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Extraction, physicochemical characteristics and functional properties of Mung bean protein

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ABSTRACT

The physicochemical characteristics, functional properties and amino acid composition of protein extracts from Mung bean were determined and analyzed for discovering its potential in the food industry. The highest efficiency (73.25%) of extracting the Mung bean protein isolate (MPI) was achieved under the buffer-to-sample ratio of 15.0 mg/mL, pH value of 9.0 and extraction temperature of 40 °C. Results showed that the extracted protein had a good solubility, water holding capacity (WHC), oil absorption capacity (OAC), emulsifying and foaming properties. Comparing to the albumin, MPI showed a similar solubility profile with the minimum solubility at pH 4.0–5.0 and the maximum solubility at pH 6.2–7.2; and MPI also exhibited a better functional properties, particularly the capacity and stability of emulsifying. MPI also showed comparable water holding capacity (WHC) and oil absorption capacity (OAC) to commercially available soy protein isolate. The main structure of MPI and albumin contained intramoleculara-helix, β-sheet by Fourier transform infrared spectroscopy (FTIR) and circular Dichroism (CD) spectra analysis. In addition, MPI and albumin were predominant with the Pro, Glu, Arg, Leu and Phe, which could be considered as a high quality of natural protein. These results indicated that MPI and albumin could be used as potential nutraceutical or ingredient of functional and health-promoting foods.

1. Introduction

Mung bean (*Vigna radiate* (L.)) belongs a leguminous plant which has a cultivation history exceeding more than 2000 years in China. It is consumed as a whole snack, bean sprouts, or bean noodles in Asia. Mung bean is one kind of prevalent food in China because of its biological function, such as detoxifying, cholesterol-lowering, anti-tumor and anti-inflammatory activities (Dahiya et al., 2015; Kudre, Benjakul, & Kishimura, 2013; Reddy & Yang, 2011). It is rich in vitamins, minerals, proteins, and essential amino acids (Zhong, Lin, & Wang, 2012). However, the use of Mung bean as a raw material in snacks has been documented (Park, Choe, & Kim, 2012) and much focus has been placed on researching the properties of Mung bean starch rather than Mung bean proteins (Ahmed, 2012). People's interest about natural plant-derived proteins continuously increases as the as the growth of the consumers' anxieties about the food security and the rising cost of animal-derived proteins. It was reported that dietary proteins in seeds were considered to be a significant biologically active protein (Henry and Kettlewell, 1996).

In Asia, the product extracted from Mung bean is mainly starch instead of protein (Wang et al., 2016). In starch process, protein is thrown away as a by-product, which results in a waste of protein resources. However, as a plant-derived protein, Mung bean protein is the second most abundant component (25–28%) in bean grain after starch (Khaket, Dhanda, & Jodha, 2015). It contains sufficient quantities of all amino acids including lysine, the levels of which quite approach to the egg (Siemensma, Weijer, & Bak, 1993), except methionine, cystine, and tryptophan (Coffmannil & Garciaj, 1977). MPI, mainly contains globulin, albumin, gliadin and glutelin, with high content of albumin. And albumin amino acid pattern is closed to human body, called a kind of high-quality natural plant protein (Wang et al., 2011). Nevertheless, as a protein complement, the bean smell and dark color restrict its use. Using MPI and full peeling

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the beans before milling them into flour partly overcome this problem (Thompson, Hung, & Wang, 1976).

Functional characteristics of protein play a significant role in foodstuff processing and food product formulation. However, to get a better understanding of the physicochemical characteristics of proteins is still a necessity. Previous studies have showed that functional properties may be responsible for their full utilization (Meyer & Meyer, 1966). These characteristics mainly including emulsification, water/oil combination, foam formation are influenced by the physicochemical characteristics of protein like molecular size and structure, including the separation method of protein, ionic strength, pH, and other ingredients during food processing system (Yu, Ahmedna, & Goktepe, 2007). The characteristics of proteins are closely related to their utilization and functions, which is necessary to make it possible to be used successfully as ingredients in food systems (Yu et al., 2007). MPI has been shown desirable functions, such as emulsibility, foaming and water absorption (El Adawy, 1996). Nevertheless, there is limited available information concerning the development and function of albumin. Improvements in those functions would make MPI and albumin more desirable as a food supplements. In this regard, it is necessary to study the physicochemical properties of the MPI and albumin, to provide a clearer interpretation of its properties and potential utilization in functional food products.

In the present study, Mung bean protein is mainly extracted by alkaline extraction and acid precipitation Response surface methodology (RSM) can be employed to optimize the extraction factors (mainly buffer-to-sample ratio, initial pH and extraction temperature), so a maximum yield of MPI would be obtained. The physicochemical characteristics and functional properties of Mung bean protein extracts were characterized using UV spectrum, circular dichroism (CD) and fourier transform infrared spectroscopy (FTIR). The functional properties, mainly the solubility, water holding capacity (WHC), oil absorption capacity (OAC), emulsifying and foaming properties of the extracted protein were analyzed. In addition, the quality and nutritional value of the amino acid composition of the protein extract were also assessed.

2. Materials and methods

2.1. Materials

Mung beans were from the Grain and Oil Processing Company (Jilin, China). Briefly, Mung beans were cleaned by removing foreign matter, then they were peeled and milled into flour, which was went through a screen to separate the coarse fibre particles.

Soybean oil was obtained from Yihai Kerry Oils & Grains Industries Co., Ltd (Shanghai, China) and was used to prepare oil-in-water emulsions. Dialysis bag was purchased from Sinopharm Company (Shanghai, China). Bovine serum albumin (BSA) was obtained from Sigma Chemical Company (St. Louis, MO, USA). Other chemicals and solvents utilized in this research were analytical reagent.

2.2. Preparation of Mung bean protein

2.2.1. Preparation of MPI

The MPI was extracted according to the method of alkaline extraction and acid precipitation described by Johnson and Brekke (Johnson & Brekke, 1983). Briefly, dispersions of Mung bean flour in water (5%, w/v) were adjusted to pH 9.0 at 25 °C, then it was centrifuged (4000 rpm, 10 min), then the supernatant was obtained. The extracts were combined followed by the adjustment in pH to 4.5 with 1 M HCl to deposit the protein. After removing the supernatant by decantation and freeze-dried, the proteins were recovered by centrifugation (4000 rpm, 15 min). After that, the freeze-dried protein was weighed and stored well until further analysis.

2.2.2. Preparation of albumin

Mung bean flour was mixed with water at a ratio of 20: 1 (v/w) and permitted to keep still for 1 h with mild mixing at 15 min intervals. It was centrifuged (4000 rpm, 10 min), and the supernatant was precipitated at pH 4.6 with 1 M HCl. The supernatant was centrifuged as before, and the precipitate was collected, dispersed in water and then dialyzed against ultrapure water at 4 °C for 24 h using dialysis tubing (1400 Da molecular weight cut off). Dialyzed protein extracts from the dialysis bag were freeze-dried (freeze-drier AL-PHA1-2, Germany) and used vacuum sealed and stored in vacuum dryer until further analysis.

2.3. Protein determination

The nitrogen contents of protein in Mung bean flour, MPI flour and albumin flour were measured by using Kjeldahl Apparatus (KDY-9820, Beijing, China), and then the protein contents were calculated according to the standard micro-Kjeldahl method (Helrich, 1990).

2.4. Experimental design

Box–Behnken experimental design (BBD) was applied to statistically evaluate main interaction and quadratic effects of the formulation ingredients on response and to optimize the combination of variables in this study. Three extraction variables, buffer-to-sample ratio (mL/g) (X_1), initial pH (X_2) and extraction temperature (X_3) and three levels, coded as 1, 0 and −1 for high, intermediate and low level. Coded levels and actual values of the independent variables were showed in Table 1.

The dependent variables were the yield of protein. The triplicates were conducted at all design points in ran-domized order. The levels of the independent variables utilized in the experimental design were presented in Table 1. Design Expert software package version 8.0.6 (State-Ease, Inc., Minneapolis, MN, USA) was utilized

Table 1
Box-Behnken design of the levels of factors, program and test results of RSM.

Independent variables	Coded symbols	Levels		
		−1	0	+1
Buffer-to-sample ratio (mL/g)	X_1	10	15	20
Alkali liquid pH	X_2	8.5	9.0	9.5
Extraction temperature (°C)	X_3	30	40	50

Run	Std	X_1 (mL/g)	X_2	X_3 (°C)	Y (actual values) (g protein/100 g extract)[a]	Y (predicted values) (g protein/100 g extract)[a]
1	4	20	9.5	40	73.35	75.37
2	8	20	9.0	50	75.04	74.03
3	2	20	8.5	40	73.97	72.51
4	12	15	9.5	50	69.62	70.51
5	14	15	9.0	40	73.32	73.30
6	7	10	9.0	50	66.49	65.82
7	15	15	9.0	40	74.32	75.02
8	3	10	9.5	40	68.51	67.16
9	1	10	8.5	40	63.51	64.30
10	9	15	8.5	30	67.75	67.63
11	5	10	9.0	30	64.59	65.82
12	11	15	8.5	50	66.84	67.63
13	6	20	9.0	30	73.59	74.03
14	13	15	9.0	40	77.32	77.67
15	10	15	9.5	30	72.05	70.50

[a] Values are Mean of three individual determinations.

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to analyze the experimental data (mainly analysis of variance, regression analysis, contour plot, and optimization of the data). All the experiments were carried out three times and the extraction efficiency was taken as the response, Y. In the present work, the extraction efficiency of proteins was expressed according to Eq. (1):

$$\text{Protein content}\ (\%) = [(V_1 - V_2) \times 0.0014 \times C \times F]/m \times 100 \quad (1)$$

where, V_1 = mL alkali back-titration of sample, V_2 = mL alkali back-titration of blank, C = mol/L normality of alkali, m = g quality of sample, F = conversion coefficient of nitrogen. The F value of mung bean flour is 5.71 (Du, Qiao, Wang, Lv, & Wang, 2016; Mosse, 1990; Tomé & Mirand, 2008) and that of mung bean protein flour is 6.25.

The experimental data were suitable for the second-order polynomial model below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} x_i x_j \quad (2)$$

where Y is the measured response variable association with each factor level combination; β_0, β_i, β_{ii} and β_{ij} are the model constant; x_i and x_j are the independent variables.

2.5. Physicochemical characteristics

2.5.1. UV spectrum

After dissolved in 100 mL deionized water, the proteins were scanned with an using UV–vis spectrophotometer (TU-1900, Purkinje General Instrument Co., Beijing, China) in the range of 200–800 nm.

2.5.2. Fourier transform infrared spectroscopy (FTIR)

Protein samples were desiccated prior to FTIR analysis, ground with potassium bromide at a 1/100 ratio (w/w), and pressed at high pressure into KBr pellet. FTIR spectra of the samples were recorded using Fourier-transform infrared spectrophotometer (Thermo Electron, USA) from 400 to 4000 cm^{-1} at room temperature. The measuring resolution was 4 cm^{-1} and iterations were conducted for 32 times.

2.5.3. Circular dichroism (CD)

CD spectra were gathered employing a Jasco J-810 spectrometer (Jasco Spectroscopic Co., Japan), for protein solutions diluted in 0.02 M sodium diphosphate without sodium azide. A quartz cuvette with a path length of 1 mm was utilized for 0.1 (mg/mL) protein solutions. Ellipticity (mdeg) data were obtained at 25 °C in continuous scanning mode in the wavelength of 190–250 nm. On the basis of at least two scans, the average spectrum for each sample was drew employing the Spectra Manager software. Secondary structure was predicted from the deconvolution of the average spectrum by the online server Dichroweb (Whitmore & Wallace, 2004).

2.6. Functional properties

2.6.1. Protein solubility

Following the method of Tsumura et al. (2005) with slight modifications, the solubility of the MPI and albumin were determined at various pH values (from 3.0 to 8.0). Briefly, 0.5 g of MPI and 0.3 g of albumin was added to 100 mL of distilled water, respectively, and the pH value was adjusted to 3, 4, 5, 6, 7, 8 using 1 M HCl, respectively, followed by centrifugation at 4800×*g* for 10 min after oscillating. Then, the supernatant was diluted 5 times. The protein level of the resulting solution was immediately recorded according to the method described by Grintzalis, Georgiou, & Schneider (2015) use bovine serum albumin (BSA) as the standard.

2.6.2. Water holding capacity (WHC)

Water holding capacity (WHC) of protein was determined following the method of Chandi and Sogi (2007). In brief, after adding 0.5 g (W) of protein to 10 mL (V_1) distilled water, the mixture was kept still for 80 min and then centrifuged. The free water was poured out, and its volume V_2 was recorded, and WHC was determined as follows:

$$\text{WHC}(g/mL) = (V_1 - V_2)/W \quad (3)$$

2.6.3. Oil absorption capacity (OAC)

A modified method of Chandi and Sogi (2007) was used to determine the oil absorption capacity (OAC) of the protein concentrate. Briefly, 0.5 g (W) of protein sample was dissolved in 5 g (W_1) of soybean oil, and stirred evenly with static set 30 min and the mixture was centrifuged at 4000*g* for 20 min after mixing every 5 min. Free oil was dislodged, and the quantity of free oil (W_2) was recorded. OAC was defined as weight of oil adsorbed per gram of sample, and determined by the following equation:

$$\text{OAC}(g/g) = (W_1 - W_2)/W \times 100 \quad (4)$$

2.6.4. Emulsion activity index (EAI) and emulsion stability

Emulsification activity of protein was determined based on the method of Surangna and Anal (2016). Protein sample (30 mL 5% (w/v) concentration) was dispersed in 10 mL soybean oil. The mixture was homogenized at 10,000 rpm for 2 min. Then 200 μL emulsion sample was mixed to 25 mL, 0.1% SDS immediately. By employing UV–vis spectrophotometer, the absorbance of the obtained solution was instantly recorded at 500 nm (TU-1900, Purkinje General Instrument Co., Beijing, China). Emulsifying activity index (EAI, expressed in m^2/g) and emulsion stability (expressed in min) were calculated using Eqs. (5) and (6) (Thiansilakul, Benjakul, & Shahidi, 2007), respectively.

$$\text{EAI}\left(m^2/g\right) = [2 \times 2.303 \times A_0/0.25 \times \text{Protein concentration}] \quad (5)$$

$$\text{Emulsion stability}(\text{min}) = [A_0 \times \triangle t/\triangle A] \quad (6)$$

where, A_0 = absorbance at 0 min, A_{10} = absorbance at 10 min, $\triangle A = A_0 - A_{10}$ and $\triangle t = 10$ min.

2.6.5. Foaming capacity and stability

Foaming properties were determined according to Kaushik et al. (2016) after minor revisions. 50 mL solution of the protein concentrate (20 mg/mL) was dispersed in a graduated cylinder. The solution was mixed with a homogenizer at 10,000 rpm for 1 min. Volumes were recorded before and after homogenization using a 100 mL graduated cylinder. Foaming capacity and foam stability were calculated using Eqs. (7) and (8) (Jain & Anal, 2016), respectively.

$$\text{Foaming capacity}(\%) = [(V_2 - V_1)/V_1] \times 100 \quad (7)$$

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$$\text{Foamstability}(\%) = [(V_3 - V_2)/V_2] \times 100 \quad (8)$$

where V_1 is the volume of the protein solution before homogenization, V_2 is the volume of the protein solution after homogenization, and V_3 is the volume of foam the protein solution remaining after 10 min at room temperature after homogenization, respectively.

2.7. Amino acid analysis

Amino acid composition can be used to measure the quality and nutritional value of a protein. Amino acid composition of the sample was identified by a Model L-8900 Amino Acid Auto-Analyzer (L-8900, HITACHI, Japan), as reported by Yin et al. (2010) after a few revisions. In brief, samples were hydrolyzed with 6 M HCl for 24 h in a sealed pipe and then the hydrolysates were eluted, ranging from 0.2 M, pH 3.25 to 0.35 M, pH 5.25. Total analysis time was 3.5 h per sample. The amino acid composition of g/100 g protein was reported. Each analysis was carried out three times.

2.8. Statistical analysis

All experiments were conducted three times and the data was reported as means of three values. Design-Expert (Version 8.0.6, Stat-Ease) and Origin Pro (version 8.0) software (Stat-Ease, Inc., Minneapolis, USA) were utilized for analyzing results. Analysis of variance was carried out by ANOVA Duncans new multiple range test was carried out to define the significance differences using SPSS 13.0 software (SPSS Inc., Chicago, USA). Significant differences were defined at the $P \leq 0.05$ level between the samples.

3. Results and discussion

3.1. Optimization of the MPI extraction conditions

Effects of three extraction parameters: the ratio of material to solution (X_1), initial pH value (X_2) and extraction temperature (X_3) on the protein production of MPI were investigated by BBD design. Based on the single factor experiment, the scope of each factor was determined by employing steepest ascent techniques. Table 1 showed the experimental matrix design, as well as the obtained results of protein production. Results indicated that the experimental protein yields ranged from 63.51% to 77.32% in various test conditions. The protein production appeared to be different according to the given extraction conditions.

3.1.1. Model fitting

For the sake of assessing the significance of the coefficient of the models, analysis of variance (ANOVA) was completed. From Table 2, it could be seen that the contribution of quadratic model was extremely significant ($p < 0.001$), which indicates that it is very similar with the experimental data.

Hence, this equation could be utilized to forecast the actual values of MPI extraction yield. Coefficient of determination (R^2) was 90.4% which indicated the adequacy of the applied model. Earlier studies had reported R^2 ranging from 71.00% to 95.20% (Mizubuti, Biondo, Souza, da Silva, & Ida, 2000; Sogi, Arora, Garg, & Bawa, 2003; Wanasundara & Shahidi, 1996) for flaxseed, pigeon pea and tomato seed. The adjusted coefficient of determination (R^2_{Adj}, 0.8275), indicated that more than eighty percent of the variation (82.75%) exists within the three related factors. Thus, the experimental results matched this equation well. The variation coefficient (CV, 2.38%) of this model showed it was suitable for the regression

Table 2
ANOVA for response surface quadratic model after optimization results.

Source	DF	Sum of squares	Mean square	F value	P value
Model	6	222.50	37.08	12.19	0.0012**
X_1	1	134.89	134.89	44.35	0.0002**
X_2	1	16.42	16.42	5.40	0.049*
X_3	1	1.25×10^5	1.25×10^{-5}	4.11×10^{-6}	0.998
X_1X_2	1	7.90	7.90	2.50	0.175
X_1X_3	1	0.05	0.05	0.02	0.904
X_2X_3	1	0.58	0.58	0.18	0.687
X_1^2	1	16.98	16.98	5.58	0.046*
X_2^2	1	33.39	33.39	10.98	0.011*
X_3^2	1	31.37	31.37	10.31	0.012*
Residual	8	24.33	3.04		
Lack of fit	6	15.66	2.61	0.6	0.733
Pure error	2	8.67	4.33		
Cor total	14	246.83			
$R^2 = 0.9014$					
$R^2adj = 0.8275$					
$cv = 2.38$					

* Significant ($p < 0.05$).
**Very significant ($p < 0.01$).

equation. Besides, the design of level intervals of the correlation factors is feasible according to the Adeq Precision of 10.232. The fitted quadratic model for MPI production incoded variables is shown in Eq. (9).

$$Y = 74.12 + 4.11\,X_1 + 1.43\,X_2 + 0.00125\,X_3 - 1.41\,X_1X_2 - 0.11\,X_1X_3 - 0.38\,X_2X_3 - 1.71\,X_1^2 - 2.57\,X_2^2 - 2.48\,X_3^2 \quad (9)$$

where Y represent protein yield (g) of extract obtained from 100 g of Mung bean flour (g protein/100 g extract), X_1, X_2 and X_3 are the actual values of the independent variables.

The linear effect of X_1 on protein extraction yield was extremely significant ($P < 0.001$), and those of X_2 was also significant ($P < 0.05$), but X_3 was not significant. The camber effects of X_1^2, X_2^2 and X_3^2 are all significant ($P < 0.05$). These results showed that the relation between experimental factors and MPI extraction yields were not linear. Both quadratic items and interactions have a huge effect on the dependant value. By comparing the effect of the monomial variance on protein extraction yields, the important order of relevant factors could be obtained and it was listed below: X_1> X_2 > X_3, ratio of material to liquid > initial pH value > temperature. That it may be short extraction time makes the influence of temperature on the experiment not significant.

3.1.2. Three-dimensional surface response graphs and contour plots analysis

To clearly understand the effect of the independent variables on the response of interest, three-dimensional (3D) surface response and contour plots for protein productions as a function of buffer-to-sample ratio and initial pH value at varying extraction temperature were illustrated in Fig. 1.

It can be seen that the pH value showed a quadratic effect on the protein production no matter what the extraction temperature was (Fig. 1). At extraction temperature of 30 °C, it was found the protein yields were increased with the increase of pH value from 8.5 to 9.1, and then slightly increased thereafter (Fig. 1A). pH value showed a very similar response surface pattern, but the protein production reached maximum when the extraction temperature was 40 °C. These remarkable effects suggested that the reduction of the extraction efficiency could be caused by the increase of pH value. It also showed that denaturation of the protein was likely to happen

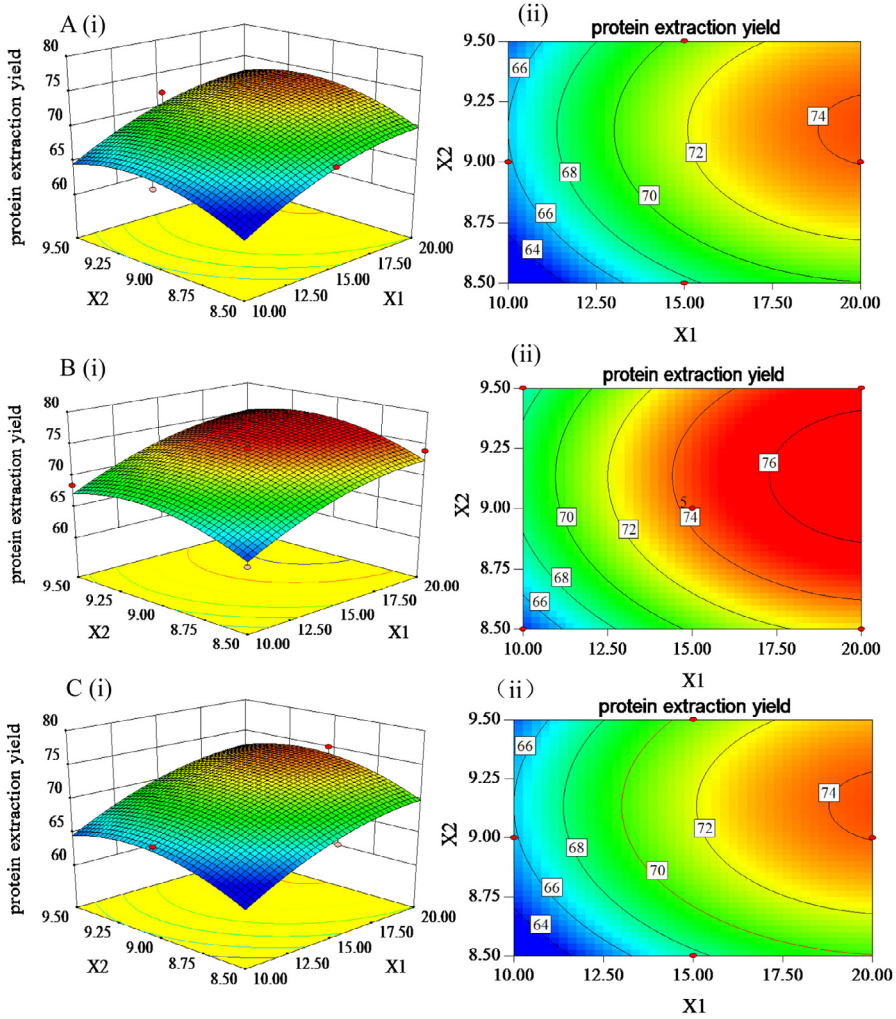

Fig. 1. Response surfaces: (i) three-dimensional plots and (ii) contour plots for extraction yield as a function of buffer-to-sample ratio (X1) and initial pH (X2) time at extraction temperature of (A) 30 °C, (B) 40 °C and (C) 50 °C.

at higher pH (>9.0). Above all, the pH value reaching 9.0 can obtain a higher protein yield.

As to the effect of buffer-to-sample ratio on protein production, its marked quadratic effect was presented along the pH value (varying from 8.5 to 9.5) in Fig. 1. It was found that protein yields first increased significantly and then decreased with improved buffer-to-sample ratio. According to the report, the improved extraction pH value did not help to raise protein production (Eromosele, Arogundade, & Eromosele, 2008; Mengru, Chen, Wang, Li, Li & Hong, 2016). From the above, it could be concluded that the extraction condition of pH 9.0 and a proper buffer-to-sample ratio would all result in a higher production of protein.

Changing of the extraction temperature didn't result in a significant ($p > 0.05$) changes in protein production. The range of protein yields were approximately between 64% and 72%, 67.50%–75.01%, 64.90%–73.50% under an extraction temperature of 30 °C, 40 °C and 50 °C, separately. Therefore, it suggested that extraction temperature of 40 °C was suitable for the extraction of protein.

3.1.3. Verification of predictive models

The optimum conditions for MPI were determined by a typical analysis of an experimental model, and obtained the following results: buffer-to-sample ratio 19.79 mL/g; initial pH value, 9.12; extraction temperature, 40 °C. Under the optimum conditions, the highest protein yield could reach 77.12 (g protein extract/100 g). A verification test was performed for confirming the feasibility of RSM. The optimum parameters with practical operational factors and convenience taken into consideration were modified as follows: buffer-to-sample ratio, 20 mL/g; initial pH value, 9.0; extraction temperature, 40 °C. The results of the validation test showed that protein production could reach 76.25 (g protein/100 g extract) closed to the predicted value 77.12 (g protein/100 g extract) with deviation below 3%. Therefore, it can be seen that the model was valid to optimize the process of protein extraction.

3.2. Determination of MPI and albumin from Mung bean

Total protein content of Mung bean was 23.73% on a dry weight basis measured by the standard micro-Kjeldahl method. The total protein content of Mung bean was in the range of 3–10% previously reported by Coffmann and Garciaj (1977). It is also comparable to reported values for other bean species such as *Vigna angularis* cv Takara (Adzuki bean) (21.60%) (Tjanjadi, Lin, & Breene, 1988), and *Phaseolus angularis* (Red bean) (24.76%) (Meng & Ma, 2002). Mung bean protein content in total protein powder measured by the kjeldahl determination was 86.94%. Albumn protein content also

detected by kjeldahl determination indicated that protein content was 92%. Mung bean protein content in total protein powder is very close to the 36–44% protein similar with rapeseed (Salunkhe, 1992; Shahidi, 1990).

The results indicated that Mung bean contained significantly high amounts of protein and its protein isolates could be added as nutritional and functional components to a great deal of food products.

3.3. Physicochemical characteristics

3.3.1. Ultraviolet analysis

Fig. 2A showed ultraviolet spectra of MPI and albumin in the range of 200–800 nm. It could be seen that the spectra of MPI and albumin were quite similar, with a weak absorption at 280 nm. This indicated that the solution contains protein.

3.3.2. FTIR spectrum analysis

FTIR spectroscopy, as an important tool to estimate the secondary structure of protein (Kong & Yu, 2007), could be used for providing information about the structural composition of protein. Fig. 2B shows FTIR spectra of MPI and albumin from Mung bean. It could be seem from that FTIR spectra of MPI and albumin were very similar, only a few characteristic peaks had changed (Fig. 2B), which indicated that MPI and albumin had some different amino acid residues. The spectrum of MPI and albumin presented characteristic peaks in the region of 1510–1650 cm^{-1} which might corresponded to C-O, N-H stretching caused by flexural vibration frequencies of the intra- and inter-molecular hydrogen bonds.

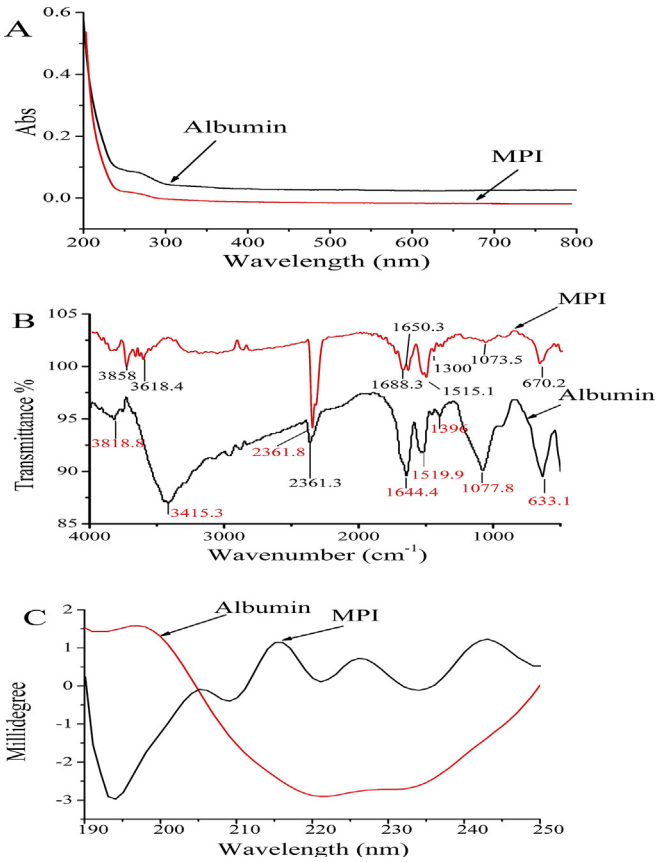

Fig. 2. Ultraviolet spectra of MPI and albumin (A), Fourier transform infrared (FTIR) spectra of MPI and albumin (B), Circular dichroism (CD) spectra of MPI and albumin (C).

Report displayed that hydrogen bond between functional groups of C-O and N-H could form a helical formation in the obvious peak (~1640 cm^{-1} and ~1550 cm^{-1}) (Gupta et al., 2006).

In the IR spectrum of MPI and albumin, there were typical protein bands: amide I (1600–1700 cm^{-1}), amide II (1500–1580 cm^{-1}) and amide III (1200–1400 cm^{-1}) (Silverstein, Bassler, & Morrill, 1981) were observed (Fig. 2B). Report showed that the typical protein bands corresponded to particular stretching and bending vibrations of the protein backbone (Siow & Gan, 2014). Predominantly, amide I was arise from a-helix (1650–1658 cm^{-1}) and β-sheet (1638 cm^{-1}, 1687 cm^{-1}), while N-H bending vibrations (60%) coupled to C-N stretching vibrations (40%) attributed to amide II. For amide III, it was obtained from a complex mix of a-helix (1290–1340 cm^{-1}) and β-sheet (1181–1248 cm^{-1}) along with random coil (1255–1288 cm^{-1}). Therefore, it was suggested that the peaks (1300 cm^{-1}, 1650 cm^{-1}, 1650 cm^{-1}, and 1688 cm^{-1}) found in the spectra of MPI corresponded to a-helix and β-sheet mentioned above. The spectrum of albumin showed two absorption peaks (1644 cm^{-1} and 1396 cm^{-1}), which suggested albumin only contains a-helix.

3.3.3. Circular dichroism (CD)

Circular dichroism spectra (CD) of the soluble fraction of MPI and albumin dispersions were presented in Fig. 2C. Albumin emerged the typical spectra of α+β proteins reported by Galazka, Dickinson, and Ledward (2000), which agreed with the measurement by Puppo et al. (2004). Intensity of CD peaks reflected the magnitude of ellipticity in the protein (Venyaminov & Yang, 1996).

The spectra of albumin showed a positive band near 190 nm with a zero crossing around 210 nm, and a negative band near 220 nm. These characteristic showed that secondary structures of albumin predominantly contain a-helix, with a positive band near 190 nm and β-sheet, with a negative band near 220 nm due to the appearance of the a-helix around 192 nm and β-sheet around 216 nm. It might be complicated composition of MPI which resulted in unanalyzable in CD spectra.

3.4. Analysis of physicochemical properties

3.4.1. Solubility

The most important functional property, owing to its effect on other functionalities like emulsification, foaming and gelation, is protein solubility. Therefore, protein solubility could have an influence on creating flavor, texture and nutritional value (Kinsella, 1982).

Fig. 3A shows the pH-dependent solubility profile of MPI and albumin. In pH range of 3–5, the solubility ability of albumin was obviously better than that of MPI at other pH value ($p < 0.01$). So, the pH range of solubility of albumin was wider than that of MPI. But MPI application will have better effect than albumin in vegetable protein beverage production because of the pH value of ordinary vegetable protein beverage at about 6.8–7.0, which is consistent with that of MPI (Constantinides & Adu-Amankwa, 1980). The results showed that the increase of pH value first decreased and then raised the solubility at pH 4–5 for MPI and albumin. The minimum solubility of MPI and albumin appeared at pH 4.6, 4.4, respectively. This result was similar to those reported for defatted peanut flour and various peanut protein isolates (Wu, Wang, Ma, & Ren, 2009), black bean protein isolates (Kudre et al., 2013), chickpea flours and protein isolates (Kaur & Singh, 2007).

At the isoelectric point (pI), protein is in an electrically neutral state because of the equal positive and negative charges on the molecular surface. Hydrophobic interactions between proteins are much larger than hydrophilic and hydration repulsive force produced by charged residues (Constantinides & Adu-Amankwa, 1980;

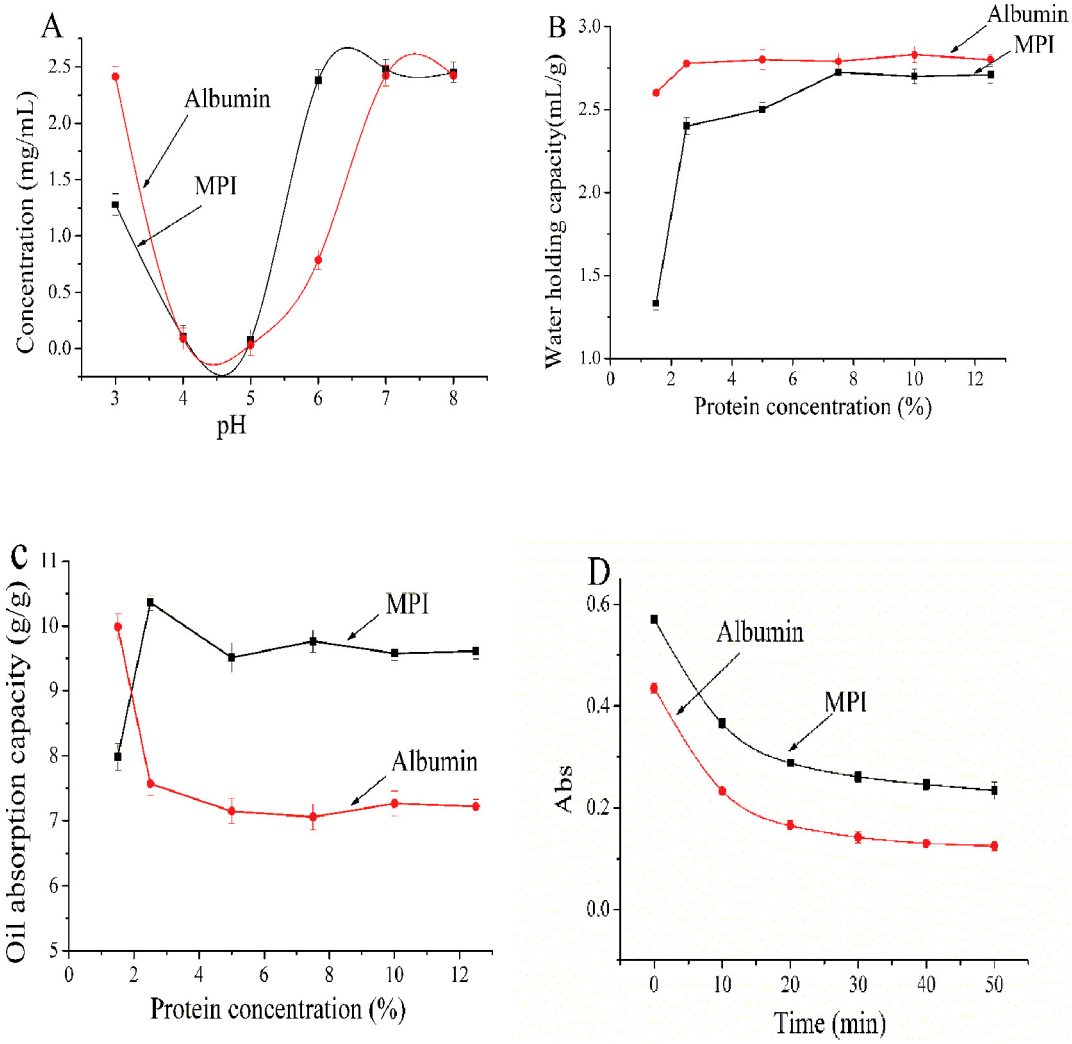

Fig. 3. Physicochemical properties of MPI and albumin extracted from Mung bean (A: Solubility, B: Water holding capacity (WHC), C: Oil absorption capacity (OAC), D: Emulsification and emulsion stability. Values were means ± SD (n = 3).

Eromosele, Arogundade, & Ademuyiwa, 2008). Therefore, the lowest solubility value obtained and aggregation occurs at pI. More far from pI the pH value was, more positive and negative charges appeared on the surface of the protein molecule. The hydrophobic interaction could be overcome by hydrophilic and hydration repulsion force, which maybe make MPI and albumin maintain a high solubility.

3.4.2. Water holding and oil absorption capacity (WHC and OAC)

Water binding capacity (WBC) could be affected by amino acid composition, protein conformation and the ratio of surface polarity to hydrophobicity (Seena & Sridhar, 2005). WBC is an important factor in viscous foods like soups, confectionery products, and in bakery products such as breads and cakes. In such products, imbibing water is needed without protein solubility, so that the desired viscosity is provided.

The WHC of MPI and albumin under various protein concentrations are displayed in Fig. 3B. The highest WHC of albumin and MPI were 2.75 mL g^{-1} and 2.62 mL g^{-1}, which was higher than fenugreek protein concentrate (1.56 mL g^{-1}) (El Nasri & El Tinay, 2007) and commercial soy protein isolate (1.26 g g^{-1}) (Amza, Amadou, Zhu, & Zhou, 2011), but less than that reported for fenugreek protein isolate (3.52 mL g^{-1}) (Abdel-Aal, Shehata, El-Mahdy, & Youssef, 1986). One possible reason for higher WBC in albumin could be its high phosphate groups and other polar groups, which could interact with water molecules to enhance the hydration of proteins (Ahmedna, Prinyawiwatkul, & Rao, 1999; Sathe, Deshpande, & Salunkhe, 1982). Moreover, albumin in this study had more amino acids containing phosphate, which could lead to higher WBC.

Fat absorption capacity is the binding of fat by nonpolar side chains of proteins (Sathe et al., 1982). The OAC of MPI and albumin under different protein concentrations are presented in Fig. 3C. The OAC of MPI was significantly higher than that of albumin except at the protein concentrations 1.5% ($p < 0.01$), although the OAC of two samples decrease a little bit with the increase of concentration. The OAC value was significant as a measure of the protein absorption capacity of oil, which can reflect the hydrophobic capacity of protein (Xu, Yu, & Jiang, 2014). The process of protein adsorbing oil can form a protein-oil complex (Adebowale & Lawal, 2004). As the protein concentration raised within certain limits, the protein side chain hydrophobic group increased and more protein–oil complexes were generated (Xu, Yu, Xu, Miao, & Jiang, 2014). When the protein concentration was increased, the OAC of two samples decreased a little bit in the concentration of 1.5–3% in our study. This finding was in accordance with the results of many other

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proteins, such as peanut protein obtained from *Arachin Conarachin* L (Yu et al., 2015) and Cowpea and Bambara Bean (Mune, Martin & Sogi, 2015). It was reported that with the increasing concentration of the protein, neighboring molecules can generate space steric effect that can prevent further oil adsorption by the protein and cause the OAC to decrease (Yu et al., 2015).

3.4.3. Emulsification and emulsion stability

The emulsion activity index (EAI) and emulsion stability are two key parameters of the emulsifying properties of protein. EAI is a measure of the capacity of formation of the emulsion created by protein, while emulsion stability is a measure of the ability of the protein to form a stable emulsion within a prescribed time period (Boye et al., 2010; Kumar, Ganesan, Selvaraj, & Rao, 2014). The emulsion activity and emulsion stability of MPI and albumin at different time are illustrated in Fig. 3D. At first, Mung bean protein had a good emulsion activity, then decreased with the increase of time. Especially after 10 min, Mung bean protein emulsification straightly declined, and the emulsification stability of MPI was relatively poorer than albumin.

3.4.4. Foaming capacity (FC) and foaming stability (FS)

Bubble is a kind of more porous membrane dispersion system with gas and liquid mixed. The bubble of 13 mL was generated after beating protein solution. The volume of the bubble got to 10 mL after 10 min; Protein solution appeared to be potent foaming agents with 26% FC, 76.9% FS. The FC value of Mung bean protein was lower than that of *Pisum sativum L.* protein isolates which was reported to have exhibited 270.8% FC (Moreno et al., 2011). This lower FC value of might be caused by the high levels of hydrophobic amino acid. On the other hand, the strength of protein film and its permeability for gasses affected the stability of foam forming (Moreno et al., 2011).

3.5. Amino acid profile of protein

The amino acid composition of MPI and albumin taken from Mung bean are listed in Table 3. According to the amino acid profile, Glu were the major component of amino acids in MPI and albumin, at approximately 16.21%, 20.78%, respectively (Table 3). A relatively greater quantity of Asp, Leu and Phe were demonstrated in the result, too. Conversely, the contents of val were lowest in MPI and albumin, at approximately 0.14%, 0.10% respectively, followed by sulphur-containing amino acids (i.e. methionine and cysteine). Besides, the contents of hydrophobic and uncharged amino acids in MPI (38.32%) were higher, followed by albumin (32.13%), than that reported for black bean and bambara groundnut protein isolate (Kudre et al., 2013). A report showed that hydrophobic amino acids definitely played an important part in the conformation of globulins and thermal stability (Gorinstein, Zemser, & Paredes-López, 1996). Except for val and total sulfur amino acids, MPI and albumin satisfied FAO/WHO (FAO, 1991) requirements.

Table 3
Amino acid composition of MPI and albumin from Mung bean.

Coding	Amino acid	Concentration (mol/kg)		Relative amount (%)	
		MPI	Albumin	MPI	Albumin
1	Aspartic acid (Asp)	0.488	1.004	7.49	12.91
2	Threonine (Thr)	0.345	0.266	4.74	3.06
3	Serine (Ser)	0.316	0.490	3.83	4.97
4	Glutamic acid (Glu)	0.956	1.462	16.21	20.78
5	Glycine (Gly)	0.335	0.461	2.90	3.34
6	Alanine (Ala)	0.356	0.451	3.66	3.88
7	Valine (Val)	0.010	0.009	0.14	0.10
8	Cysteine (Cys)	0.454	0.469	6.34	5.49
9	Methionine (Met)	0.053	0.065	0.91	0.94
10	Isoleucine (Ile)	0.357	0.374	5.40	4.74
11	Leucine (Leu)	0.646	0.687	9.77	8.71
12	Tyrosine (Tyr)	0.163	0.175	3.4	3.06
13	Phenylalanine (Phe)	0.402	0.421	7.65	6.72
14	Lysine (Lys)	0.459	0.480	7.74	6.78
15	Histidine (His)	0.182	0.192	3.25	2.88
16	Arginine (Arg)	0.387	0.416	7.77	7.00
17	Proline (Pro)	0.663	0.417	8.80	4.64
18	Totals			100	100

Table 4
Chemical score of the essential amino acid extracted from Mung bean.

Coding	Essential amino acid	Standard protein (mg/g)	Chemical score (%)	
			MPI	Albumn
1	Lys	55	140.7	123.3
2	Ile	40	135	118.5
3	Leu	70	139.6	124.4
4	Met+Cys	35	207.1	183.7
5	Phe+Tyr	60	184.2	163
6	Thr	40	118.5	76.5
7	Val	50	2.8	2

Table 4 shows that the chemical score of the essential amino acid of MPI and albumin is very high, and the chemical score of the essential amino acid of MPI is higher than that of albumin. The chemical score of the essential amino acid of the sample when compared to previous report (DRI, 2002) suggested a good comparison with most food sources. Based on the amino acid profile, chemical score of the Val were the lowest in MPI and albumin, at approximately 2.80%, 2.00%, respectively. However, chemical score of other six amino acids were far over recommended value. The chemical score of Met+Cys in MPI (207.1%) and in albumin (183.7%) were highest, followed by Phe, Tyr, Leu, Ile, which were far higher than the recommended value of FAO/WHO requirements. They could be used as a supplement in cereals to obtain the nutritional balance of these protein isolates, which are rich in val and sulfur-containing amino acids (Boye, Zare, & Pletch, 2010).

Mung bean protein has been identified as a effectively excellent plant protein for rich source of amino acids, and the essential amino acids in particular, in which many cereals are deficient (Kudre et al., 2013). In some situations, it contains a higher amount of amino acids than some conventional foods high in protein (DRI, 2002). Its essential amino acid composition was compared favourably with that of soybean, kidney bean and FAO/WHO reference protein, indicating that they could be added as a hopeful nutritional and functional components to a great deal of food products.

4. Conclusion

The extraction conditions were optimized by RSM successfully, and the best conditions were as follow, a buffer to sample ratio of 20 mL/g, pH 9.1, extraction temperature of 40 °C, and the highest yield of protein was 77.32%. The physicochemical properties of MPI and albumin showed that MPI was more superior and could be considered as a high quality of natural protein. In addition, MPI and albumin were the rich source of amino acids, which indicated that they could be used as a promising nutraceutical and food ingredient.

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