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Effect of different pre-boiling treatment on *in vitro* protein and amino acid digestibility of mung beans [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Introduction: Mung beans [*Vigna radiata* (L.) Wilczek] are good sources of protein. Nevertheless, its protein quality is still questionable. This study aimed to determine the effect of different processes prior to boiling, on the *in vitro* protein and amino acid digestibility of mung beans by using a 6-hour enzymatic digestion. **Methods:** This study was based on the household method of the processes before boiling including unsoaking, soaking, and dehulling. Products from all treatment methods were analysed for proximate composition (moisture, crude protein, crude fat, ash, and dietary fibre) on a dry basis, naturally occurring anti-nutritional factors, amino acid composition, and digestibility of protein and amino acids. The amino acid composition and amino acid digestibility were used to calculate the dietary protein quality. **Results:** The treatments prior to the boiling of mung beans such as dehulling, soaking and without soaking, improved protein digestibility significantly by 10.8%, 10.3%, and 12.0%, respectively, when compared with that of raw mung beans (37.9%). Of the different mung bean pre-treatments, soaking seems to have the highest value of average indispensable amino acid (IAA) digestibility (55.4%), in particularly branched-chain amino acids (66.4%). However, there was no difference in the protein quality in terms of digestible indispensable amino acid score (DIAAS) across different treatment groups. **Conclusion:** The different processes performed on mung bean before boiling had only a slight impact on its amino acid digestibility and they rarely affected DIAAS values.

Keywords: Protein digestibility, protein quality, amino acid digestibility, DIAAS, pre-cooking treatment, mung bean

INTRODUCTION

Most developing countries meet their protein requirements by consuming mainly plant-based protein from sources such as legumes, seeds and pulses. These sources have recently received attention as they are environmentally friendly with a low-fat content and are cheaper than animal protein. However, the quality of plant protein is questionable (Henchion *et al.*, 2017). Protein quality is important for human life and it is likely to have

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several positive effects on growth and development as well as for promoting optimal health (FAO, 2013). High protein quality is needed to reduce childhood stunting which is associated with increased risk of metabolic diseases in later life (Arsenault & Brown, 2017). The protein quality of food depends not only on the content of its indispensable amino acids (IAAs) but also on its digestibility and, therefore, its availability (FAO, 2013).

Legumes are the important sources of protein, energy and dietary fibre. Mung bean [*Vigna radiata* (L.) Wilczek] is one of the major economic crops and is widely consumed in Asia, particularly in South, East and Southeast areas of the continent. It can be consumed as both dehulled and whole bean to make a variety of main dishes, snacks, and desserts. Generally, they are used to complement local staple foods such as rice which is deficient in lysine (Singh, D'souza & Yogitha, 2015). However, legumes have been reported to have low nutritive value because of the limited amounts of the sulphur-containing amino acids. It is also known that legumes contain several naturally occurring anti-nutritional factors, which are capable of inhibiting nutrient digestion and absorption (Gilani, Xiao & Cockell, 2012).

At the household level, soaking and dehulling are common processing techniques that are used before cooking legumes. Thermal cooking, i.e. boiling, improves the edibility and palatability of legumes. Several studies have shown that the elimination of anti-nutrients through thermal cooking treatments improve protein digestibility (Mubarak, 2005; Gilani *et al*, 2012). In addition, boiling can increase protein content in mung beans, kidney beans, chickpeas, and faba beans (El-Moniem, 1999; Wang *et al.*, 2010). With respect to amino acid composition, some studies have demonstrated increased concentrations of essential amino acids after cooking, while others have found reduced contents of methionine, tyrosine, and threonine (Candela, Astiasaran & Bello, 1997; Alajaji & El-Adawy, 2006). Although the boiling method has shown a reduction in anti-nutritive factors, a direct investigation of the impact of this process on the protein quality of mung beans has yet to be performed. In particular, studies of mung bean protein quality in terms of amino acid digestibility are scarce.

The Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation on Protein Quality Evaluation has recommended the use of the Digestible Indispensable Amino Acid Score (DIAAS) method, which is based on the true ileal digestibility of each amino acid (FAO, 2013). It is necessary to consider amino acids as individual nutrients due to the differences in their bioavailability. Nevertheless, the collection of ileal fluid from the last part of the small intestine in human studies is very challenging and this invasive approach is the reason for the limited number of such studies. *In vitro* amino acid digestibility is one of the techniques that has been proposed to replace the time-consuming, costly, and invasive procedure to collect digesta from the terminal ileum, used in the *in vivo* evaluation of amino acid digestibility (Brulé & Savoie, 1988). A previous study showed that processing methods such as extrusion, baking, and cooking had impacted differently on the amino acid digestibility and DIAAS values of red and green lentils (Nosworthy *et al.*, 2018). Furthermore, soaking mucuna beans in different solutions followed by autoclaving also improved amino acid and protein digestibility (Siddhuraju & Becker, 2005). However, to our knowledge, there is no study that has investigated the effect of different

preparatory processes prior to boiling, which is a household preparation method, on the amino acid digestibility of mung beans.

The aim of the present work was to determine the effect of different treatment methods prior to boiling on the *in vitro* protein and amino acid digestibility of mung beans.

MATERIALS AND METHODS

Materials

The seeds of mung beans [*Vigna radiata* (L.) Wilczek] were purchased from a local market of Thailand for the various treatments. Prior to the processing and cooking treatments, the seeds were cleaned, and the immature seeds, dust, and unwanted particles were manually removed.

Pre-thermal cooking treatment methods

No pre-cooking treatment

Untreated seeds were boiled in distilled water ratio 1:10 (weight/volume) at 100±2°C for 25 min on a hot plate, according to the procedure used in households.

Soaking

Seeds were soaked in distilled water for 7 h at room temperature, in accordance with the method of Devi *et al.* (2018). The soaked seeds were washed twice with distilled water and then boiled in the same way as the untreated mung beans.

Soaking and dehulling

Seeds were soaked in distilled water for 7 h at room temperature. After 7 h, seed coats were manually removed and the dehulled mung beans were then boiled in the same way as the untreated mung beans.

Preparation of samples

The thermally cooked samples were oven-dried at 50°C for 24 h. The unprocessed raw seeds and dried cooked samples were ground in an electric grinder at 3500 rpm for 1 min, passed through a 120-mesh screen and stored at 4°C in sealed plastic containers for further analysis.

Proximate composition

All samples were analysed for proximate composition (moisture, crude protein, crude fat, ash, and dietary fibre) in triplicate, by using standard methods of the Association of Official Analytical Chemists (AOAC) (AOAC International, 2016) and expressed on a dry basis. Moisture content was determined in accordance with AOAC method No. 990.19, by drying using a hot air oven at 100±2°C until a constant weight was achieved. Total nitrogen was analysed according to Kjeldahl's method and calculated into protein content (Nx6.25) (AOAC method No. 991.20). The factor of 6.25 was used to convert nitrogen to protein, based on a report of a Food and Agricultural Organization Technical Workshop (FAO, 2002). Total fat was analysed by acid digestion prior to continuous extraction using ether in Soxtec system (AOAC method No. 948.15, 945.16). Ash content was determined by incinerating all organic matter at 550±5°C (AOAC method No. 945.46). Carbohydrate per 100 grams was calculated using the following formula: 100-moisture-protein-fat-ash; energy was calculated using the Atwater factor (4 for protein and carbohydrate and 9 for total fat). Dietary fibre was analysed using the enzymatic gravimetric method (AOAC method No. 985.29).

Naturally occurring anti-nutritional factors

All samples were analysed in triplicate for phytic acid content, trypsin inhibitor activity, and tannin content.

Phytic acid

Phytic acid content was determined using the colorimetric method described by Gao (2007) with slight modification. Briefly, a 0.5 g sample was extracted with 10 mL of 2.4% hydrochloric acid (HCl) by using shaker at 500 rpm for 16 h and then centrifuged at 1000 g at 10°C for 20 min. One gram of sodium chloride (NaCl) was added to the crude acid extract and shaken at 500 rpm for 20 min. The extract was allowed to settle at -20°C for 20 min and then centrifuged at 1000 g at 10°C for 20 min. The clear supernatant was diluted with distilled water, following which 3 mL of diluted sample was added with 1 mL of Wade reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid). The resulting solution was mixed and centrifuged at 1000 g at 10°C for 10 min. The absorbance of colour reaction products was read at 500 nm on UV-visible spectrophotometer. Phytic acid content was calculated against the standard curve.

Trypsin inhibitor activity

Trypsin inhibitor activity was determined using a colorimetric method (AACC International, 1999). One gram of sample was extracted in 50 mL 0.01 N sodium hydroxide (NaOH) by stirring for 3 h. The extraction solution was diluted with distilled water and trypsin solution was added at 37°C, following which benzoyl-DL arginine-p-nitroanilide (BAPA) was added as a substrate. After 10 min, the reaction was stopped by adding 1 mL acetic acid. The final solution was filtered through Whatman® filter paper No.2, before measurement of absorbance at 410 nm using spectrophotometer. Trypsin inhibitor activity was reported in terms of trypsin units inhibited. One trypsin inhibitory unit (TIU) was defined as an increase of 0.01 absorbance units per 10 mL of the reaction mixture.

Tannin

Tannin was determined colorimetrically by the vanillin-HCl method (Burns, 1971). All samples were extracted with methanol at 28°C for 12 h. The decanted methanol extract was made up to 25 mL and filtered with Whatman® filter paper No.1. One mL of the extract was then treated with 5 mL of reagent mixture (1:1, 4% vanillin in methanol and 8% concentrated HCl in methanol). The absorbance of the resultant colour was read on a spectrophotometer at 500 nm after 20 min, using catechin as the reference standard. The tannin content was then calculated from a standard curve.

Amino acid analysis

The amino acid composition was determined by AOAC method No. 994.12 (AOAC International, 2016). Briefly, protein sources and dialysates were suspended in 10 mL of 6 M HCl with nor-leucine as an internal standard, in vacuum hydrolysis tubes at 121-123°C for 3 h. The suspension was diluted to 20 mL with 2 M NaOH and then filtrated through Whatman® filter paper No.42 and syringe filter with pore size 0.45 µm; 100 µL of filtrate was derivatized by N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) prior to injection to gas chromatography-mass spectrometry (GC-MS/MS).

The instrumental conditions for the GC-MS/MS (mass spectrometer) analyses were as follows: helium carrier gas was passed at a flow rate of 3.0 mL/min through the Hewlett-Packard HP-5MS column (30 m x 0.25 mm (5%-phenyl)–dimethyl-polysiloxane column, film thickness 0.25 µm). The

inlet temperature was set at 250°C. Two µL of prepared sample was injected using split mode in the ratio 3:1, with split flow rate of 3 mL/min. The GC oven temperature was increased from 100°C to 300°C at 15°C /min. The MS source and quadrupole temperatures were set at 230°C and 150°C, respectively. The MS was run in the multiple-reaction monitoring acquisition mode. Data were obtained in the full scan mode with a scan range from m/z 50 to 550. Data were collected and integrated with a personal computer using MassHunter Software.

The contents of amino acids were analysed in duplicate and presented as mg/g protein. The IAAs of each sample were compared with the recommended reference patterns for children aged 6 months to <3 years, older children aged ≥3 years, adolescents, and adults (FAO, 2013).

In vitro protein and amino acid digestibility evaluation

The *in vitro* digestion procedure was performed in triplicate and based on a two-step proteolysis with pepsin and pancreatin as described by Brulé & Savoie (1988), with minor modification. Each sample containing 250 mg protein (40 mg nitrogen) was suspended in 16 mL of 0.1 M HCl and stirred for 10 min at 37°C. The pH was adjusted to 1.9, and the hydrolysis reaction was initiated by adding 1 mL pepsin solution (1 mg/ml in 0.1 M HCl) and carried out for 30 min. The enzymatic reaction was stopped by adjusting the pH to 7.5 with 1 M NaOH and then poured into the dialysis bag [Spectra/Por® 6, molecular weight cut-off (MWCO) of 1 kDa, Spectrum Laboratories, Inc., LA, CA] of the digestion cell. The pancreatin solution (10 mg/ml in 0.01 M sodium phosphate buffer solution, pH 7.5) was added to the mixture. The digestion products were dialysed to the outer compartment of the cell and collected by circulating 0.01 M sodium phosphate buffer solution (pH 7.5) at a rate of 1.4 mL/min with a peristaltic pump for 6 h at 37°C.

The collected solution was evaporated by a rotary evaporator (Model R-124, BÜCHI, Switzerland) and adjusted to a final volume. Amino acid contents of the collected solution were determined according to the procedure mentioned earlier. Nitrogen contents of the dialysate were measured by Kjeldahl's method. The *in vitro* protein and amino acid digestibility were calculated by the following formulae:

$$\text{Protein digestibility (\%)} = \frac{\text{protein in dialysed sample} \times 100}{\text{protein in sample}} \quad (1)$$

$$\text{Amino acid digestibility (\%)} = \frac{\text{amino acid in dialysed sample} \times 100}{\text{amino acid in protein sample}} \quad (2)$$

Dietary protein quality determination

On the basis of protein quality, Amino Acid Score (AAS) and DIAAS were calculated as follows:

$$\text{AAS} = \frac{\text{amino acid content in 1 gram of test protein}}{\text{amino acid content in 1 gram of reference protein}} \quad (3)$$

$$\text{DIAAS} = \text{lowest digestible indispensable amino acid reference ratio} \quad (4)$$

The recommended reference pattern of IAA profile was the amino acid requirement pattern for children aged 6 months to <3 years (FAO, 2013).

Statistical analysis

All data were expressed as mean±standard deviation (SD). Statistical differences between treatments were analysed by using one-way analysis of variance (ANOVA) and Tukey's test where

Table 1. Proximate composition and naturally occurring anti-nutritional factors of mung bean (per 100 g dry weight)

Component	*Raw*	*Cooked*		
		Unsoaked	*Soaked*	*Soaked & dehulled*
Moisture (g/100 g fresh sample)	10.36[a]	61.76[b]	71.85[c]	72.40[c]
Proximate composition				
Energy (kcal)	394[a]	399[b]	397[c]	404[d]
Crude protein (g)	25.95[a]	27.37[b]	27.66[b]	29.04[c]
Crude fat (g)	1.62[a]	1.53[a]	1.15[b]	2.18[c]
Total carbohydrate (g)	68.97[a]	68.91[a]	69.05[a]	67.09[b]
Ash (g)	3.47[a]	2.18[b]	2.14[b]	1.69[c]
Dietary fibre (g)	12.15[a]	12.86[b]	15.16[c]	13.60[d]
Natural anti-nutritional factors				
Phytic acid (mg/g)	1.28[a]	0.86[b]	0.79[b]	0.71[b]
% reduction		32.38	37.85	43.95
Trypsin inhibitor (TIU/mg protein)	15.47	ND	ND	ND
% reduction		100	100	100
Tannin (mg/g)	2.40[a]	0.76[bc]	0.64[c]	0.97[b]
% reduction		68.23	73.15	59.36

Data were expressed as mean. The values within the same row with different superscript letters showed significantly differences between treatments at $p<0.05$, by one-way ANOVA with Tukey test. ND-Not detected

$p<0.05$ was considered as statistically significant. Statistical analysis was performed using SPSS software version 17.0 (IBM Corp., Armonk, NY).

RESULTS AND DISCUSSION

Proximate composition and naturally occurring anti-nutritional factors of mung bean

The proximate composition of raw and cooked mung bean seeds is presented in Table 1. The protein content of mung beans ranged from 26 to 29 g/100 g dry matter in this study. These are similar to the results reported by Dahiya *et al.* (2015). Boiling dehulled mung beans resulted in significantly higher protein and fat content, but significantly lower ash content on a dry basis ($p<0.05$) compared to raw beans. This was similar to what was reported by El-Moniem (1999). Removal of seed coat, which contained less protein and fat, would proportionally increase protein and fat in the dehulled seeds. Furthermore, the loss of soluble solids through boiling into water might improve the protein, fat, and total dietary fibre concentration in cooked seeds. Our findings are consistent with those of Wang *et al.* (2010) who demonstrated that cooking various beans and chickpeas in boiling water could significantly enhance protein, fat, and total dietary fibre content. The decrease in ash content might be attributed to the diffusion of certain minerals into the cooking water (Mubarak, 2005).

Cooking or boiling of beans is the most common method of preparation in a home setting. It decreases most of the naturally occurring anti-nutritional factors as shown in Table 1. Trypsin inhibitor activity was found to be 15.47

TIU/mg protein in raw mung beans, a value which was similar to that reported by Dahiya *et al.* (2015). Due to its thermolabile nature, trypsin inhibitor was completely destroyed by the boiling (Mubarak, 2005). It has been suggested that trypsin inhibitor was inactivated and destroyed by moist heat but not dry heat. Decreases in trypsin inhibitor levels would also be due to leaching that occurred during soaking and cooking in water (Dahiya *et al.*, 2015).

Boiling significantly decreased tannin and phytic acid content by 60-73% and 32-44 %, respectively. Similarly, Mubarak (2005) reported that boiling reduced the tannin and phytic acid content of mung bean seeds by 46% and 25%, respectively. The reduction in phytic acid content during cooking or autoclaving might be due to the loss of divalent metals (potassium, calcium, phosphorus, magnesium, iron, and manganese) which bind as the phytate-cation protein complex demonstrated by Mubarak (2005). It is possible that mung beans that are pre-soaked and dehulled prior to undergoing hydrothermal treatment may reduce that complex even further. The present study showed that the combination of soaking and dehulling of mung beans lowered the phytic acid content compared with all other treatments. However, the reduction in the tannin content was higher in cooked whole mung beans than in dehulled mung beans. These slight differences were likely caused by the distribution of condensed tannin, which is more abundant in the cotyledons of mung beans than their seed coats (Luo *et al.*, 2016). The protein digestibility can be improved when the anti-nutritional factors are decreased by cooking methods (Mubarak, 2005).

Amino acid composition

GC-MS has been widely used for amino acid analysis in food due to its high-resolution simplicity of operation and speed of analysis (Jimenez-Martin *et al.*, 2012). The amino acid composition of raw and treated mung beans is presented in Table 2, along with the amino acid profiles of casein and the patterns of IAA requirements for children and adults for comparison, as suggested by the FAO (2013). Mung beans were abundant in all IAAs except sulphur amino acids, when compared with the FAO (2013) reference. The methionine content of raw mung beans in the present study was 0.95 g/100 g protein which was within the range of 0.5-1.9 g/100 g protein that was reported by Dahiya *et al.* (2015). The content of other amino acids, except cystine, histidine and aspartic acid, were also in agreement with this report. These differing results may be attributed to the different methods used for amino acid analysis by GC-MS in this study.

Casein, which was used a reference food protein, matched with all IAAs when compared with the FAO (2013) reference pattern for adults. However, when compared with reference pattern for children, it did not match with sulphur amino acids. The foremost amino acids in casein were histidine, glutamic acid, and proline. This study showed that casein contained significantly higher amounts of isoleucine, valine, methionine, tyrosine, threonine, glutamic acid and proline than all mung bean treatments. Nevertheless, the contents of lysine, phenylalanine, alanine, aspartic acid, and glycine in casein were lower than treated mung bean.

The results of this study indicated that boiling either soaked or unsoaked beans caused a slight increase in total IAAs. They were consistent with the results of the study by El-Moniem (1999) for mung bean seeds, and that of Alajajai & El-Adawy (2006) for chickpea seeds. The small increase in total amino acid content after boiling is similar to the increase in protein content after

Table 2. Amino acid composition of raw and cooked mung bean, casein and FAO (2013) recommended allowances (g/16 g N)

Amino acids	*Raw*	*Cooked*			*Casein*	*FAO (2013)*	
		Unsoaked	*Soaked*	*Soaked & Dehulled*		*Child*	*Adult*
Indispensable amino acid (IAA)							
Branched-chain amino acid (BCAA)							
Isoleucine	3.68[a]	3.62[a]	3.68[a]	3.79[a]	4.25[b]	3.20	3.00
Leucine	8.50[a]	8.75[a]	8.84[a]	8.75[a]	8.90[a]	6.60	6.10
Valine	4.71[a]	4.74[a]	4.65[a]	4.69[a]	6.04[b]	4.30	4.00
Sulphur amino acids (SAA)						2.70	2.30
Methionine	0.95[a]	1.11[a]	1.04[a]	1.16[a]	2.11[b]		
Cystine	ND	ND	ND	ND	0.37		
Aromatic amino acids (AAA)						5.20	4.10
Phenylalanine	5.23[a]	5.46[ab]	5.54[b]	5.66[b]	4.04[c]		
Tyrosine	2.76[ab]	2.63[a]	2.72[ab]	2.81[b]	4.93[c]		
Lysine	7.34[a]	8.15[b]	8.38[b]	7.96[b]	5.78[c]	5.70	4.80
Threonine	4.34[a]	4.15[a]	4.17[a]	4.09[a]	5.68[b]	3.10	2.50
Histidine	12.38[a]	12.07[a]	12.16[a]	12.74[b]	12.12[a]	2.00	1.60
Total IAA	49.90	50.67	51.18	51.66	54.22		
Dispensable amino acids (DAA)							
Alanine	4.30[a]	4.20[a]	4.22[a]	4.40[a]	2.88[b]		
Arginine	6.28[a]	6.22[a]	6.26[a]	6.16[a]	4.03[b]		
Aspartic acid	11.79[a]	11.85[a]	11.92[a]	11.76[a]	6.30[b]		
Glutamic acid	11.53[a]	11.34[a]	10.84[a]	10.73[a]	14.94[b]		
Glycine	4.31[a]	4.11[b]	4.08[bc]	4.00[c]	1.72[d]		
Serine	5.59[a]	5.51[a]	5.45[a]	5.30[a]	5.31[a]		
Proline	6.31[a]	6.09[a]	6.05[a]	5.99[a]	10.60[b]		
Total DAA	50.10	49.33	48.82	48.34	45.78		

Data were shown as mean of two independent analyses. The values within the same row with different superscript letters showed significantly differences between treatments at $p<0.05$, by One-way ANOVA with Tukey test. ND-Not detected.

cooking. These results varied from that of Mubarak (2005) who reported that all thermal processes such as boiling, autoclaving, and microwave cooking did not increase in total IAAs.

Several studies have shown that cooking decreased lysine and total aromatic amino acids, which may be explained by destruction, Maillard reaction, and cross-linkage reactions (Alajaji & El-Adawy, 2006). However, the present study found that boiled mung bean seeds were still higher in lysine and total aromatic amino acids which were similar to the results from study of El-Moniem (1999) which revealed that cooking mung beans at 100°C for 38.6 min increased the phenylalanine and tyrosine by 5.01-10.91% when compared with raw seeds. Consistent with this result, Nosworthy *et al.* (2018) reported that boiling red and green lentils for 25-35 min had higher lysine and phenylalanine content when compared with untreated samples. In contrast, Mubarak (2005) and Alajajai & El-Adawy (2006) reported that boiling at 100°C for 90 min slightly decreased lysine and total aromatic amino acids contents. Igwe *et al.* (2012) showed that boiling at 100°C for 12 h significantly (p<0.05) reduced lysine in *Prosopis africana* and *Ricinus communis*. This may imply that the length of boiling treatment or prolonged cooking also contributed to the alteration of amino acid composition in mung bean seeds.

Protein and amino acid digestibility

Digestibility is a critical major factor affecting the quality of dietary plant proteins. When certain peptide links are not hydrolysed in the digestive process, part of the protein is either excreted in faeces, or transformed into a metabolic product by gut microorganisms present in the large intestine (van der Wielen, Moughan & Mensink, 2017). Protein quality evaluation measures the proportion of amino acids that can be absorbed from the diet and utilized in the body. An *in vitro* digestion procedure was used to determine protein digestibility in this study. This method was compared with true faecal protein digestibility in rodents and found to have a significantly high correlation (p<0.001) (Rozan *et al.*, 1997).

The preparation step of oven-drying and grinding of samples before *in vitro* digestion, which used in our current study, was similar to that used in other studies (Mubarak, 2005; Ghavidel & Prakash, 2007; Kalpanadevi & Mohan, 2013). The hot air oven-drying at 50°C during the sample preparation should not have any effects on protein digestibility since the drying at 50°C does not result in conformational changes of the protein. Based on the study of Bax *et al.* (2012), protein will change its conformation at a temperature of 70°C and that will, in turn, increase pepsin hydrolysis. The grinding of samples was performed to simulate the mechanical digestion at the mouth by teeth. Thus, oven-drying and grinding of samples may be used for the sample preparation in the *in vitro* digestibility studies.

The raw mung beans had the lowest protein digestibility among all treatments (Table 3), whereas casein presented the highest protein digestibility. The domestic cooking methods of mung beans improved protein digestibility by approximately 11%. Similar results were obtained by Barroga, Laurena & Mendoza (1985) and Mubarak (2005) who showed that protein digestibility improved by about 8-13%. The improvement in protein digestibility of mung beans by thermal cooking may be attributed to the destruction or removal of anti-nutritional factors, resulting in the easier release of nutrients than raw beans and the alteration of protein structure through denaturation. Protein denaturation by thermal treatment

Table 3. Amino acid digestibility (%) and *in vitro* protein digestibility (%) of raw and cooked mung bean after a 6-hour enzymatic digestion

Protein and amino acids	*Raw*	*Cooked*			*Casein*
		Unsoaked	*Soaked*	*Soaked& Dehulled*	
Indispensable amino acids (IAA)					
Branched-chain amino acid (BCAA)					
Isoleucine	50.2^{ab}	53.7^{b}	60.0^{c}	54.8^{bc}	47.5^{a}
Leucine	60.6^{a}	63.1^{ab}	69.3^{ab}	65.7^{ab}	74.9^{b}
Valine	64.5^{ab}	63.5^{ab}	69.8^{ab}	71.9^{b}	59.8^{a}
Sulphur amino acids (SAA)					
Methionine	52.4^{a}	42.0^{bc}	48.0^{ab}	36.8^{c}	51.6^{a}
Cystine	ND	ND	ND	ND	ND
Aromatic amino acids (AAA)					
Phenylalanine	36.8^{a}	46.8^{b}	39.5^{a}	44.7^{b}	60.6^{c}
Tyrosine	ND	ND	ND	ND	79.2
Lysine	57.9^{a}	52.9^{ab}	53.4^{ab}	49.3^{b}	70.2^{c}
Threonine	45.3^{a}	56.4^{b}	52.7^{bc}	54.1^{bc}	50.5^{ac}
Histidine	26.4^{a}	53.1^{b}	50.1^{c}	48.8^{c}	70.4^{d}
Mean of BCAA	58.4^{a}	60.1^{ab}	66.4^{b}	64.1^{ab}	60.7^{ab}
Mean of IAA	49.3^{a}	53.9^{bc}	55.4^{c}	53.3^{b}	62.7^{d}
Dispensable amino acids (DAA)					
Alanine	52.3^{a}	63.7^{cd}	54.7^{ab}	59.5^{bc}	66.6^{d}
Arginine	ND	ND	ND	ND	ND
Aspartic acid	23.4^{a}	34.1^{b}	27.0^{c}	32.0^{d}	39.5^{e}
Glutamic acid	28.1^{a}	35.9^{b}	29.3^{a}	35.0^{b}	35.9^{b}
Glycine	39.7^{a}	54.7^{b}	50.9^{b}	55.5^{b}	70.1^{c}
Serine	18.7^{a}	40.1^{b}	37.8^{b}	38.7^{b}	41.6^{b}
Proline	44.4^{a}	54.7^{b}	61.5^{bc}	57.0^{b}	64.0^{c}
Mean of DAA	34.4^{a}	47.2^{b}	43.5^{c}	46.3^{b}	52.9^{d}
Mean of total amino acids	42.9^{a}	51.1^{b}	50.3^{b}	50.3^{b}	58.8^{c}
Protein digestibility	37.9^{b}	49.9^{a}	48.2^{ab}	48.7^{a}	58.4^{a}
Relative protein digestibility (compared with casein)	65.2^{b}	85.4^{c}	82.2^{c}	83.3^{c}	100.0^{a}

Data were shown as mean of three independent analyses for protein digestibility and mean of two independent analyses for amino acids. The values within the same row with different superscript letters showed significantly differences between treatments at $p<0.05$, by one-way ANOVA with Tukey test.

could also increases polypeptide chain flexibility and accessibility to attack by proteolytic enzymes (Negi, Boora & Khetarpaul, 2001).

Soaking and/or dehulling mung beans before cooking did not further improve protein digestibility. The protein digestibility of cooked mung beans was 48-50%, which was around 82-85% relative to that of casein. These results were consistent with those of Embaby (2010) who showed that soaking and dehulling did not affect the protein digestibility of cooked sweet lupin. However, that study showed improvement in protein digestibility of soaked and dehulled bitter lupin. Similarly, Kalpanadevi & Mohan (2013)

showed that there was no difference in protein digestibility after cooking unsoaked and soaked seeds. By contrast, soaking and/or dehulling of moth beans prior to pressure cooking improved protein digestibility by 3-6% when compared to the pressure cooking of unsoaked beans (Negi *et al.*, 2001). Deol & Bains (2010) found that the protein digestibility of pressure-cooked cowpea pods was slightly higher than boiled peas. These results may be attributed to the difference in cooking methods such as boiling and pressure-cooking.

For the determination of biological availability of protein in foods, the FAO/WHO Expert Consultation on Protein Quality Evaluation report recommended the use of the DIAAS by considering the true ileal digestibility of individual amino acids (FAO, 2013). To determine the amino acid digestibility, we simulated the intestinal absorption by using *in vitro* dialysis digestion. Amino acid digestibility of mung beans is shown in Table 3. The amino acid digestibility of mung beans also reflects the quality of its protein. When the amino acid digestibility of cooked mung beans was compared with that of casein, it was observed that they had lower the total amino acid digestibility than casein. Among mung bean treatments, soaked and boiled mung bean seemed to have the highest IAAs digestibility, which was 55.4%.

All mung bean treatment methods had similar branched-chain amino acid digestibility when compared to that of casein. Soaked mung beans and those that were soaked and dehulled exhibited isoleucine digestibility of 60.0%, and 54.8% and valine digestibility of 69.8%, and 71.9%, respectively, which were higher than those available from casein. These results were consistent with those of Brulé & Savoie (1988) who reported that isoleucine and valine digestibility of field peas, rapeseeds, and soybeans were higher than that of casein. However, phenylalanine and lysine digestibility of cooked mung beans was lower than those of casein even though the former contained more phenylalanine and lysine. Lysine and methionine digestibility tended to decrease after boiling whereas phenylalanine, threonine and histidine digestibility slightly increased after hydrothermal treatments.

In heat-processed food, methionine, cysteine, and lysine can be oxidized or react with compounds to form unavailable derivatives such as methionine sulphoxide, methionine sulphone, as well as cysteic acid. Rutherfurd & Moughan (2012) also reported that the available amino acid content determined by the difference between ingested amino acid and remaining undigested amino acid at terminal ileum may not be accurate because the unabsorbed amino acids contained in foods can revert to available forms during hydrolysis step of analysis leading to overestimation of available amino acids. Furthermore, for determining true ileal amino acid digestibility, the endogenous losses of amino acids need to be considered. These losses depend on the contents of dietary fibre, body weight of animal models and the alteration of bacterial nitrogen. Endogenous ileal amino acid losses are significant especially in malnourished people, elderly and patients with inflammatory bowel diseases (Gaudichon *et al.*, 2002).

Consequently, these factors may cause changes in the amino acid composition of ileal fluid and lead to a biased estimation of the endogenous amino acid loss. Therefore, an *in vitro* digestion procedure might overcome the downside of true ileal digestibility method due to the difference in the sample collection from dialysate (available) and digesta (unavailable), and reduce the bias in endogenous losses of amino acids.

Protein quality determination

The AAS and the DIAAS for the determination of protein quality are presented in Figure 1. The AAS and DIAAS values of soaked and cooked mung beans were 0.39 and 0.19, respectively for the sulphur amino acids. These scores indicated that AAS was higher than DIAAS, which was due to the digestibility correction. It may imply that the digestibility measurement of each amino acid is very important especially for plant protein. Similar results were obtained by Nosworthy *et al.* (2018) for red and green lentils. Our results indicated that the value of AAS did not reflect the quality of plant-based protein. According to the recommendation of the FAO (2013), DIAAS is a recent method that is used to measure the protein quality; however, this approach is based on ileal digestibility, which is very invasive rather than faecal digestibility.

The DIAAS values of raw, unsoaked, soaked, soaked and dehulled boiled mung beans were 0.18, 0.17, 0.19, and 0.16, respectively for the sulphur amino acids, which had no difference among all mung bean treatments. These results were in agreement with those of the study by Hodgkinson *et al.* (2018)

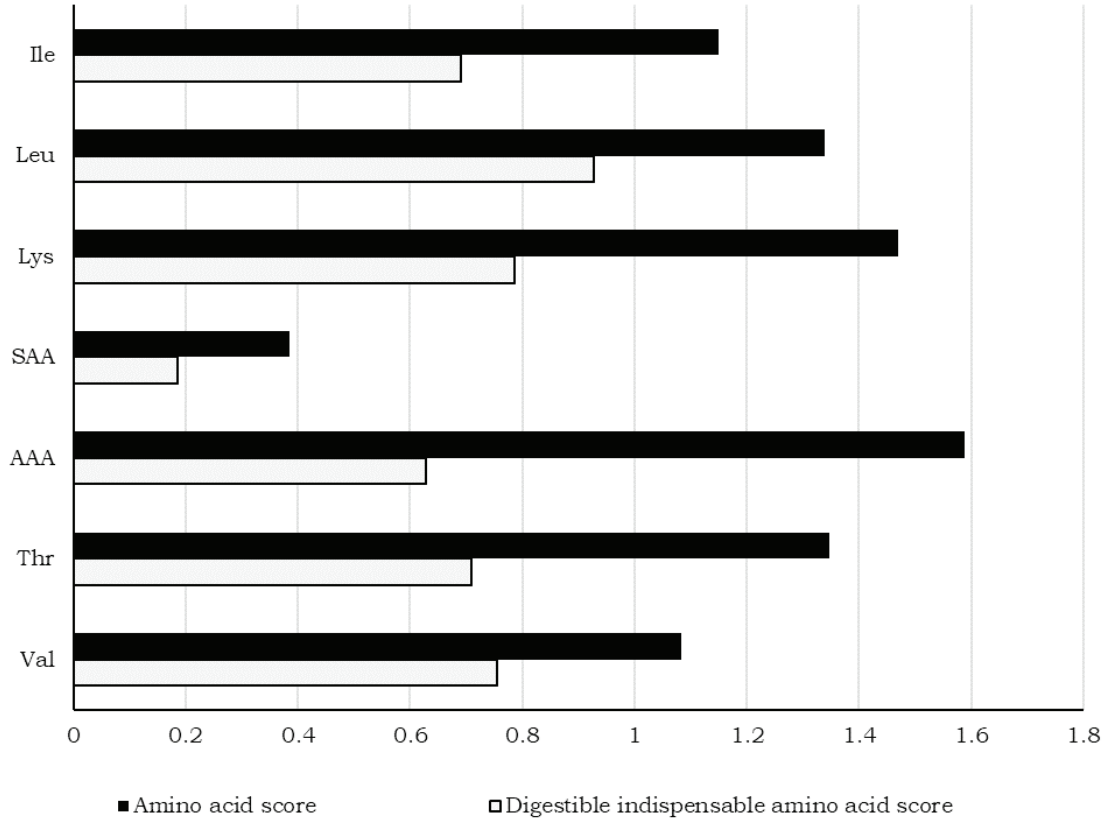

Figure 1. Protein quality determination of soaked cooked mung bean

Data were calculated using the reference pattern for children aged 6 months to <3 years. Ile: Isoleucine, Leu: Leucine, Lys: Lysine, SAA: Sulphur Amino Acid, AAA: Aromatic Amino Acid, Thr: Threonine, and Val: Valine.

who determined the effect of cooking processes on DIAAS of beef. They found that the DIAAS did not differ between raw and boiling meat but was lower in grilled and roasted beef. Consistent with these results, the previous study of Nosworthy *et al.* (2018) revealed that baking exhibited lower DIAAS than boiling and extrusion of legumes. It may be concluded that DIAAS would differ when the severe thermal cooking processes such as baking, grilling, and roasting are performed, but would not differ after boiling.

CONCLUSION

The processing techniques of soaking and dehulling prior to boiling increased the *in vitro* amino acid digestibility of mung beans, especially those of branched-chain amino acids. However, they did not further improve the overall quality of proteins determined by DIAAS. It could be due to the fact that boiling was not a severe thermal cooking method. The present findings concluded that boiling, where the cooking temperature does not exceed 100°C, is a good hydrothermal cooking procedure and it does not affect the protein quality. Mung beans were shown to have a small amount of sulphur-containing amino acids. As such, complementary amino acids from other food sources are needed for ensuring adequacy of these amino acids. This study did not determine the tryptophan concentrations of all samples due to the limitation of the amino acid analysis method. The *in vitro* digestion method should be further validated in terms of amino acid digestibility and compared with *in vivo* method to determine true ileal amino acid digestibility. However, the *in vivo* technique in animals or humans is invasive, costly, requires specific instruments and is time-consuming.

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Authors' contributions

AP, performed the study, analysed the data and drafted the manuscript; WK, designed the study, conducted the data interpretation, and reviewed the manuscript; JK, designed the methodology and analysed the data; AK, advised on the methodology and the data analysis; PC, advised on the methodology.

Conflict of interest

The authors declared no conflict of interest.

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