IN- VITRO PROTEIN DIGESTIBILITY, CHEMICAL AND FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES FROM RAW, DEFATTED AND UNDEFATTED FERMENTED MUNG BEAN

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ABSTRACT: *Protein isolates (PI) was extracted from the raw mung bean , defatted fermented mung bean and undefatted fermented mung bean by using alkaline extraction-isoelectric precipitation. In this study, the In-Vitro protein digestibility, chemical composition and functional properties of all PI were investigated. The result for In-Vitro protein digestibility showed that all PI have digestibility ranged from 85.22 to 95.08%.The results of the chemical composition revealed a significant difference (P<0.05) for some nutrients. Defatted fermented mung bean protein isolate (DFMPI) showed the highest crude protein (89.48%) and the lowest crude fat (0.29%) as well as carbohydrate (3.89%). Undefatted fermented mung bean protein isolate (UFMPI) showed the highest ash content (2.43%) compared to other PI. The moisture content of all PI's ranged from 4.07 to 5.05%, while crude fibre in all PI was undetectable. Certain functional properties of all protein isolates were significantly different (p < 0.05). DFMPI exhibited the highest oil absorption capacities (3.30 ml/g) and the lowest bulk density (0.25 g/ml). The water absorption capacities for all PI ranged from 2.19 to 2.97 ml/g. The result of foam capacity and stability revealed that all PI had highest ranged from 59.62 to 70% and 19.23 to 35% respectively in acidic condition (pH 2). Meanwhile, all PI showed a higher emulsion capacity and stability in alkaline condition (pH 8) ranged from 59-68% and 47.50 to 67.50% respectively. The protein solubility of DFMPI was significantly different (p < 0.05) from that of UFMPI and raw mung bean protein isolate (RMPI).The RMPI, UFMPI and DFMPI showed decreasing in solubility at pH 4, confirming the isoelectric of mung bean protein. This study suggested that the DFMPI could be used in food formulations requiring high oil absorption capacity, foaming capacity and emulsifying capacity. The DFMPI also can be considered as potential functional food ingredients.*

Keywords: mung bean, fermented mung bean, protein isolates, In- Vitro protein digestibility, chemical properties, functional properties

1. INTRODUCTION

Mung bean (*Vigna radiata*) is locally known as *kacang hijau* in Malaysia. Mung bean is inexpensive legumes and is readily available in the market. The crude protein content of mung bean are the second major component after carbohydrate falls within the range of 24.26% to 28.50% [1] In recent years, interest has increased in the utilization of beans in different forms rather than as the whole beans, thus, they are also processed into flour, starch and proteins [2]. Fermentation has often been proposed as a processing method which the nutrient composition and functional properties of legumes might be improved. The use of bean protein isolates is increasing as a functional ingredients to improve the nutritional quality of the products [3].The most widely used procedure to prepare protein isolates is isoelectric precipitation. After alkaline solubilization of the proteins in the ranged from pH 8–10 and removal of the insoluble material by centrifuging, proteins are precipitated by adding acid from pH 4–6 until reaching the isoelectric point [4].There is limited information available in the literature on the functionality of mung bean protein isolate as affected by fermentation. The purpose of the research is to the evaluate the effect of fermentation and defatting process on the chemical properties, In-Vitro protein digestibility and functional properties of the protein isolate of *Vigna radiata.* The data obtained from this study could be an important step towards extending the utilization of mung bean protein isolate and fermented mung bean protein isolate in different food systems.

2. EXPERIMENTAL DETAILS

Mung bean (*Vigna radiata*) were purchased from local market in Kota Kinabalu, Sabah. Mung bean were dehulled manually after soaking in water overnight. Then, dehulled mung bean were dried using universal oven at 50 °C for 12 hours and was grounded to pass all through a sieve with 250 mm aperture widths to prepare flours. Raw mung bean flour defatted by using method of Stone et al.[5].

2.1 Fermentation of Mung bean

Fermented mung bean was prepared after soaking mung bean overnight and dehulled manually. Dehulled mung bean was autoclaved at 105 °C for 15 min to inactivate microorganisms initially present in the mung bean and utensil used. After cooling at 37 °C, the dehulled mung bean was inoculated with 1% (v/w) *Aspergillus oryzae*, and spread thinly in a sterilized tray. Tray was covered with muslin cloth to allow respiration of fungus during fermentation and incubated at 37 °C for 48 hours. Fermented mung bean was dried at 50 °C in universal oven for 12 hours. Then, fermented mung bean was grounded. 1kg of the fermented flour were defatted using the method mentioned previously[5]. Then, the defatted fermented mung bean flour and undefatted fermented mung bean flour were stored at −20 °C for further use.

2.2 Preparation of Protein Isolate

The method described before [5] was used for the alkali extraction–isoelectric precipitated (AE-IP) protein. After the isolation, all the pellet was stored at−40 °C. All protein isolate were dried using freeze dryer.

2.3 Chemical Analysis

Moisture, fat, ash and crude fibre contents were determined according to the methods of AOAC[6] and are expressed in g/100 g. The micro-Kjeldahl method was used to determine

the protein content [6]. An estimate of the carbohydrate content was determined by difference [100- (moisture +ash +fat +protein)].

2.4 Functional properties of protein isolates

2.4.1 Bulk Density

Bulk density was measured according to Mohamed et al.[7].

2.4.2 Water and Oil absorption

Water and oil absorption capacity were determined by using the method of Bencini and Khalid et al.[8 and 9] with a slight modification. 0.5g of protein isolate was suspended in 5ml of distilled water or vegetable oil. Each sample was vortexed for 1 min and standing for 30 minutes. Samples were centrifuged at 2200 x g for 30 min. The volume of the supernatant was measured. Water and oil absorption capacity was calculated by dividing the volume of water/oil absorbed by the weight of the protein sample. Density of the oil was found to be 0.92 g/ml.

2.4.3 Foaming Capacity and Stability

Foaming capacity and foaming stability were evaluated according to Ogunwolu et al.[10]. The total sample volume was monitored at 0 min for foam capacity (FC) and up to 60 min for foam stability (FS). Volume increase (%) was calculated according the following equation:

$$\text{Foam capacity, FC (\%)} = \frac{(\text{Volume after whipping} - \text{volume before whipping})\ \text{ml}}{\text{Volume before whipping (ml)}} x100$$

$$\text{Foam stability, FS (\%)} = \frac{(\text{Volume after standing} - \text{volume before whipping})\ \text{ml}}{\text{Volume before whipping (ml)}} x100$$

2.4.4 Emulsion Activity and Emulsion Stability

Emulsion activity and emulsion stability were determined by the method of Chau et al. [11] with a slight modifications. 2% (w/v) protein isolate suspended in 10 mL distilled water. The pH of the suspension adjusted to 2, 4, 6, 8, and 10. Then, the suspension was homogenized at 11 000 rpm for 30 s using a Polytron homogenizer. 10 mL of vegetable oil was then added and homogenized for another 1 min. The emulsions were centrifuged in 50 mL screw cap centrifuged tubes at 1200 x g for 5 min, and the volume of the emulsion left was measured. To determine the emulsion stability (ES), emulsions prepared previously were heated at 80 °C for 30 min, cooled to room temperature, and centrifuged at 1200 x g for 5 min. Emulsion activity (EA) and emulsion stability (ES) calculated as follows:

$$\text{Emulsion activity, EA (\%)} = \frac{\text{Volume of emulsified layer (ml)}}{\text{Volume of whole layer (ml)}} X100$$

$$\text{Emulsion stability, ES (\%)} = \frac{\text{Volume of remaining emulsified layer (ml)}}{\text{Volume of original emulsion (ml)}} X100$$

2.4.5 Protein Solubility

Protein solubility was carried out according to [7] with slight modifications. 100 mg of protein isolates were added in 10 mL of deionized water. The pH of the suspension were adjusted to 2, 4, 6, 8, and 10 using either 0.1 M HCl or 0.1 M NaOH. These suspensions were shaken using shaking incubator for 30 min at 25°C. Then, all the suspensions were centrifuged at 4000 × g for 30 min. The protein content in the supernatant was determined by the Kjeldahl method and percent protein solubility was calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{Protein in supernatant}}{\text{Protein in sample}} x100$$

2.4.6 *In-Vitro* Protein Digestibility

and In vitro digestibility was carried out according to the method described by Ali et al. [12].

2.5 Statistical analysis

Data were analyzed by ANOVA using SPSS (version 23).Mean differences were judged at the 5% significance level. Tukey test was used for pair-wise comparison of outcome variable mean.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition

The proximate composition of raw mung bean protein isolate (RMPI), undefatted fermented mung bean protein isolate (UFMPI) and defatted fermented mung bean protein isolate (DFMPI) is shown in Table 1. It is apparent that UFMPI had highest ash contain (2.43%) and carbohydrate contain (15.42%) when compared to RMPI and DFMPI. Ash content was found between 1.27 and 2.43%. This result is much lower compared to the result obtained by Li et al.[1] which in ranged between 2.19% and 3.04% . However, ash content of UFMPI was higher than other protein isolates in this study. The moisture content of all protein isolates was ranged between 4.07 -5.05%. According to [1] the varies of moisture content in the protein isolates may associated with protein isolation procedure and the relative humidity during storage. RMPI and DFMPI contained 0.44 and 0.29% of crude fat which is significantly lower than UFMPI. This result indicates that most of the fat of RMPI and DFMPI were removed during defatting process. Previous study done by [1] shown that the crude fat content in protein isolate of mung bean ranged between 0.36–0.64%. Results for protein content demonstrated significantly higher amount (89.48%) in DFMPI followed by MBPI (79.12%) and UFMPI (76.25%), respectively. The relatively lower protein content of UFMPI could be mainly explained by its high crude fat and carbohydrate contents ($P<0.05$) [13]. The results for protein content in all protein isolate were in concordance with criteria of protein isolates from legumes, in which protein content must be higher than 70% [14]. Protein content of raw mung bean protein isolate has been reported by [1] ranged between 69.22% and 74.85%. Meanwhile, Kudre et al. [13] reported the protein content of mung bean much higher than present study; 87.8%. Qayyum et al. [15] had reported the protein content of protein isolates from chickpea (80.67%), lentil(84.66%), broad bean(77.64%) and kidney bean (72.69%). Carbohydrate contain in all PI ranged from 3.89 – 15.42%. Crude fibre in all protein isolates were not detected in this study. This result indicated that the crude fibre is largely removed during dehulling process and protein isolate preparation

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Table 1. Chemical composition of Protein isolates

Parameter (%)	Sample		
	RMPI	UFMPI	DFMPI
Moisture	4.07 ± 0.28[a]	5.05 ± 0.06[b]	4.47 ± 0.22[a,b]
Ash	1.27 ± 0.04[a]	2.43 ± 0.12[c]	1.87 ± 0.08[b]
Crude fat	0.44 ± 0.01[a]	0.85 ± 0.18[b]	0.29 ± 0.03[a]
Protein	79.12 ± 0.34[b]	76.25 ± 0.33[a]	89.48 ± 0.50[c]
Crude fibre	ND	ND	ND
Carbohydrate	15.10 ± 0.61[b]	15.42 ± 0.44[b]	3.89 ± 0.76[a]

Notes: Means ± standard deviation followed by different letters in the same column are significantly ($p<0.05$) different.
RMPI: Raw mung bean protein isolate; UFMPI: Undefatted fermented mung bean protein isolate; DFMPI: Defatted fermented mung bean protein isolate; ND: not detected

3.2 Functional properties of protein isolates

3.2.1 Bulk Density

The functional properties of protein isolate will determined the potential of the protein as a food ingredients. According to Ogumwolu et al.[10], bulk density (BD) is related to the packaging of the products and mainly depends on the several factors such as the intensity of attractive inter-particle forces, particle size and number of contact points. Results for BD demonstrated significantly lower amount (0.25g/ml) in DFMPI followed by UFMPI (0.38 g/ml) and RMPI (0.43 g/ml) respectively. All the BD of protein isolates in this study are lower than BD obtained by Eltayeb et al.[16] for Bambara groundnut protein isolate (0.56 g/ml). The differences in BD of the protein isolates possibly be attributable by packing behaviour and particle size [17].

3.2.2 Water and Oil Absorption Capacity

The result for water and oil absorption capacity of RMPI, UFMPI, DFMPI are shown in table 2. RMPI shown significantly higher in water absorption capacity compared to UFMPI and DFMPI. This present study obtained higher water absorption capacity (WAC) of RMPI (2.97 ml/g) compared to result reported by [1] which WAC of mung bean protein isolate ranged between 1.03 and 2.78 g/g. There are several factors may causes the higher result in this study; difference of protein structure and the high availability of polar amino acids which have been shown to be primary sites for water interaction of proteins [1]. Giami and Udensi et al. [18 and 19] reported that fermentation significantly decrease the WAC of protein isolates from fluted pumpkin seeds protein concentrate and Mucuna bean protein isolate, respectively. WAC of UFMPI was higher than DFMPI. The WAC of UFMPI was enhanced, as the carbohydrate content of UFMPI was significantly higher ($p < 0.05$) than DFMPI. Oil absorption capacity (OAC) of all protein isolates in this study ranged between 2.80 and 3.30 g/g. This result fall in the ranged obtained by [1] which is in ranged between 1.00 to 3.38 ml/g. The result of OAC in this present study are was comparable to lupin protein isolate, 2.80 ml/g [20]. OAC of DFMPI in this present study had higher value than soy bean isolate (3.2 ml/g) reported [21]. Fermentation significantly enhanced the OAC of DFMPI ($p < 0.05$). Increased OAC in UFMPI and DFMPI seem to parallel the higher protein solubility and could be explained by proteolytic activity of fungal enzymes [22]. DFMPI had highest OAC (3.30 ml/g) but lowest WAC (2.19 ml/g) might be due to interaction between high availability of non-polar/hydrophobic amino acids with hydrocarbon chains of the fats while lesser of polar amino acids which are the primary sites for water interaction [17].OAC is one of the important functional property of flours because it plays an important role in enhancing the mouth feel and retaining the flavor [23,24]. High OAC of the protein is useful in application of baked goods, soups, ground meat formulations, meat replacers and extenders [24].

3.2.3 Foaming Capacity and Stability

Foam is a colloid of many gas bubbles trapped in a liquid or solid. Small air bubbles are surrounded by thin liquid films. Foam can be produced by whipping air into liquid as much and as fast as possible [25]. The effects of pH on foaming capacity (FC) of all PI are presented in Fig. 1. FC of all PI in this study had the highest value at pH 2 (59.62 – 70%) compare to other pH, which is similar with FC of Bambara groundnut PI obtained by previous study [16]. The lowest of FC for all PI were 15.38 – 30% and it occurred at isoelectric point (pH 4).Fermentation increase the FC of DFMPI compared to RMPI. This result supported by Udensi & Okoronkwo[19] where fermentation of *Mucuna cochinchinensis* PI increased the FC . FC is closely related to the values of soluble protein, because soluble proteins can reduce surface tension at the interface between air bubbles and surrounding liquid, and protein molecules can unfold and interact with one another to form multilayer protein films with an increased flexibility at the air–liquid interface [26]. This present result of FC for RMPI are in agreement with [1] who reported FC for mung bean PI ranged from 33.0 to 67.5%. The UFMPI had the lowest FC in every ranged of pH, possibly due to the high fat content. Fat acts as an inhibitor of foaming [21]. The foaming stability (FS) of all PI are presented in figure 2. The lowest FS values of all PI were 0 to 3.85% at pH 4. The highest FS values in all PI occurred at pH 2, 19.23-35% and RMPI had the highest stability. The FS in this study is lower than obtained by [1] which in ranged from 20 to 56%. The decreased of FC and FS at pH 10 were observed in all PI, this might be due to the repulsion of peptides via ionic repulsion [10].

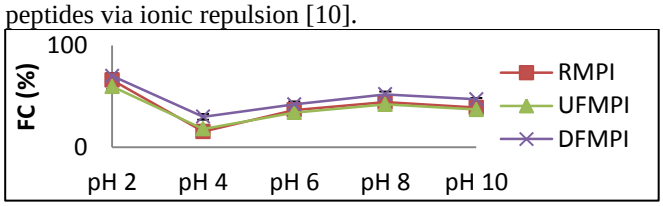

Figure 1. Foam capacity (FC) for all PI at pH 2 -10

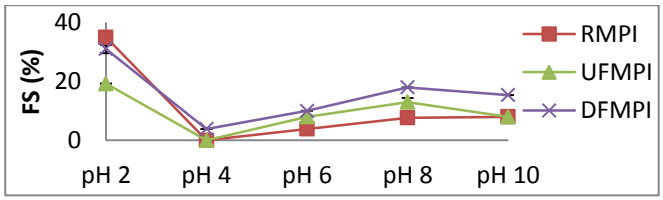

Figure 2. Foam stability (FS) for all PI at pH 2-10

3.2.4 Emulsion Activity and Emulsion Stability

Figure 3 and 4 shows the emulsifying activity (EA) of RMPI,UFMPI and DFMPI at pH 2-10. The EA of all PI decreased from pH 2–4.with the lowest EA obtained around the isoelectric point (pH 4). Thereafter, increased with increasing pH until pH 8 and slightly decreasing at pH 10. The highest EA for all PI is at pH 8 which the values ranged at 59-68%. The result are higher than obtained by previous researcher[14] where EA for pigeon pea protein isolates (49.50%),cowpea (47.50%), peas (45.50%) and mung bean (41.10%). Emulsion activity of proteins depends basically on two effects; a substantial decrease in interfacial energy due to the adsorption of the protein at the oil–water interface and the electrostatic, structural and mechanical energy barriers caused by the interfacial layer that oppose destabilization processes [27]. Lower EA of all PI at pH 10 is due to low solubility of proteins.

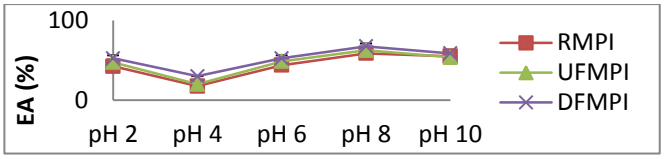

Figure 3. Emulsion activity (EA) for all PI at pH 2 -10

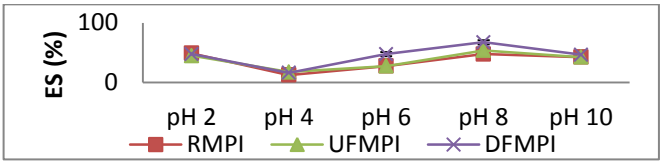

Figure 4. Emulsion stability(ES) for all PI at pH 2 -10

The result of EA in DFMPI and UFMPI are higher than RMPI, previous study also obtained significantly higher EA in fermented *Mucuna* isolates [19]. The EA of DFMPI higher than UFMPI at every range of pH values. Teh et al.[28] showed the defatting process resulted in the highest EA of Hemp (*Cannabis sativa*) and this result proven that chemical treatments such as defatting process will increase the EA as protein conformation and protein–protein interaction changed during the treatment. The emulsion stability (ES) of the PI was also affected by pH, as the lowest ES for all PI were ranged from 12.50-17.50% (pH 4) and the highest ES were ranged from 47.50-67.50% (pH 8).

3.2.5 Protein Solubility

Figure 5 shows the protein solubility (PS) of RMPI, UFMPI and DFMPI at different pH levels between 2 and 10. The results presented in this study showed that the PS is pH dependent. The minimum PS of all protein isolate was found in ranged of 17.56 to 33.18% at pH 4, while the maximum PS was 76.44 to 88.87% at pH 8, respectively. The lowest PS at pH 4 may due to isoelectric point of mung bean. There is no net charge on the protein at the isoelectric point; So, there are no repulsive interactions and the protein–protein interactions disfavor solubility [29].At pH 10, there are decreasing of PS in all protein isolates (69.98-82.16%), respectively. This might be attributed by the presence of more aggregated or denatured proteins [17 and 30]. The PS values for all PI were comparable with PS of cowpea PI obtained by Shevkani et al.[17].

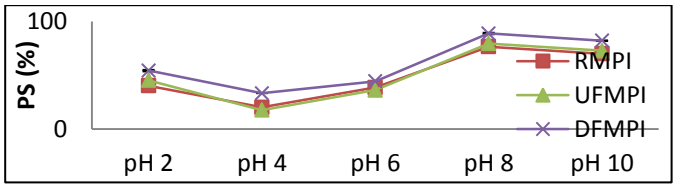

Figure 5. Protein solubility (PS) for all PI at pH 2 -10

Table 2. Some functional properties and In- Vitro protein digestibility of protein isolates

Parameters	Sample		
	RMPI	UFMPI	DFMPI
BD (g/ml)	0.43 ± 0.0007^{c}	0.38 ± 0.0001^{b}	0.25 ± 0.017^{a}
WOC(ml/g)	2.97 ± 0.06^{b}	2.32 ± 0.10^{a}	2.19 ± 0.01^{a}
OAC (ml/g)	2.80 ± 0.27^{a}	$2.94 \pm 0.04^{a,b}$	3.30 ± 0.02^{b}
IVPD (%)	85.22 ± 0.70^{a}	87.33 ± 0.15^{b}	95.08 ± 0.59^{c}

Notes: Means ± standard deviation followed by different letters in the same column is significantly ($p<0.05$) different.
BD: bulk density; WAC: water absorption capacity; OAC: oil absorption capacity; IVPD: *In-Vitro* protein digestibility

3.3 *In-Vitro* Protein Digestibility

The present study demonstrated that DFMPI had significantly higher protein digestibility followed by UFMPI and RMPI as shown in table 2. IVPD of DFMPI had similar value with soy protein isolate (95.3%) and chickpea protein isolate (94.1%) obtained by aprevious study[31]. Result for IVPD obtained by Giami et al.[18] proved that IVPD of fermented fluted pumpkin seeds concentrate significantly higher (77.3%) compared to raw fluted pumpkin seeds concentrate (69%). Fermentation could enhance the IVPD of protein isolates due to the partial degradation of complex storage proteins to more simple and soluble products [32] and the degradation of tannins, polyphenols and phytic acid by microbial enzymes [33]. According to Hag et al.[33], dehulling process decreases the anti-nutrients that interfere with the IVPD.

4. CONCLUSIONS

DFMPI has a high protein content (89.48%) and in vitro protein digestibility (95.08%) which compares with other PI. DFMPI also showed very good functional properties like oil absorption capacity, foaming and emulsifying properties. RMPI also had good water absorption capacity and foaming properties in acidic condition (pH 2). UFMPI also had good functional properties as comparable as DFMPI and RMPI. The good functional properties exhibited by all PI could make it good sources of protein ingredients for use in food systems.

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