American Journal of Food Science and Technology, 2017, Vol. 5, No. 6, 265-269
Available online at http://pubs.sciepub.com/ajfst/5/6/7

DOI:10.12691/ajfst-5-6-7

Physico-chemical Properties of Extracted Mung Bean Protein Concentrate

Zar Zar Oo[1,*], Thwe Linn Ko[2], Soe Soe Than[3]

[1]Industrial Chemistry Department, Yadanabon University, Mandalay, Myanmar
[2]Industrial Chemistry Department, University of Mandalay, Mandalay, Myanmar
[3]Industrial Chemistry Department, University of Yangon, Yangon, Myanmar
*Corresponding author: mazarzaroo99@gmail.com

Abstract Many protein concentrates have been developed for providing different functional or physical properties to meet the requirement of various food systems. The main purpose of this research work was to evaluate the most refined form of protein from mung bean and to combat the problem of malnutrition. In this research work, mung bean (*Vigna radiata* L.) was collected from Monywa Township, Sagaing Region and nutritional values of mung bean flour like moisture content, ash content, protein content, crude fiber content, fat content and carbohydrate content were determined. The fat from mung bean flour was removed by soaking in ethanol and also by soxhlet extraction using ethanol as solvent before isolating the protein. The fat removal efficiency of these two methods was investigated. Moreover, combined effect of these two methods on the removal percentage of fat from mung bean was studied. 51.37±0.03% protein content (defatted mung bean) was obtained by soaking in ethanol solution for 16 hr and followed by soxhlet extraction (meal to solvent ratio were1:5). The morphological nature and elemental compositions of the mung bean protein concentrate were characterized by Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Fluorescence (ED-XRF) spectrophotometer respectively.

Keywords: *mung bean flour, soxhlet extraction, protein concentrate, functional properties*

Cite This Article: Zar Zar Oo, Thwe Linn Ko, and Soe Soe Than, "Physico-chemical Properties of Extracted Mung Bean Protein Concentrate." *American Journal of Food Science and Technology*, vol. 5, no. 6 (2017): 265-269. doi: .12691/ajfst-5-6-7.

1. Introduction

World demand for plant protein is rising [1] because animal proteins are more expensive and rare [2]. Legumes are utilized throughout the world [3] because they are a rich source of protein [4] and also amino acids [5]. They are the second largest source of human food [6] and play significant role in diminishing protein- energy nutrition [7]. Human beings should depend on the legume proteins to meet the protein requirement in their diet [8]. Beans are one of the most consumed legume worldwide. Beans include 17.96-23.62 % proteins, 1.27-3.62 % fat 2.86-5.00 % ash and 56.53-61.56 % carbohydrate [9] .They have a balanced amino acid composition while they are low in sulfur-containing amino acids (methionine and tryptophan) [10].

Mung bean (*Vigna radiate* L.*)* [11-16] is important pulse crop belonging to the family Fabaceae [5]. It is also called green gram [2,12], a tropical grain legume [2], widely cultivated in Asia. It is a rich source of protein and amino acids especially lysine and thus can supplement cereal based human diet [2].

There are three principal methods to concentrate protein depend on heat, acid or alcohol treatment [18]. They were applied beef sausages and beef burger, especially under a local conditions of meat shortage and high price [17]. The objectives of the present study were to remove the fat from mung bean flour and to determine the protein content of defatted mung bean flour for enhancement of protein isolation.

Figure 1. Mung Bean

2. Materials and Methods

2.1. Raw Materials

Mung bean was collected from Monywa Township, Saging Region. Ethanol was purchased from (BDH Chemicals Ltd), Able Chemical Store, Mandalay Region.

2.2. Preparation of Mung Bean Flour

Mung bean seeds 300 g were washed with distilled water to remove foreign materials and then the seeds were

soaked in 1000 mL of distilled water using automatic water distiller (LWD-3004, DAIHAN LABTECH Co., LTD, KOREA for 12 hr and dehulled. After that, the seeds were crushed to smaller fragments with a blender and then dried in an oven (J.P.SELECTA,s.a, SPAIN) at 60°C for 12 hours. And then, they were powered and sieved with 80 mesh screen using vibratory sieve shaker (J-VSS, NANOVA Ltd, KOREA) and then stored in an air tight container.

2.3. Defatting the Mung Bean Flour

2.3.1. Soaking in the Solvent Ethanol

Mung bean flour (80 mesh) 100 g was soaked in 600 mL of 95 % ethanol for (4 hr, 8 hr, 12 hr, 16 hr and 20 hr) respectively. After soaking, the solvent was decanted and defatted mung bean was dried in an oven at 60°C for 12 hours. After that, it was ground in the grinder and sieved with 200 mesh screen. Then, defatted mung bean flour powder was packed with air- tight plastic bags.

2.3.2. Soxhlet Extraction Method

Mung bean flour (80 mesh) 100 g was placed inside a thimble and loaded into the main chamber of the soxhlet extractor. 600 mL of 95 % ethanol was placed in a round bottom flask and extraction was started at different temperatures 50°C, 55°C, 60°C, 65°C and 70°C respectively. The temperature provided the highest fat removal percentage was decided for that bean flour to solvent ratio 1:6. The extraction was again conducted for following bean flour to solvent ratios: 1:3, 1:4, 1:5, 1:6, and 1:7 at extraction temperature 60°C. The defatted mung bean flour powder were then prepared as described above.

2.3.3. Preparation of Mung bean Protein Concentrate

Mung bean flour 100 g was soaked in 600 mL of 95 % ethanol for 16 hours and followed by soxhlet extraction (meal to solvent ratio were 1:5) at extraction temperature 60°C. In order to remove all ethanol, defatted mung bean flour was dried in an oven at 60°C for 12 hours. After that, it was ground in the grinder and sieved with 200 mesh screen. Then, mung bean protein concentrate powder was packed with air- tight plastic bags.

2.4. Methods of Analysis

Physico-chemical properties of mung bean flour and defatted flour such as protein content, moisture, ash, fiber, carbohydrate, fat content (AOAC-Method, 2000) [19] and also fat removal percentage were determined. The ED-XRF, Energy Dispersive X-ray Fluorescence Spectrometer (SPETRO XEPOS, Benchtop XRF Spectrometer) was used for the determination of elemental composition of mung bean protein concentrate and SEM, Scanning Electron Microscope (JSM 5610, JEOL Co. Ltd, Japan) was measured the surface of the mung bean flour and protein concentrate.

2.4.1. Determination of Protein Content

(2) g of sample was transferred to a digestion flask followed by the addition of 3 g of catalyst mixture (K_2SO4:CuSO4:SeO_2 in 100:20:2.5) and 20 mL of concentrated sulphuric acid. The content was then digested till transparent liquid was obtained. The volume of digested material was made up to 100 mL with distilled water. Carry out a blank digestion without the sample and make the digest to 100 mL. Measured aliquot of digested material was distilled with excess of 40% NaOH solution and the liberated ammonia was collected in 20 mL of 2% boric acid solution containing 2-3 drops of mixed indicator (10 mL of 0.1 percent bromo cresol green + 2 mL of 0.1 percent methyl red indicator in 95 percent alcohol). The entrapped ammonia was titrated against 0.01 N hydrochloric acid. A reagent blank was similarly digested and distilled. Nitrogen content in the sample was calculated as follows and a factor of 6.25 was used to convert nitrogen to protein [19].

$$\%N_2 = \frac{\text{Sample titre} - \text{Blank titre} \times \text{Normality of HCl} \times 14}{\text{Aliquot of the digest taken} \times \text{Weight of sample} \times 1000} \times \frac{\text{vol.made of digest} \times 100}{}$$

$$\text{Protein content} = \%\ \text{Nitrogen} \times 6.25.$$

2.4.2. Determination of Moisture Content

3 g of sample was weighed in a petri dish and dried for 4 hours at 110°C in hot air oven and it was cooled in a desiccators and weighed. The process of heating, cooling and weighing was repeated. Moisture content was calculated as follows: [19]

$$\text{Moisture}(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

where, W_1= weight (g) of sample before drying, W_2= weight (g) of sample after drying

2.4.3. Determination of Ash Content

Accurately weighed 1g of sample was introduced into the porcelain crucible. The crucible and sample were carefully ignited over hot plate and heated until the sample was thoroughly charred. Then, it was placed in the muffle furnace at 550°C for 5 hours until residue was free from carbon. The crucible and ash were then cooled in the desiccator and weighed. The weighing, heating in the furnace and cooling were repeated until the constant weight was obtained. The ash content of sample was calculated as follow: [19]

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100.$$

2.4.4. Determination of Crude Fiber Content

The sample was weighed into 500 mL beaker and 200 mL of boiling 0.255 N sulphuric acid (1.25 percent w/v) was added. The mixture was boiled for 30 min keeping the volume constant by the addition of hot water at frequent intervals (a glass rod stirred in the beaker helps smooth boiling). At the end of this period, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200 mL of boiling 0.313 N (1.25 percent w/v) NaOH was added. After boiling for 30 min.,

the mixture was filtered to a crucible, dried overnight at 80-100°C and weighed. The crucible was kept at in a muffle furnace at 550°C for 3 hours. Then it was cooled in desiccators and weighed again. The difference in residue weights and ash represents the weight of crude fiber [19].

2.4.5. Determination of Fat Content

Accurately weighed (5) g of sample was introduced inside the thimble and a piece of cotton was placed at the open and of the thimble. The thimble containing the sample was kept inside soxhlet apparatus fixed with round bottom flask (500) mL containing petroleum ether (B.P 40-60°C) 250 mL. The extraction flask was heated on the heating mantle for 14 hours at the boiling point of petroleum ether. After the extraction was completed, the ether dissolving oil was transferred into the beaker. Then, the ether was removed by evaporation. Fat content was calculated as follows: [19]

$$\text{Fat}(\%) = \frac{\text{Fat weight}}{\text{Sample weight}} \times 100.$$

2.4.6. Determination of Carbohydrate Content

Carbohydrate value of the sample was determined by using the following formula:

$$\text{Carbohydrate } (\%) = 100 - (\text{protein} + \text{fat} + \text{fiber} + \text{ash} \pm \text{moisture}).$$

2.4.7. Determination of Fat Removal Percentage

The fat removal percentage of mung bean protein concentrate was determined.

$$\text{Fat Removal Percentage} = \frac{A - B}{A} \times 100$$

where, A= initial Fat content, B= final fat content.

2.4.8. Statistical Analysis

Statistical analysis was carried out using a one way analysis of variance (ANOVA) and the significant difference between the samples was determined using LSD test at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Composition

Proximate composition of mung bean flour was determined and presented in Table 1. The protein content, 22±0.03% of local mung bean flour was lower than that of the 22.5±0.24 % [5] due to species of mung bean, cultivation area and soil condition. Fat content of local mung bean flour, 1.08 ± 0.01 was lower than that of the 1.35 ±0.048 [5]. The moisture content of local mung bean flour was 12.72±0.04%. Excess of moisture content in mung bean flour can provide greater danger of bacteria action and mold growth which produce undesirable changes. Furthermore, the ash content of mung bean flour was 3.20±0.02% and it is an approximate measure of mineral and inorganic matter. However, the crude fiber of local mung bean flour, 0.74±0.01 % was significantly different from the 2.9±0.061% [5]. The high fiber content in [5] may be due to bean's hulls. Thus, dehulling can reduce the fiber. The proximate composition of mung bean flour can be varied depending on the weather and soil conditions, cultivation area, and species of mung bean, harvesting time and storage condition. The remaining lipids, mainly non polar compounds may still interact with proteins. High fat content may interfere protein isolation and protein may be denatured. So, fat should firstly be removed to isolate the protein.

Table 1. Proximate Composition of Mung Bean Flour

Composition (Dry Basis) (%w/w)	Mung Bean Flour (%w/w)
Protein content	22±0.03
Moisture content	12.72±0.04
Ash content	3.20±0.02
Fiber content	0.74±0.01
Carbohydrate content	59.77±0.02
Fat content	1.08±0.01

3.2. Effect of Soaking Time on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

Figure 2 shows the effect of soaking time on the percentage of fat removal and protein content from mung bean flour. The protein content slightly increased from 22.41±0.02 % to 23.22±0.02 % and fat removal percentage of mung bean flour increased from 16.67 ±0.05% to 26.85 ±0.03 % by soaking the mung bean flour in 95 % ethanol for 16 hr. There was no sharp change in the percentage of protein content and fat removal between 16 hr and 20 hr soaking time. So, the most suitable soaking time was found to be 16 hr.

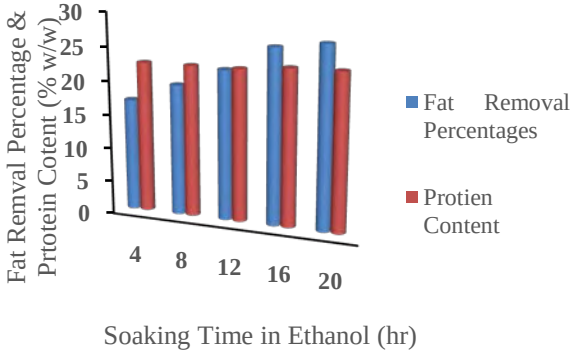

Figure 2. Effect of Soaking Time on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

3.3. Effect of Extraction Temperature on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

Figure 3 shows the effect of extraction temperature on the fat removal percentage, protein content of defatted mung bean flour by soxhlet extraction. It can be seen from the figure 3 that, steadily increase in protein content from 31.43±0.02% to 33.79 ± 0.02 % whereas fat removal percentages increased from 32.07 ±0.03 % to 39.81 ±0.01 %

with increase in extraction temperature at extraction time of 6 hr. Increasing temperature from 60°C to 70°C did not bring about the increase on fat removal and protein content. Moreover, high temperature may cause protein denaturing. Thus, 60°C was found to be most suitable temperature for extraction of fat from mung bean flour.

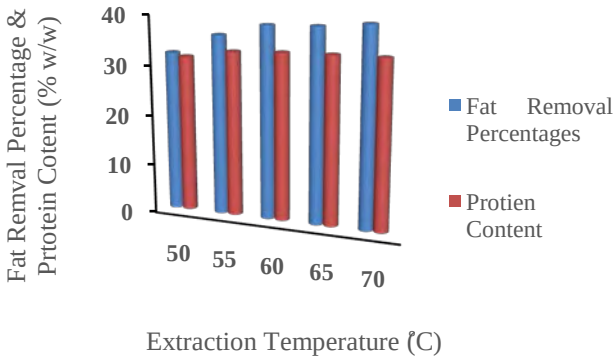

Figure 3. Effect of Extraction Temperature on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

3.4. Effect of Material to Solvent Ratio on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

Furthermore, to decide the most suitable bean to solvent ratio on the fat removal content and protein content of mung bean flour by soxhlet extraction, the bean to solvent ratio were varied from 1:3 to 1:7 at extraction temperature of 60°C. The resultant data is shown in Table 2. The protein content gradually increased by changing the material to solvent ratio and the highest protein content, 33.47±0.04% with the highest fat removal, 39.81 ±0.02% was observed for 1:6 of bean to solvent ratio.

Table 2. Effect of Material to Solvent Ratio on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

Material to Solvent Ratio	Fat Removal Percentage (% w/w)	Protein Content (%w/w)
1:3	30.55±0.03	32.45±0.02
1:4	33.33±0.01	32.86±0.03
1:5	36.11±0.04	33.15±0.05
1:6	39.81±0.02	33.47±0.04
1:7	40.74±0.01	33.50±0.01

3.5. Effect of Ratio of Ethanol Soaked Bean Flour to Solvent on the Percentage of Fat Removal and Protein Content from Mung Bean Flour

Table 3 describes the effect of ratio of ethanol soaked bean flour (partially defatted mung bean flour) to solvent on the percentage of fat removal and protein content from mung bean flour**.** It has been observed that combined effect of bulk soaking and soxhlet extraction influenced on the maximum removal of fat content as well as the higher yield of protein concentrate. The most suitable meal to solvent ratio was 1:5 at the extraction temperature 60°C. By combining the two processes, the highest fat removal of 57.14 ±0.02% was achieved with relatively high protein content of 51.37±0.03 %.

Table 3. Effect of Ratio of Ethanol Soaked bean flour to Solvent on the Percentage of Fat Removal and Protein Content from Mung Bean flour

Material to Solvent Ratio	Fat Removal Percentage (% w/w)	Protein Content (%w/w)
1:3	45.71±0.06	49.75±0.02
1:4	52.86±0.03	50.88±0.04
1:5	57.14±0.02	51.37±0.03
1:6	58.57±0.02	51.46±0.02
1:7	58.71±0.03	51.59±0.01

3.6. Characteristics of Mung Bean Protein Concentrate

Characteristics of mung bean protein concentrate were determined and the data was presented in Table 4. Mung bean protein concentrate was characterized by a protein content 51.37±0.02 % and low content in fiber, respectively 0.23±0.04 % and in ash, represented by 1.93±0.03%. By refinement, the carbohydrate level was substantially diminished to 37.63 ±0.01% level which is characteristic of the protein concentrate.

Table 4. Characteristics of Mung Bean Protein Concentrate

Characteristics (%w/w)	Mung Bean Protein Concentrate (%w/w)
Protein content	51..37 ±0.02
Moisture content	8.54 ±0.01
Ash content	1.93±0.03
Fiber content	0.23±0.04
Carbohydrate content	37.63±0.01
Fat content	0.3±0.02

3.7. Elemental Compositions of Mung Bean Protein Concentrate

The elemental compositions of mung bean protein concentrate were analyzed by ED-XRF. The data was presented in Table 5. It shows potassium, sulfur, iron, Zinc and copper. These minerals can effectively contribute towards the daily recommended allowances [20] for all groups. It was observed that mung bean protein concentrate is used for protein source but it can fulfill the micro nutrients deficiency as well.

Table 5. Elemental Composition of Mung Bean Protein Concentrate Analyzed by ED-XRF Method

Elements	Compositions (%)
Potassium (K)	0.304±0.02
Sulfur (S)	0.058±0.03
Iron (Fe)	0.003±0.04
Zinc (Zn)	0.001± 0.03
Copper (Cu)	0.001±0.01

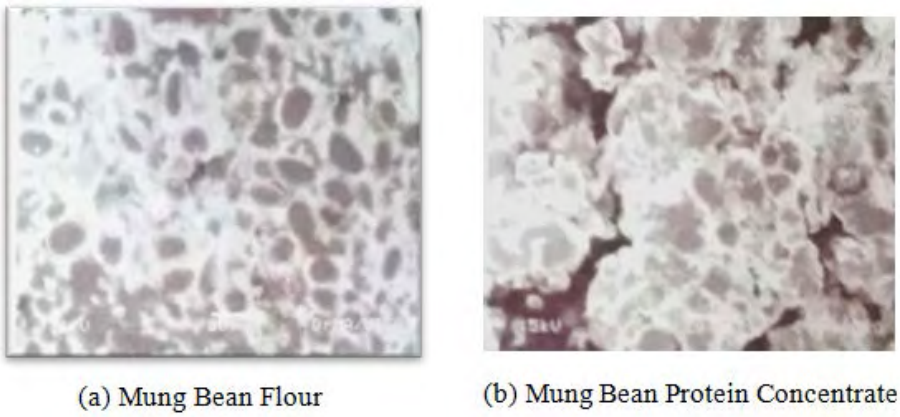

Figure 4. Morphological Nature of Mung Bean Flour and Mung Bean Protein Concentrate

3.8. Surface Morphologies of Mung Bean Flour and Mung Bean Protein Concentrate

Surface morphologies of mung bean flour and mung bean protein concentrate were illustrated in Figure 4. It as examined by scanning electron microscopy (SEM) using magnification of 550. The microstructure of mung bean flour had typical kidney and ellipse shape and the surface of the mung bean flour appeared smooth, without pores and fissures. The SEM image of the ethanol leached mung bean protein concentrate showed the agglomeration into cluster or mass due to characteristic of less fat content.

4. Conclusions

Combination of bulk soaking and soxhlet extraction accelerated the fat removal from mung bean. Isolation of protein from mung bean was interrelated to fat removal.

Acknowledgements

I wish to acknowledge to my supervisor Dr.Soe Soe Than, Professor, Industrial Chemistry Department, University of Yangon and co-supervisor Dr.Thwe Linn Ko, Professor, Industrial Chemistry Department, University of Mandalay, Myanmar, for their invaluable guidance, and kind advice throughout the research period.

References

[1] Eltayeb, A.R.S.M, Ali. O.A, A.A. Abou-Arab and F.M. Abu-Salem, "Chemical Composition and Functional Properties of Flour and Protein Isolate Extracted from Bambara Groundnut (*Vigna subterranean)", African Journal of Food Science*, 5 (2), 82-90, February, 2011.

[2] Habibullah, M.Abbas and H. U. Shah, "Proximate and Mineral Composition of Mung Bean, *Sarhad Journal of Agriculture*", 23 (2), 1-4, 2007.

[3] Tharanathan, R.N. and S. Mahadevamma, "Grain legumes-a boon to Human Nutrition". *Trends in Food Science and Technology*, 14, 507-518, 2003.

[4] Kenawi, M.A, R.R. Abdelsalam and S.A .EI-Sherif, "The Effect of Mung Bean Powder, and Low Fat Soy Flour as Meat Extender on the Chemical, Physical and Sensory Quality of Buffalo Meat Product", *Biotechnology in Animal Husbandry*,25 (5-6), 327-337, 2009.

[5] Shaheen, S , N. Harun , F. Khan , R.A. Hussian, S.Ramzan, S. Rani, Z. Khalid, M. Ahmad and M. Zafar, "Comparative Nutritional Analysis Between *Vigna radiata* and *Vigna mungo* of Pakistan", *African Journal of Biotechnology*, 11 (25), 6694-6702, March, 2012.

[6] Berrios, J.D.J, "Extrusion cooking of legumes: Dry bean flours". *Encyclopedia of Agricultural, Food and Biological Engineering*, 1-8, 2006.

[7] Kudre, T.G, S. Benjakul and H. Kishimura, "Comparative Study on Chemical Compositions and Properties of Protein Isolates from Mung Bean, Black Bean and Bambara Groundnut", *Journal of Sciences and Food Agriculture*, 93 (10), 2429-2436, Feburary, 2016.

[8] Abbas, S., M.S.B, M.T.S, M.K. S, A.N.A & R.B, "Nutritional and Functional Properties of Protein Isolates Extracted from Defatted Peanut Flour" *International Food Research Journal*, 22 (4), 153-1537, 2015.

[9] Siddiq. M, R. Ravi, J. B. Harte and K.D Dolan, "Physical and Functional Characteristics of Selected Dry Bean (*Phaseolus vulgaris* L.)", *Food Science and Technology*, 232-237, 2010.

[10] Wani, I.A, D.S Sogi, U.S Shivhare and B.S. Grill., "Physico-chemical and Functional Properties of Native and Hydrolyzed Kidney Bean (*Phaseolus vulgaris* L.) Protein Isolates", *Food Science and Technology*, 53 (1), 278-284, 2013.

[11] Amponsah, G, Z .Ma and J.I .Boye, "Crop-legume", *Food Processing: Principles and Applications*, 305-337, 2014.

[12] Okweche, S. Idoko and T. Avav, "Yield Evaluation of Some Cultivators of Mungbean (Vigna radiata L. Wilczke in Southern Giunea Savanna Location of Nigeria", *International Journal of Plant, Animal and Environmental Sciences*, 3 (3), 85-88, May, 2013.

[13] Akaerue, B.I, G.I .Onwuka, "Evaluation of the Yield, Protein Content and Functional Properties of Mung Bean [*Vigna radiate* (L.) Wilc zek] Protein Isolates as Affected by Processing", *Pakistan Journal of Nutrition*, 9 (8), 728-735, August, 2010.

[14] Wanialw, A and N.W.H. Talwana, "Morphological and Agronomic Traits Variations for Mungbean Variety Selection and Improvement in Uganda", *African Crop Science Journal*, 22 (2), 123-136, May, 2014.

[15] Campillo E.D, L.M .Shannon and C.N. Hankins, "Molecular Properties of the Enzymic Phytohemagglutinin of Mung Bean", *The Journal of Biological Chemistry*, 256 (14), 7177-7180, February, 1981.

[16] Wijaya, H, F.R .Zakaria, D. Syah and E. Prangdimutri,"Isolation of Mung Bean(Vigna radiate (L.)R.Wilczek Proteins To Create A Skin Prick Test Reagent To the Diagnosis of Mung Bean Allergy", *IOSR Journal of Pharmacy*,5 (1), January, 52-58.

[17] Bahlol, H.E.I.M. and I.M.A.E, "Beef Sausages and Beef Burger Production by Adding Treated Mung Bean", Moshtohor, Faculty of Agriculture, Moshtohor, Zagazing University, 1-22.

[18] Hu lu li, "*Chickpea Protein for Food Applications*", China, 1996, 1-3339.

[19] AOAC, "*Official Methods of Analysis of AOAC International*", 17th ed, Washington, DC: , 2000, 5-15.

[20] Recommended Daily Dietary Allowances, "Food and nutrition board", *National Academy of Sciences National Research Council*, USA, 1980.