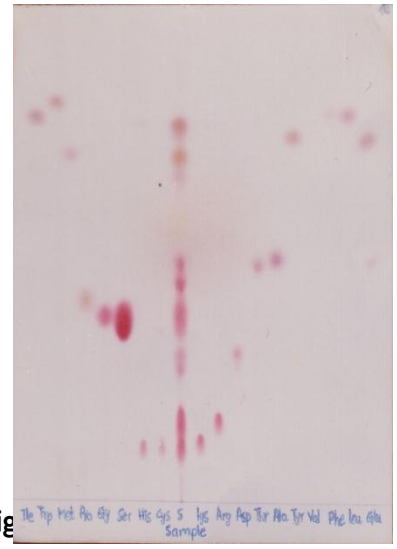


Figure 3. Eig and Meal Cake Hydrolysate of Sesame Seed

The presence of these amino acids was further determined by carrying out the two–dimensional paper chromatography and one–dimensional thin–layer chromatography.

Two–dimensional paper chromatogram

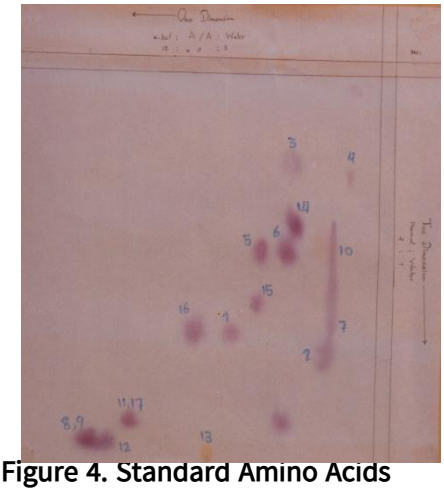


Figure 4. Standard Amino Acids Mixture (10 µL)

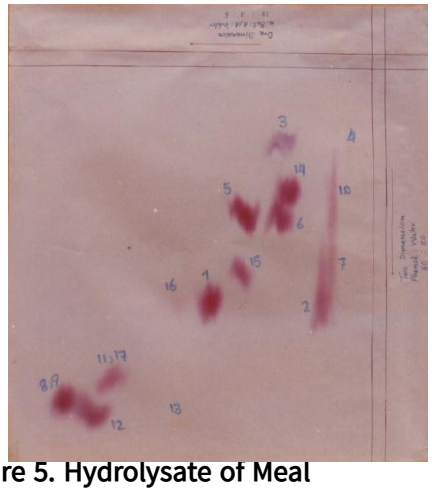


Figure 5. Hydrolysate of Meal Cake of Sesame Seed (10 µL)

Solvent systems : (1) n–Butanol : Acetic Acid : Water (120 : 30 : 50 v/v)
(2) Phenol : Water (160 : 40 v/v)

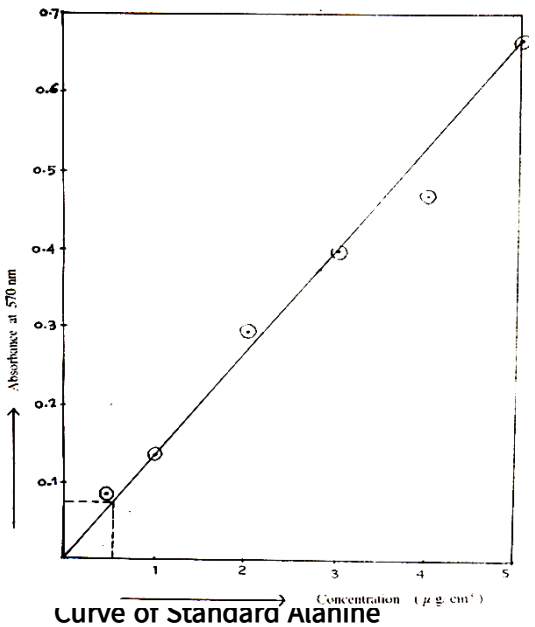
1. Alanine
7. Histidine
13. Proline

2. Arginine
3. Aspartic acid
4. Cystine
5. Glutamic acid
6. Glycine
8. Isoleucine
9. Leucine
10. Lysine
11. Methionine
12. Phenylalanine
14. Serine
15. Threonine
16. Tyrosine
17. Valine

The better separation of amino acids in the protein hydrolysate was obtained in two–dimensional paper chromatogram (Figure 5) so that the quantitative determination of amino acids in the sample hydrolysate was carried out by using elution method. The contents of amino acids can be evaluated from the respective calibration curve. In the research, eight amino acids, namely alanine, arginine, histidine, glycine, lysine, serine, phenylalanine and threonine were determined by two–dimensional paper chromatography.

Table (1) Percentage of Respective Amino Acids Present in the Sample Hydrolysate by Two Dimensional Paper Chromatography

No.	Amino Acids	Percent (%)
1.	Alanine	3.30
2.	Arginine	2.40
3.	Histidine	1.74
4.	Glycine	3.00
5.	Lysine	2.28
6.	Serine	3.24
7.	Phenylalanine	2.76
8.	Threonine	1.68



Figur Curve of Standard Alanine

Conclusion

In the present investigation, sesame seeds were collected from Kyauk–Pan–Taung Township, Mandalay Region. Studies were made on the meal cake obtained from the sesame seeds after defatting using petroleum ether (bpt. 60°C–80°C). The yield percent of extracted oil and meal cake were 36.2 % and 63.7 % respectively. The protein content of meal cake was determined by Micro–Kjeldahl method. To determine the amino acids, the sesame seed meal cake was hydrolyzed by 6 N HCl. The hydrolysate obtained was analysed by paper chromatography and thin–layer chromatography, using with different solvent systems. From protein hydrolysate, seventeen amino acids, namely alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, phenylalanine, proline, serine, threonine, tyrosine, leucine, isoleucine, lysine, methionine

and valine were isolated. The contents of eight selected amino acids, namely alanine (3.30 %), arginine (2.40 %), glycine (3.0 %), histidine (1.74 %), phenylalanine (2.76 %), serine (3.24 %), threonine (1.68 %) and lysine (2.28 %) were determined. These results indicated that sesame seed meal cake is high in protein content and healthy oil. So, sesame seeds have many potential health benefits and are used for folk medicine. The seed cake provides as a protein concentrate for humans.

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Online Materials

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