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“EVALUATION OF THE FUNCTIONAL PROPERTIES OF MUNG BEAN PROTEIN ISOLATE FOR DEVELOPMENT OF TEXTURED VEGETABLE PROTEIN”

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Abstract

Mung bean is considered a 'green pearl' for its relatively high protein content; however, it has limited application as a raw material for industrial food products. As the potential use of mung beans relies on its protein behavior, this study characterized the functional properties of mung bean protein isolates and the results were compared with soy protein isolates. The protein isolates were prepared from mung bean and soy bean flours via extraction with 1 N NaOH, precipitated at pH 4, and subsequently freeze-dried. The amino acid profile as well as the hydrophilic and hydrophobic ratio of mung bean protein isolate, had been comparable with soya protein isolate. The water and oil absorption capacities as well as the denaturation temperature of mung bean protein isolate, were found to be similar with those of soy bean protein isolate. However, foaming capacity (89.66%) of mung bean protein isolate was higher than that of soya protein isolate (68.66%). Besides, least gelation concentration (LGC) of mung bean protein isolate (12%) was also close to LGC of soy protein isolate (14%), while the protein solubility was comparable between both the isolated proteins. The physical features of the textured mung bean were close to the commercial textured soy protein, which showed a heterogeneous and porous network like matrix when the mung bean flour was extruded to measure its potentiality to produce textured vegetable protein.

Introduction

Mung bean (*Vigna radiata*), a plant species of the legume family, was originally cultivated in India. Since then, it has been widely produced in Southeast Asia, Africa, South America, and Australia. Mung bean is a store house of nutrients and it is rich in vitamins B and C, manganese, iron, and calcium as well. Mung beans are low in fat content; therefore, they are an attractive food for those who are health conscious. Its calorific value is low, while its protein content is relatively high at around 20-27%, and has excellent digestibility (Fan and Sosulski, 1974). Hence, it can be used in a wide variety of food preparations as one of the richest and cheapest sources of protein (Adsuleet al., 1986). In addition, its essential amino acid composition matches satisfactorily with soy bean and FAO/WHO reference protein (Fan and Sosulski, 1974; Thompson et al., 1976). Furthermore, mung bean is a diabetic friendly food as its glycemic index is low and it is highly effective in inhibiting LDL oxidation. Its protein hydrolysates and bioactive peptides inhibit the angiotensin converting

enzyme (ACE) to modulate blood and heart disease (Aluko, 2008). Additionally, mung bean has been shown to offer some pharmacological benefits, such as antitumor effect, as well as antioxidant and antifungal activities (Wang et al., 2004).

It has been shown that mung bean protein isolate has different functional and physicochemical properties in various solutions and environments. Thompson (1977) conducted a study where mung bean protein isolate was mixed with wheat flour for bread preparation. The results showed that mung bean protein isolate performed as a protein supplement in bread flour mixtures and was comparable with soy protein isolate. Meanwhile, El-Adwy (2000) prepared mung bean protein isolate and modified it via acylation to various degrees by acetic and succinic anhydrides. Acylated mung bean protein isolates displayed good functional properties when compared with untreated protein isolate. On the other hand, Kudre et al. (2013) evaluated and compared the chemical properties of protein isolate from mung bean, black bean, and bambara groundnut. He recommended that mung bean protein isolate can be used as an ingredient where heat treatment is necessary as it has high thermal stability. However, limited studies have looked into the complete physicochemical profile of mung bean protein isolate and evaluated its potential to produce textured vegetable protein (TVP) for application in meat-based products.

Textured vegetable protein (TVP) is a processed vegetable product usually made from high (50%) protein flour or its protein concentrate through an extrusion process at high temperature and pressure to produce a fibrous, insoluble and porous network textured product, which could be used as a meat substitute or incorporated into meat-based products to enhance their textural and sensory qualities. Until now, soybean protein has been used widely to produce TVP. As mung bean is relatively high in protein, inexpensive to cultivate and profusely produced in Asia, it could be a potential plant source to develop into textured vegetable protein as well, which could be a healthy and economical diet for most of people.

Therefore, a complete profiling of functional properties of mung bean protein isolate is of paramount importance to product formulation. Functional properties are those desirable characteristics, which food protein ideally possesses, such as solubility, water and oil holding capacities, emulsifying activity and stability, foaming capacity and stability, gelation and texturizability. The full utilization of protein products depends mainly on these functional characteristics as these functional features influence the final product's characteristics.

Therefore, the present study was aimed to evaluate the functional properties of mung bean protein isolates and comparing with functional properties of soy protein isolates as a potential protein source for development of textured vegetable protein.

Materials and Methods

Raw materials

The mung bean and soy bean flours were purchased from local supermarkets.

Chemicals

Hydrochloric acid, sodium hydroxide, sulfuric acid (97%) and boric acid were purchased from Sigma- Aldrich (Munich, Germany). Phenylisothiocyanate (PITC) and Triethylamine (TEA) were purchased from Schuchardt, Germany. Methanol was obtained from Merck (Darmstadt, Germany) and α -amino butyric acid (AABA) was obtained from Sigma (St. Louis, USA).

Preparation of protein isolate

The preparation of protein isolates was done according to the method described by Thompson (1977) with slight modification. Briefly, 100 g of mung bean and defatted soy bean flours were dispersed in 1000 mL of distilled water. The dispersions were adjusted to pH 9 with 1 N NaOH, shaken for 1 h at 30°C and centrifuged for 15 min at 2000 \times g. In order to obtain increased protein yield, the extraction and the centrifugation processes were further carried out on the residue. To extract protein from the residue, it was re-dispersed in 1000 mL of distilled water, adjusted to pH 11, shaken at 30°C, and centrifuged for 15 min at 2000 \times g. Afterwards, all the supernatants were combined and adjusted to pH 4 with 1 N

HCl to precipitate the protein. The proteins were then recovered via centrifugation at $2000 \times g$ for 20 min. The protein curds were washed twice with distilled water and then freeze-dried.

Proximate analysis

Proximate composition (moisture, ash, protein and fat contents) of mung bean was investigated according to the AOAC methods [17]. As for the percentage of crude protein, total nitrogen content of mung bean determined by Kjeldahl method was used to calculate crude protein by multiplying it with a conversion factor of 6.25.

Amino acids profile

The amino acid content was analyzed with the Pico-tag method (Cohen, 1989) with slight modification using the HPLC (Jasco UV-2075 HPLC system, Jasco Pu-2080 Plus Intelligent HPLC Pump, CO-2065 plus intelligent column oven, PDA detector). About 50 mg of mung bean protein isolate was weighed in a test tube and 15 mL of 6 N HCl was added. Then, the test tube was sealed rapidly and hydrolyzed in an oven at 110°C for 24 h and after that allowed to cool at room temperature. After cooling, the mixture was transferred to a 50 mL volumetric flask and 10 mL of α -aminobutyric acid (AABA) was added as an internal standard. By adding deionized water, the volume was increased up to 50 mL. The mixture was then filtered through a whatman filter paper No.1. Before the derivatization process, the mixture was filtered again through $0.2 \mu\text{m}$ nylon type nitrate membrane filter. About $10 \mu\text{L}$ of filtered sample was transferred into a derivatization tube and dried under vacuum for 30 min. Immediately after drying, $20 \mu\text{L}$ of re-drying reagent (Methanol+water+Triethylamine, at a ratio of 2:2:1, w/w/w/) was added and dried again under vacuum for 30 min. After drying, $20 \mu\text{L}$ of the derivatization reagent (methanol+Triethylamine+ water+phenylisothiocyanate, 7:1:1:1, v/v/v/v) was added and allowed to stand at room temperature for 20 min. It was then dried for another 30 min. About $100 \mu\text{L}$ of sample diluent (ammonium acetate) were added to the sample. From the prepared sample, $20 \mu\text{L}$ of aliquot and $8 \mu\text{L}$ of blank solution were injected into C18 reversed phase column (C18, $250 \text{ mm} \times 4.6 \text{ mm}$). The quantification of each amino acid was determined from a standard calibration.

Water absorption capacity (WAC)

Water absorption capacity was determined by following the method described by Bencini (1986) with slight modification. 3 g of protein isolate was thoroughly mixed with 50 mL of distilled water in pre-weighed centrifuge tubes. Then, the tubes were centrifuged at $3000 \times g$ for 30 min to separate the protein from the supernatant. After centrifugation, the supernatant was decanted carefully and the centrifuge tubes containing the protein isolate were re-weighed. Water absorption capacity was expressed as grams of water absorbed per gram of sample. The WAC was calculated by using the following equation:

$$\text{WAC (g H}_2\text{O)} = \frac{W_2 - W_1}{W_0} \times 100$$

$$W_0$$

where W_2 = Weight of tube plus the sediment (g), W_1 = Weight of the tube plus dry sample (g), and W_0 = Weight of the dry sample (g)

Oil absorption capacity (OAC)

Oil absorption capacity was determined by following the method described by Beuchat (1977) with minor modification. One gram of protein isolate was mixed with 10 mL of corn oil in a pre-weighed 20 mL centrifuge tube. The tubes were agitated for 2 min to get a complete dispersion of the sample in oil, allowed to stand at 28°C for 30 min, and then, centrifuged at $3000 \times g$ for 30 min. The clear supernatant was decanted and the tubes were inverted for 25 min to drain the oil before re-weighing. The oil absorption is expressed as gram of oil absorbed per gram of protein isolate. The OAC was calculated by using the following equation:

$$\text{OAC (g Oil)} = \text{O2} - \text{O1} \times 100$$

$$\text{O0}$$

where O2 = Weight of tube plus the sediment (g), O1 = Weight of the tube plus dry sample (g), and O0 = Weight of the dry sample (g) Emulsion activity (EA) and emulsion stability (ES) Protein isolate (3.5 g) was homogenized for 30 sec in 50 mL of distilled water using a homogenizer. Corn oil (50 mL) was added to the protein solution and homogenized again for 30 sec. The emulsion obtained was divided evenly and centrifuged at $1100 \times g$ for 5 min (Yasumatsu et al., 1972). The Emulsion activity was calculated by using the following equation:

$$\text{EA} = \text{H1}/\text{H0} \times 100$$

Where H1= Height of the emulsified layer in the tube, and H0= Height of the total contents in the tube

Furthermore, in order to measure the emulsion stability, the emulsions were heated at 80°C for 30 min and centrifuged again. Emulsion stability was calculated by using the same equation as above (Yasumatsu et al. 1972). Similar procedure was employed to determine the emulsifying activity and the stability of protein isolates in 3% NaCl solution. By

Foam capacity (FC) and Foam stability (FS)

The protein isolate 1% (w/v) was dispersed into 50 mL of distilled water using Waring blender for 5 min and then immediately transferred into a graduated cylinder. The sample volume was recorded before and after whipping (Lawhon et al., 1972). The foaming capacity was determined with the equation given below

$$\text{FC} = \text{V2} - \text{V1} \times 100$$

$$\text{V1}$$

where V1 = volume of protein solution before whipping, and V2 = volume of protein solution after whipping

The foam stability was determined by measuring the change in foam

volume after 15, 30, 45, and 60 min of standing time at room temperature (Naczki et al., 1985). The stability was calculated by using the following equation:

$$\text{FS} = \text{Vt} \times 100$$

$$\text{V0}$$

where Vt = foam volume at time t, and V0 = initial volume of the dispersion

Least gelation concentration (LGC)

Protein isolates in different concentrations (2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, and 20%, w/v) were dispersed in 50 mL of distilled water. Then, the dispersions were adjusted to $\text{pH } 7.0 \pm 0.05$ by drop wise addition of 1 N NaOH or 1 N HCl solution. Next, 5 mL aliquot of each dispersion was poured into a clean and dry glass test tube. Then, the test tubes were heated at 100°C for 1 hour in a water bath (Mettler, Germany). After heating, the test tubes were promptly cooled under running tap water for 10 min and then kept in a refrigerator at 4°C for an additional 2 hours. The strength of the coagulum formed was assessed by turning the tube upside down. The lowest concentration of protein, which formed a stable gel, was considered the LGC (Circle et al., 1964).

Protein solubility

The solubility tests of protein isolate were carried out at different pH values ranging from 2 to 12. Two grams of protein isolate was dispersed in 50 mL of distilled water and then, the pH of the dispersion was maintained constant by adding 1 N NaOH or 1 N HCl and stirred continuously with a magnetic stirrer for 30 min. The supernatant extract was centrifuged at $1300 \times g$ for 30 min, filtered through Whatman No. 1 filter paper, and analyzed for total protein content by the micro-kjeldal method. The results were expressed as the percentage of protein isolate soluble at a certain pH (Coffmann and Garcia, 1977).

Differential scanning calorimetry (DSC)

The thermal properties of the protein isolates were determined by using a differential scanning calorimetry (DSC-823E, Mettler Toledo, GmbH, Switzerland). The samples were sealed in hermetic aluminum TA pans, weighed (8–10 mg) using a precision balance (± 0.01 mg, Analytical Plus, Mettler Toledo). The TA pans with samples were heated from 25 to 300°C at a heating rate of $10^{\circ}\text{C}/\text{minute}$. The calibration was carried out with indium. The onset temperature (T_o), the peak temperature (T_d), the enthalpy (ΔH), and the endset temperature (T_e) were computed by the Mettler stare software system, version 9.x.

Extrusion of mung bean to produce textured vegetable protein

In order to prepare vegetable protein (TVP), a single screw extruder (Brabender Single screw, Stand Alone Extruder, KE-19) was employed. Mung bean flour (500 g) with particle size 15-20 mesh size was used for TVP preparation (Riaz 2000). The mung bean flour was preconditioned at 40°C and moisture content at 25%. Then, the extruder was operated at barrel temperatures of $120\text{--}140^{\circ}\text{C}$ and screw speed at 100 rpm.

Microstructure analysis

Scanning electron microscope (Joel, JSM 5200, Tokyo, Japan) was used at an accelerating voltage of 15 KV to view the extrudate and to determine the surface feature of the extrudate. Photographs were taken at $\times 100$, $\times 50$, and $\times 35$ magnification.

Statistical analysis

All results are shown as means of three replicates and the data are expressed as means \pm standard deviations. Besides, the results were subjected to one way anova and independent sample T Test using SPSS (version 17). The sample means were separated by comparing the means at $p \leq 0.05$.

Results and Discussion

Proximate composition and amino acid profile Proximate composition plays an important role in determining the overall nutritional quality, as well as the potential use of a food commodity. As shown in

Table 1, protein, fat, and moisture contents of mung bean flour were found to be 23.84%, 1.53%, and 10.21%, respectively, which are similar to the previous findings obtained by Blessing and Gregory (2010). As for the protein, Butt and Batool (2010) and Mubarak (2004) reported higher protein contents at 25% and 27.5% respectively. Variations in the protein content of mung bean flour reported could be due not only to the differences in production environment and agriculture practices, but more importantly, the cultivars (Habibullah et al.,

2007). The extractability of protein from mung bean flour was then compared

Table 1. Proximate compositions of mung bean and soy bean protein isolates

	Mung bean Flour	Mung bean Protein Isolates	soya bean protein isolates
Protein (%)	23.84 ± 0.04a	81.53± 0.02b	86±0.86c
Fat (%)	1.53 ± 0.04a	0.14±0.01b	0.11±0.01b
Fiber (%)	4.95 ± 0.08a	0.73±0.02b	0.10±0.00c
Ash (%)	3.02 ± 0.03a	4.38±0.28b	3.47±0.05c
Moisture (%)	10.21 ± 0.06a	4.56±0.11b	4.56±0.05b
Carbohydrate (%)	56.43 ± 0.16a	8.66±0.26b	5.76±0.88c

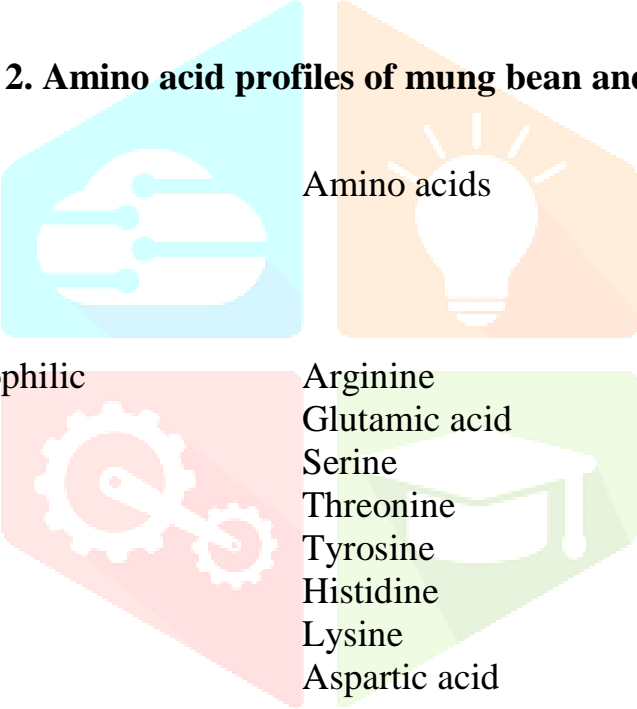
#Each value in the table represents the means; ±SD of triplicate determinations a-c Means with the different superscript letters in the same row indicated significant difference ($p < 0.05$).

with soy bean flour. Although the protein content of mung bean protein isolate (81.53%) was significantly ($P \leq 0.05$) lower than the protein content of soy protein isolate (86%), the amount is still considered high in comparison to other legumes. Moreover, there were no significant differences in fat and moisture contents between mung bean protein isolate and soy protein isolate, while carbohydrate, fiber, and ash contents of mung bean and soy bean isolates were significantly different (Table 1). Similarly, a broad range of proximate compositions has been reported for proteins (81-88%), fat (0.39-0.92%), moisture(5.08-6.52%), ash (3.05-5.55), and carbohydrate (0.2- 9.00%) derived from protein isolates of chickpea, faba bean, lentil, and pea (Asli *et al.*, 2011).

As the behavior of protein is largely influenced by its amino acid composition so the amino acid profile of mung bean protein isolate was determined. The amino acid profile of mung bean protein isolate compared favorably with the soy protein isolate. The total hydrophilic (653.68 mg/g) and hydrophobic amino acid content (493.4mg/g) of mung bean protein isolate (1147.08 mg/g) was significantly ($P \leq 0.05$) higher than the total hydrophilic (568.16 mg/g) and hydrophobic (429.47mg/g) amino acid composition of soy protein isolate (997.63 mg/g) (Table

2). However, the ratios of total hydrophilic amino acid to hydrophobic amino acid for both protein sources were similar. Nakai and Modler (1996) stated that the relative amounts of hydrophilic and hydrophobic amino acids control the solubility characteristics, the water binding possibility, and the surfactant properties of proteins. Generally, solubility of protein refers to the hydrophilic and hydrophobic interactions with water. Protein solubility can be increased if the number of hydrophilic groups are higher in protein structure. Furthermore, the distribution and percentage of hydrophilic and hydrophobic amino acids in a protein structure govern the degree of protein solubility. The overall amino acid profile of mung bean protein isolate in present study was in agreement with the results previously reported by Babu *et al.* (1988). In addition, the results showed that the essential amino acid contents of mung

Table 2. Amino acid profiles of mung bean and soy bean protein isolates



Amino acids		Mung bean protein isolate (mg/g of protein)	Soy bean protein isolate (mg/g of protein)
Hydrophilic	Arginine	46.00±1.41	54.17±2.92
	Glutamic acid	203.28±10.01	195.18±2.80
	Serine	53.22±2.92	52.53±0.73
	Threonine	48.84±1.90	53.63±2.60
	Tyrosine	26.62±0.91	33.81±0.64
	Histidine	37.54±4.39	31.26±0.44
	Lysine	140.19±3.33	48.05±1.34
	Aspartic acid	97.99±5.21	99.53±0.33
		653.68 ^a	568.16 ^b
Hydrophobic	Alanine	29.74±0.53	35.28±2.40
	Glycine	28.66±0.55	36.82±1.47
	Leucine	69.09±0.82	71.57±0.33
	Valine	32.39±1.08	31.92±0.59
	Isoleucine	64.48±0.70	33.87±0.55
	Phenylalanine	53.13±2.07	45.63±2.12
	Proline	42.96±0.16	53.60±1.25
	Cysteine	42.99±0.50	3.59±0.83
	Methionine	129.96±3.67	117.19±7.15
		493.4 ^a	429.47 ^b

Each value in the table represents the means; \pm SD of triplicate determinations a-b Means with the different superscript letters in the same row indicated significant difference ($p < 0.05$).

bean for lysine, methionine, isoleucine, phenylalanine, valine, and histidine were higher than those in soy protein isolate, whereas threonine and leucine showed higher amounts compared to mung bean protein isolate although their amounts did not differ significantly. Moreover, amino acid composition profile of mung bean protein isolate showed appropriate essential amino acids content. As shown in Table 2, cysteine as a semi essential amino acid exhibited a significantly higher amount (42.99 mg/g) compared to soy bean Table 2. Amino acid profiles of mung bean and soy bean protein isolates (3.59 mg/g), while lysine as an essential amino acids showed 140.19 mg/g in mung bean protein isolate, which it was also significantly higher than lysine amount of soy bean protein isolate (48.05 mg/g).

Water and oil absorption capacities

Water or fat absorption capacity is defined as the amount of water or fat that can be absorbed per gram of protein material. Water absorption capacity is a useful sign to predict if protein isolates can be incorporated into aqueous food products. As protein has both hydrophilic and hydrophobic properties, thus, it can interact with water and oil in foods (Butt and Batool, 2010). The water and fat absorption capacities depend on the availability of polar and non-polar amino acids. Less availability of polar amino acid is responsible for lower water absorption capability and vice versa (Kuntz, 1971). Moreover, the presence of hydrophilic and polar amino acids over the surface of the protein molecules leads to lesser fat absorption capacity (Sathe *et al.*, 1982).

As shown in Table 3, the water absorption capacity of mung bean and soy bean protein isolates were found to be 3.33 g and 3.00 g per gram protein, respectively. These values are not significantly

Table 3. Functional properties of mung bean protein isolate compared with soy bean protein isolate

Properties	Mung bean protein isolate	Soy bean protein isolate
Water absorption capacity (g H ₂ O/g protein)	3.33±0.57	3.00±0.00
Fat absorption capacity (g oil /g protein)	3.00±0.00	3.45±0.39
Emulsion activity, % (in 3% NaCl)	72.03±0.53	84.73±0.45b
% (in distilled water)	63.18±0.38	74.50±1.32b
Emulsion stability, % (in 3% NaCl)	66.50± 1.37	82.40±2.94b
% (in distilled water)	62.75±0.43	81.20±0.61b
Foam capacity (v/v), %	89.66±0.57	68.66±2.30b
Foam stability (v/v), %		
Standing time (min) 15	80.83±1.04	No Chnge
30	78.33±0.57	No Chnge
45	60.16±0.28	63.33±2.88
60	50.40±0.69	53.66±3.21
Least gelation concentration, Protein concentration, %		
2%	NG	NG
4%	NG	NG
6%	NG	NG
8%	NG	NG
10%	NG	NG
12%	GEL	NG
14%	GEL	GEL

16%	GEL	GEL
18%	GEL	GEL
20%	GEL	GEL

No gel = NG

#Each value in the table represents the means; \pm SD of triplicate determinations a-b Means with the different superscript letters in the same row indicated significant difference ($p < 0.05$)

different, but in the higher end of the range for chickpea (2.3 to 3.5 g/g protein isolates), a member of legumes family, as reported by Kaur and Singh (2007). In contrast, Fernandez-Quintela *et al.* (1997) found lower water absorption capacity for soy bean protein isolate at 1.3 g per gram of protein isolate.

The fat absorption capacity of mung bean and soy protein isolates were 3 g and 3.45 g per gram protein isolate, respectively. The fat absorption capacity of mung bean protein isolate is comparable to soy protein isolate but the value is considered high in comparison with other legumes, such as pea protein and faba bean protein isolates that had 1.2 and 1.6 g of fat absorption capacity per gram protein isolate, respectively (Fernandez-Quintela *et al.*, 1997). Generally, variation in water and oil absorption capacities is influenced not only by the protein concentration and structure, but also by their extent of interaction with water and oil, as well as their conformational characteristics. As shown in Table 1 and 3, there is no significant difference between water and oil absorption capacities of mung bean protein and soy protein isolate. This result is expected as mung bean protein isolate composed almost a similar amount of hydrophobic amino acids (493.4 mg/g) with soy bean protein isolate (429.47 mg/g). The results also imply the proteins capability of retaining water and reducing interfacial tension in an emulsion system, which play an imperative role in processing and maintaining the superiority of meat-based products, especially comminuted meat products. Thus, addition of mung bean protein isolate could contribute to the improvement of textural and sensory qualities during processing of fabricated foods (Rutkowski and Gwiazda, 1986).

Emulsification activity and stability

Emulsifying activity is the ability of protein to form emulsion. As shown in Table 3, there was a significant difference in emulsifying activity and stability of mung bean protein isolate in comparison with soy bean protein isolate. The emulsifying activity of mung bean protein isolate was found to be 72.03% and 63.18% in 3% NaCl and in distilled water, respectively. However, the emulsifying activity of soy bean protein isolate showed 84.73% and 74.50% in 3% NaCl and in distilled water, respectively. The variation in emulsifying activity could be due to the number of hydrophobic groups on the protein surface. High surface hydrophobicity enables a large quantity of protein to interact with oil or fat. This leads to the formation of stronger protein films that tend to become superior emulsifiers (Nakai and Modler, 1996).

Meanwhile, in distilled water, the emulsifying activity is less compared to the emulsifying activity in 3% NaCl solution (Table 3). This is because NaCl improves the solubility of protein, leading to high protein availability during emulsification. Similar result was revealed by Raghav *et al.* (2003). Moreover, Butt and Batool (2010) reported that the emulsification activity of mung bean protein isolate was 41.10%, whereas El-Adway (2000) reported that mung bean protein exhibited an emulsifying activity at about 65%, which is in accordance with the results obtained from the current study.

The emulsion stability is the measure of the steadiness of emulsion formed by protein. There was significant difference in emulsion stability between mung bean protein and soy protein isolate (Table 3). The emulsion stability of mung bean protein isolate was found to be 66.50% and 62.75% in 3% NaCl and distilled water which was significantly lower than emulsion stability of soy protein isolate that could be due to the high surface charge of soy protein isolate (Asli *et al.*, 2011). Butt and Batool (2010) found that the emulsion stability of mung bean protein isolate was 21%, which is not in agreement with this study. Generally, protein forms a charge layer around fat droplets, which causes mutual repulsion and hinders coalescence. The interfacial film of protein is responsible for the stability of the emulsion system (Szuhaj and Sipos, 1989). Besides, the globular nature of legume protein isolate is also a reason for high emulsion stability (Sathe *et al.*, 1982). In addition, the stability of emulsion, nonetheless, increased in 3% NaCl solution (Table 3) for both mung bean and soy protein isolates. This is because; the salt might

have stabilized the emulsion by decreasing the columbus interaction between the adjoining droplets (McWatter and Holmes, 1979).

Emulsifying activity and stability are critical factors in chopped and fabricated meat-based products. In comparison to the other legumes, such as navy bean, red kidney bean, azuki bean, the emulsifying activity and stability of mung bean protein had been rather high (Sai-Ut, 2009). Hence, mung bean protein isolate can be used in both formation and stabilization of fluid emulsion during the production of textured vegetable protein, which is heated during processing.

Foam capacity and stability

Generally, proteins that can rapidly absorb into the newly formed air-liquid interface during whipping and easy to unfold at the interface display better foamability than proteins that absorb slowly and resist unfolding at the interface (Nakai and Modler, 1996). Moreover, as depicted in Table 3, mung bean protein isolate indicated significantly higher foam capacity (89.66%) compared to soy bean protein isolate (68.66%). This might be due to the differences in the adsorption and in the degree of unfolding at the air-water interface of these two protein isolates. Other than that, findings retrieved from Butt and Batool (2010) and El-Adway (2000) showed that the foam capacity of mung bean protein isolate had been around 110% and 108%, respectively, both of which were higher than the results obtained in this study (89.66%).

Another important functional parameter of protein is foam stability, as the effectiveness of whipping agents relies on their capability to preserve the whip as long as possible. The foam stability of mung bean protein isolate was 78.33% after 30 min of standing time (Table 3). Nevertheless, no significant difference was recorded in foam stability after 30 min between mung bean protein and soy bean protein isolate. According to Butt and Batool (2010) and El-Adway (2000), the foam stability of mung bean protein isolate was 58%, which was lower than the findings in this study. This might be ascribed to the protein denaturation (Fidantsi and Doxastakis, 2001). In fact, foam stability mainly relies upon the effectiveness of the protein film and its permeability to gases. High foam stability could be due to the formation of viscous and gel-like cohesive film with high elasticity by protein. Moreover, protein that shows optimum intermolecular interactions and creates a cohesive continuous network could form stable foams at the air-liquid interface (Nakai and Modler, 1996).

Least gelation concentration (LGC)

Gel is a three-dimensional (3D) cross-linked network structure of protein molecules, which are formed by the gradual aggregation of unfolded molecules containing large quantities of trapped water. Least gelation concentration (LGC) is one of the mostly used properties to measure the gelling ability. Several factors affect gel formation, such as concentration of protein, molecular weight, ionic strength, amino acid composition, pH, and interaction with other components. LGC is the minimal protein isolate concentration in which gel forms and does not move along the test tube walls in an upside-down position (Moure *et al.*, 2006). Proteins with lower LGC exhibit greater gelling ability. As shown in Table 3, the LGC for mung bean protein isolate was 12%, whereas for soy bean protein isolate was 14%. Therefore, the gelling capacity of mung bean protein isolate was slightly better than soy bean protein isolate which is not in agreement with the finding previously reported by Coffman and Gracia (1977)., with 10% of LGC.

As legume proteins such as mung bean is globular in nature, so they intend to form a globular network structure by slightly altering their structure through heating above a minimal unfolding temperature. To develop a gel network, the main forces involved are hydrophobic and hydrogen bonding, as well as disulfide bridges, which help to form the gel structure (Tiwari and Singh, 2012). Besides, during and after heating, if protein unfolding is high, then the formation of gel matrix becomes easier by protein isolate and strong gel is developed (Berghout, 2015). This could be the reason that mung bean protein isolate develop gel easily at low concentration. Moreover, in a study conducted by the Circle and Smith (1972), 16-17% of protein concentration had been suggested to form strong and firm gel with soy bean protein isolate. Hence, mung bean protein isolate has the ability to form gel and retain water in 3D structure at a lower protein concentration than the soy protein isolate, which is particularly useful in preparing comminuted meat emulsion. The fat and water molecules are entrapped in the 3D structure of gel and stabilize the fabricated meat emulsion.

Protein solubility

The pH-solubility profile of protein is a commonly measured functional property and a vital determining factor of the sensory quality attributes of foods. The solubility of a protein is the thermodynamic index of the equilibrium between protein–protein and protein solvent interactions (Damodaran *et al.*, 2008). It is governed by pH, temperature and ionic strength, freezing, heating, and drying. In addition, protein solubility at different pH values is an important index to determine the behavior of protein isolate when they are applied to food

systems. As illustrated in Figure 1, the solubility patterns of mung bean and soy bean protein isolates are similar. As noted, at pH values of 2, 10, and 12, the solubility of both protein isolates had been higher than that of other pH values. Generally, the solubility pattern of most legumes is high in acidic and alkaline pH. Proteins possess a net positive or a net negative charge in acidic or alkaline pH, respectively. The high solubility in acidic or alkaline pH is due to electrostatic repulsion and hydration of charged residues, which enhance solubilization of the protein (Damodaran *et al.*, 2008). At pH 4, the lowest solubility was observed (Figure 1). Furthermore, the solubility of most legumes was found noticeably decreased at the isoelectric point, which is generally between pH 4 and pH 6 (Boye *et al.*, 2010). The reason behind the insolubility of most proteins at their isoelectric points owes to the neutralization of charge repulsion between the

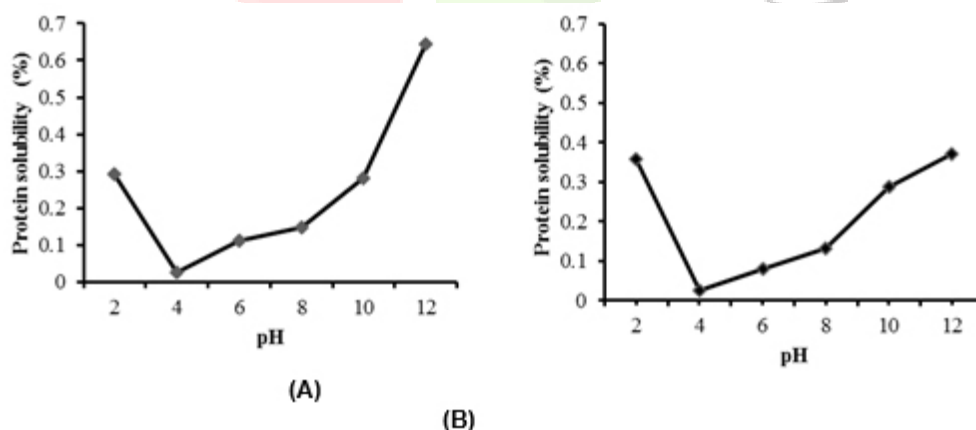


Figure 1. Protein solubility of (A) mung bean protein isolate compared to (B) soy bean protein isolate

protein molecules, which aids the aggregation via hydrophobic interactions (Nakai and Modler, 1996).

Generally, high solubility of protein isolate is associated to good foaming and emulsifying properties. For texturization of protein ingredients, the quality of protein is usually determined by measuring the level of protein solubility. The difference of protein solubility between the native and the thermally denatured extruded protein is used to measure the degree of texturization that occurs during the production of textured vegetable protein (Onwulata *et al.*, 2010).

Thermal properties

During texturization, the microfibrillar protein network is formed due to protein unfolding, orienting, and thermal cross-linking. This phenomenon takes place at a temperature that is considerably above the denaturation temperature of protein. As a matter of fact, it is important to determine the denaturation temperature of protein isolate prior to texturization or application into other heat treatment process.

On top of that, thermal analysis functions as an important tool to examine the effect of thermal processing on vegetable proteins. The differential scanning calorimetry (DSC) is commonly used to study the denaturation of protein. In DSC, denaturation of protein is observed as an endothermic peak on the thermogram (Figure 2). There was no significant difference in denaturation temperature between mung bean (157.90°C) and soy bean protein isolates (157.86°C) (Figure 2).

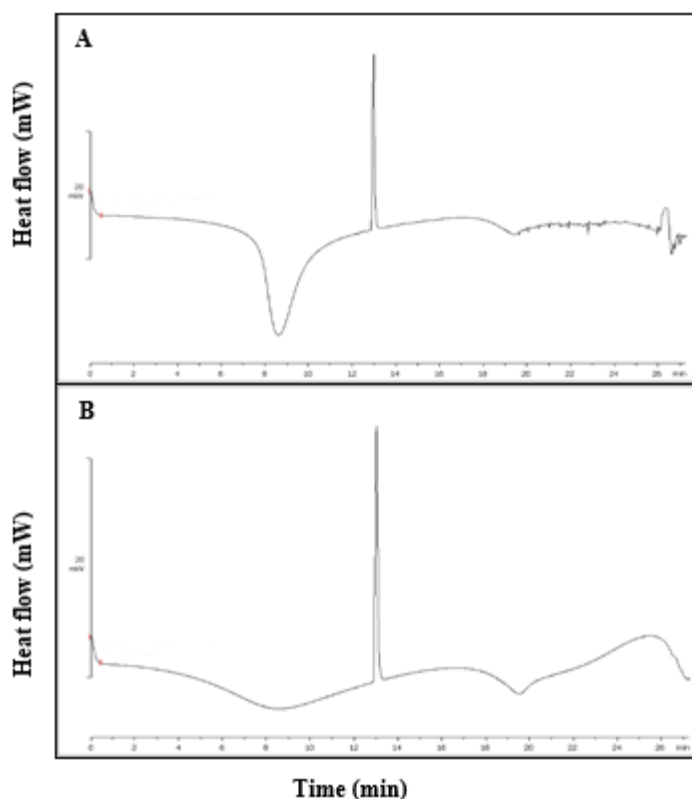


Figure 2. DSC thermogram of (A) mung bean protein isolate and (B) soy bean protein isolate

Table 4. Thermal properties of mung bean and soy bean protein isolates

Samples	Peak (Td, °C)	Onset (To, °C)	Enthalpy (ΔH , J g ⁻¹)	End set (Te °C)
Mung bean protein isolate	157.90± 0.17a	155.54±0.26a	41.64±0.64a	158.00±0.38a
Soy bean protein isolate	157.86±0.17a	155.62±0.09a	50.20±2.5b	158.36±0.87a

#Each value in the table represents the means; \pm SD of triplicate determinations a-b Means with the different superscript letters in the same column indicated significant difference ($p < 0.05$)

The structure and the conformation of proteins are stabilized by polar bonds (e.g., hydrogen bonds) and nonpolar interactions (e.g., hydrophobic bonds). When proteins are heated, they go from native to denatured state, a transition that is accompanied by rupture of

intramolecular bonds. The rupture of polar bond is responsible for the endothermic peak in DSC thermogram. Besides, higher Td value is usually related to higher thermal stability of protein. The denaturation enthalpy, as well as onset and endset temperatures of mung bean protein isolate were 41.64 J g⁻¹, 155.54°C, and 158.38°C respectively (Figure 2). According to Tang (2007), disulfide bonds within the protein molecule contributes to the thermal stability of protein. Meanwhile, Kudre *et al.* (2013) stated that the high thermal stability attribute could be due to the disulfide bonds, whereas the presence of salt bridges in the hydrophobic clefts of protein structure makes it more thermostable.

Therefore, in order to produce textured vegetable protein, food protein is heated above 100°C to bring texturization and provide meat-like texture. Besides, thermal and mechanical energy is applied on protein molecule so that denaturation takes place, involving protein cross-linking and arranging the protein matrix in a fiber-like configuration. Therefore, the thermal properties of mung bean protein isolate can serve as an important tool for selecting and ensuring optimum process temperature for extrusion and other heat treatment processes.

Microstructure analysis

One of the important desirable features of textured proteins is heterogeneous; a network-like matrix with the presence of voids. In order to evaluate the presence of such features, the microstructure of textured mung bean flour was observed under SEM and the results were compared with commercial textured soy protein. During extrusion process, under high temperature, pressure, and shear; a molten mass is formed inside the barrel and move towards the end of the extruder. The high pressure inside the barrel keeps the moisture in liquid state. When that molten mass emerges out through the end of the extruder, the heated entrapped water vaporizes rapidly due to the sudden drop in pressure to normal atmosphere and thus, generates voids over the product; resulting

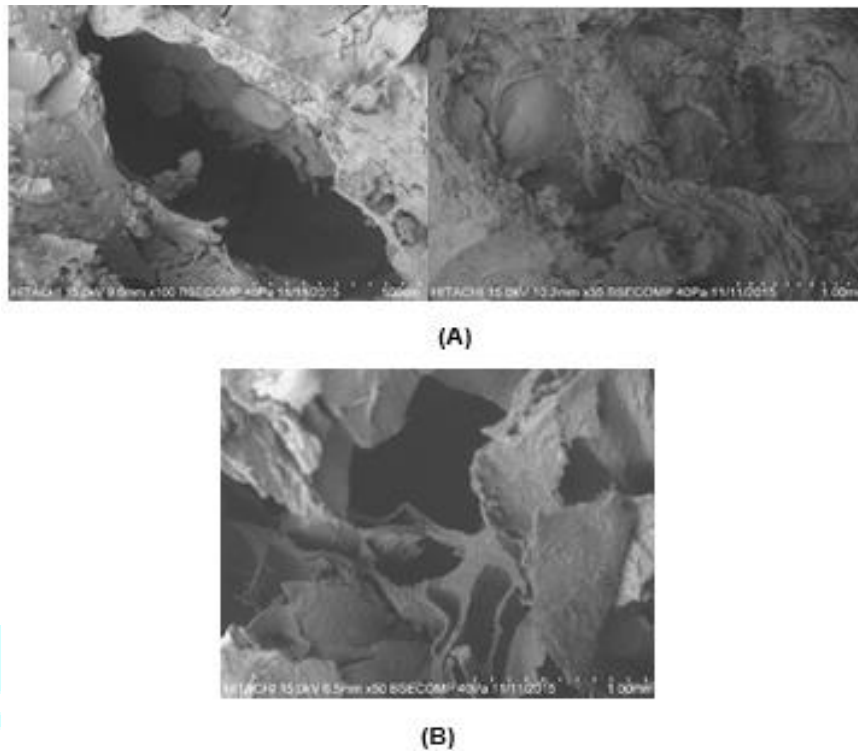


Figure 3: Scanning electron micrographs of (A) textured mung bean flour and (B) commercial textured soy bean protein

in a porous and fibrous final product (Riaz, 2000). These features had been observed in textured mung bean flour which is comparable to the textured soy bean protein isolates illustrated in Figure 3A and 3B respectively. Hence, based on the above result, it is anticipated that mung bean protein isolate will be able to produce firmer, more fibrous, and more textured product than the mung bean flour. This is because; increased protein percentage in raw material generates firmer, more fibrous, and more textured product (Yada, 2000).

Conclusion

The functional properties of mung bean protein isolate were evaluated and compared with soy protein isolate. Mung bean and soy bean protein isolates showed high protein solubility in acidic and alkaline pH (pH 2 and pH 8-12) comparing to low pH (pH 4). Besides, the protein content, the foaming capacity, the gelation, as well as the water and oil absorption capacities of mung bean protein isolate were relatively higher than soy bean. Therefore, it makes mung bean as a potential source of protein for application in food systems. Additionally, the thermal stability of mung bean protein (157.90 °C) was comparable with soy protein isolate (157.86°C). Therefore, it could be used as an ingredient in food systems where heat treatment is a vital process. Furthermore, the mung bean protein isolate displayed an adequate quantity of essential amino acids; particularly its lysine content (140.19 mg/g), which was significantly higher than that of soy bean protein isolate (48.05 mg/g). Therefore, it is hoped that the findings of numerous essential food properties of mung bean protein isolate may lead to its best utilization and exploitation for human food. Furthermore, the scanning electron microscope of the extruded mung bean flour opens a new door for the production of mung bean protein-based textured product.

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