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**Techno-functional properties and allergenicity of mung bean (*Vigna radiata*) protein
isolates from Imara and KPS2 varieties**

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Abstract

Mung bean is an increasingly cultivated legume. This study compared mung bean varieties 'KPS2' from Thailand (Th) and 'Imara' from Tanzania (T) with a focus on protein composition, allergenicity, and techno-functional properties. Two rounds alkaline-acid extraction were performed to produce mung bean protein isolate (MBPI - Th₁/T₁ and Th₂/T₂), supernatant (S) and protein-poor residue (PPR). Mass spectrometric analysis revealed high abundance of 8s-vicilin and 11s-legumin in MBPI and S. Extraction removed considerable amounts of the seed albumin allergen but increased the relative abundance of cupins in MBPI. Higher vicilin levels were found in Th₁ samples, contributed to increased protein solubility above pH 6.5. Th formed stronger gels which were more stable at higher frequencies. In contrast, T proteins were structurally more flexible, leading to its improved foaming ability. This study provides the knowledge and methods for appropriate selection of mung bean varieties for various food applications.

Keywords: *Vigna radiata*; Food processing; Plant-based food protein; Protein structure; Gelation; Rheological properties

1. Introduction

The mung bean (*Vigna radiata* (L.) R. Wilczek, also known as green gram) is a legume native to India that is commonly cultivated and consumed in Southeast Asia, Africa, America, some areas of China and Australia (Li, Shu, Yan, & Shen 2010). The plant requires very little fertilization and water and is able to grow well with a relatively short growth cycle of around 70 to 90 days in warm climates (Dahiya et al., 2015; Ganesan & Xu, 2018). It is known for its health benefits such as antioxidant, antidiabetic, antihyperlipidemic and antihypertensive properties (Ganesan & Xu, 2018; Tang, Dong, Ren, Li, & He, 2014).

The major component of mung bean is starch at around 60%, which is most commonly consumed in the form of noodles or soup, and is rich in essential amino acids such as leucine and lysine (Yao et al., 2019). Proteins form the second most abundant component at around 20 to 24% of the mung bean (Tang et al., 2014). About 60% of proteins are globulins followed by albumins with around 25%. Globulins are grouped under 8s-vicilin (89% of the total globulin content), 11s-legumin (7.6%) and 7s-basic type (3.4%) (Yi-Shen, Shuai, & Fitzgerald, 2018). Seed albumin and 8s-vicilin have also been reported as major allergens, which can trigger allergic reactions in sensitized individuals upon recognition by human Immunoglobulin E (IgE) antibodies (Turck et al., 2021). For mung bean, the allergen database of the World Health Organization and International Union of Immunological Societies (www.allergen.org) lists these two allergens as Vig r 2 and Vig r 4, respectively, and two additional allergenic proteins (cytokinin-specific binding protein as Vig r 1 and pathogenesis-related protein as Vig r 6). However, mung bean is under-investigated as a food allergen source while for the closely related legume peanut (*Arachis*

hypogaea), 17 allergens are known, some of which could be cross-reactive allergens (Dramburg et al., 2023).

Mung bean is a well-studied legume on aspects of agriculture and food production, with many articles on its nutritional properties and its functional characteristics. While the allergenicity and techno-functional properties are highly dependent on the extraction and drying methods, the effect of the protein and globulin composition is not well explored in the literature. It has been reported that crops cultivated in different locations, water availability and climates can differ in nutritional composition and techno-functional properties. One study reported that mung beans cultivated in different regions of China produced crops of different nutritional content such as varying starch and amino acid content (Shi, Yao, Zhu, & Ren, 2016); corresponding extracted protein isolates yielded a diverse variety of protein content. The only other published study comparing different mung bean cultivars described significantly different functional properties such as protein solubility and foaming capacity (Li et al., 2010). However, these previous studies did not explore the mechanisms that affect the functional properties of the proteins.

This project sought to investigate how the techno-functional protein properties and allergenicity varies between mung beans of two different varieties. Mung bean varieties investigated in this project derived from Tanzania (Imara) and Thailand (KPS2).

2. Materials and methods

2.1. Materials

Mung beans from two different countries, Tanzania (T) and Thailand (Th), were purchased and analysed with a focus on the mung bean protein isolate (MBPI). The two mung bean varieties were identified as ‘Imara’ and ‘KPS2’ for T and Th respectively and were selected for this study

based on their difference in size and colour deviation (Figure S.1). Sodium dodecyl sulphate (SDS), Coomassie Brilliant blue R-250, dithiothreitol (DTT) and bovine serum albumin were purchased from Bio-Rad (Hercules, California, USA). Sodium hydroxide (NaOH), 37% hydrochloric acid (HCl), sodium acetate, triethylammonium bicarbonate buffer (TEAB), trichloroacetic acid, tris glycerine buffer, tris hydrochloride buffer, tris(2-carboxyethyl)phosphine (TCEP), iodoacetamide, trypsin, and light mineral oil were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation of mung bean protein isolate

Alkaline protein extraction was performed based on Wang et al. (2011) with slight modifications as shown in Figure S.2. In brief, mung beans were milled into a flour using a Thermomix (TM-5). 10% (w/w) mung bean flour solution was prepared in deionized (DI) water and the pH was adjusted to 9 using 1 mol/L NaOH. The solution was magnetically stirred for 2 hours at room temperature and then centrifuged at $6,000 \times g$ for 25 minutes. The protein rich supernatant was collected, and the pH was adjusted to 4.5 using 1 mol/L HCl. The solution was then centrifuged at $6,000 \times g$ for 25 minutes. The supernatant (S) was collected and stored in freezer prior to further testing. The residue was also collected, and the pH was adjusted to 7 before undergoing freeze drying (VirTis Benchtop Pro, SP Scientific, USA) at -80°C under vacuum to form the first extract of MBPI₁ (T₁ and Th₁). The residue from the first centrifugation step then underwent another round of extraction with the same method and the second extract of MBPI₂ (T₂ and Th₂) and the protein poor residue (PPR) were collected. Due to the low yield of MBPI₂, technological property testing was limited to solubility, zeta potential and Fourier transform infrared spectroscopy as discussed below.

2.3. Determination of protein contents

The nitrogen content of the extracted MBPI (T₁, T₂, Th₁ and Th₂) was determined using Gerhardt Dumatherm (C. Gerhardt GmbH & Co. KG, Königswinter, Germany), the protein conversion factor of 6.25 was used for the calculation of protein content (Du et al., 2018). According to Table S.1, the protein content of T₁ and Th₁ samples were found not significantly different (P<0.05) at 88.14% and 87.75% respectively. The second round of extraction lowered the protein yield to 83.42% for Th₂ and 76.45% for T₂.

Proteins from PPR and dried flour, MBPIs and S were extracted in 50 mmol/L TEAB with 5% SDS as described previously (Ruethers et al., 2023). The protein concentrations were determined using the Pierce™ bicinchoninic acid assay kit with pre-diluted bovine serum albumin as standard (Thermo Fisher Scientific, Rockford, Illinois, USA). All protein extracts were diluted to the same total protein concentration for subsequent analyses.

2.4. SDS-PAGE analysis

SDS polyacrylamide gel electrophoresis (SDS-PAGE) was conducted under reducing conditions to characterize the protein profiles of all extracts as described previously (Ruethers et al., 2022). Proteins (15 µg) were mixed with DTT-containing 5x Laemmli buffer before heating at 95 °C for 5 minutes. After centrifugation at 15,700 × g for 5 minutes, supernatants were loaded into a Criterion® AnykD TGX™ precast protein gel (Bio-Rad) for electrophoresis. The proteins were fractionated at 180 V for approximately 45 minutes with a corresponding Bio-Rad system containing Tris/Glycine/SDS buffer. The gel was stained with Coomassie Brilliant Blue R-250

(Bio-Rad) prior to imaging with an Odyssey ® M system (LI-COR Biosciences, Lincoln, Nebraska, USA).

2.5. Mass spectrometry analysis

The detailed protein composition in all extracts was investigated by mass spectrometry as described previously with modifications (Ruethers et al., 2019, 2021; Taki et al., 2023). In brief, 70 µg of proteins were loaded onto S-Trap™ micro columns (ProtiFi, Fairport, New York, USA) followed by reduction with TCEP, alkylation with iodoacetamide and digestion with trypsin following the manufacturer's instructions. Recovered peptides were analysed using an LTQ Orbitrap Elite (Thermo Scientific) coupled to an Ultimate 3000 RSLC nanosystem (Dionex). Label-free quantification was conducted for all proteins in each extract using MaxQuant v2.4.12 against all 43,372 mung bean proteins listed by the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/protein, “Vigna radiata”[porgn: __txid157791]). The abundance of all proteins in each sample is expressed in relative intensity-based absolute quantification (iBAQ%).

2.6. Techno-functional properties characterisation

2.6.1. Solubility and Zeta potential

1% (w/w) MBPI solution was prepared by dissolving freeze-dried MBPI samples in 10 mmol/L sodium acetate buffer followed by overnight incubation at 4 °C for full hydration. The pH of the protein solutions was then adjusted from 3 to 7 at intervals of 0.5 pH followed by centrifugation at 10,000 × g for 10 minutes.

The solubility was determined in accordance with Zhao et al. (2023). The protein concentration of the uncentrifuged protein solution, as well as supernatant was determined via the Bradford method (Kruger, 2009), calculated as shown in equation (1).

$$\text{Solubility}[\%] = \frac{\text{Protein conc. of supernatant}}{\text{Total protein conc.}} \times 100\% \quad (1)$$

Zeta potential of the protein solutions was measured with a nanoparticle analyser using disposable electrophoresis cells (Nanosizer SZ-100, Horiba Ltd., Kyoto, Japan).

2.6.2. Particle size analysis

The particle size distribution of the 0.1% (w/w) T₁ and Th₁ solution was measured in accordance with Schlangen et al. (2023) with slight modifications, using dynamic light scattering with a nanoparticle analyser (Nanosizer SZ-100, Horiba Ltd, Kyoto, Japan). The solutions were filtered with a 0.45 µm cellulose-acetate filter before measurements were taken.

2.6.3. Protein flexibility

The protein flexibility of T₁ and Th₁ samples was determined using the method as described by Sun et al. (2022) with slight changes. The samples were dissolved in pH 8 0.05 mol/L tris-HCl buffer at a concentration of 1mg/mL. Then, 1mg/mL trypsin solution was added in a ratio of 1:16 to the protein solutions. The solutions incubated at 38 °C for 5 minutes, 5% trichloroacetic acid was then added in a 1:1 ratio to stop the trypsinisation reaction. The solutions were centrifuged at 6000 × g for 10 minutes. The supernatant was collected, and the absorbance was measured at the wavelength of 280 nm as an indicator of protein flexibility.

2.6.4. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of all MBPI samples were obtained using a FTIR spectrometer (Cary 630, Agilent Technologies, California, USA) in the range of 650–4000 cm^{-1} , with 64 scans at a resolution of 4 cm^{-1} (Zhao et al., 2023). The amide I band region of 1600–1700 cm^{-1} was examined using peak deconvolution method with OriginPro Version 2023b software (OriginLab Corporation, Northampton, MA, USA) which revealed the structural components of the proteins via the Gaussian curve fitting function and integration of the peak areas of the respective components. The respective components were identified as follows: β -sheets (1610–1640 cm^{-1} , 1670–1680 cm^{-1}), α -helix (1650–1660 cm^{-1}), turns (1660–1670, 1680–1700 cm^{-1}) and random coils (1640–1650 cm^{-1}) (Long et al., 2015).

2.6.5. Least gelation concentration

6, 8, 10, 12 14 and 16% (w/w) protein solutions were prepared as described by Schlangen, Dinani, Schutyser, & van der Goot (2022) with slight modifications, by dissolving freeze-dried T₁ and Th₁ samples in DI water. The protein solutions were magnetically stirred for 2 hours, and 700 μl of each solution were transferred to a microcentrifuge tube prior to heating at 90 °C for 30 minutes and ice bath for 2 hours followed by inverting for observation.

2.6.6. Rheological properties

10% (w/w) MBPI₁ solutions were prepared by dissolving the extracted MBPI₁ (T₁ and Th₁) in DI water. The protein solutions were magnetically stirred for 2 hours and stored overnight at 4 °C. Before testing, the sample was adjusted to room temperature and stirred gently before it was loaded onto the rheometer measuring geometry. The viscoelastic properties of the protein solutions were measured using a rheometer (MCR302e, Anton Paar GmbH, Graz, Austria), with a 4° 40 mm

cone geometry. For all rheological measurements, the storage modulus (G'), loss modulus (G'') and loss factor ($\tan \delta$) values were obtained.

2.6.6.1. Temperature sweep

Temperature sweep of the protein solution was performed using a modified method as described by Brishti et al. (2020), at a constant frequency and shear strain of 0.3 Hz and 1% respectively, with the temperature ramping from 25 °C to 90 °C at 4 °C/min, isothermal at 90 °C for 10 minutes, 90 °C to 4 °C at 4 °C/min and finally isothermal at 4 °C for 15 minutes. The crossover temperature of G' and G'' which represented the initial formation of gel was obtained and recorded (Shevkani, Singh, Kaur, & Rana, 2015).

2.6.6.2. Frequency sweep

The frequency sweep was performed according to the method described by Tojan, Kaur, & Singh (2023) with small modifications. After the temperature sweep, the formed gel underwent a frequency sweep from 0.1 to 10 Hz at a constant shear strain of 1%.

2.6.6.3. Amplitude sweep

Finally, an amplitude sweep was performed from 0.01% to 100% shear strain at a constant frequency of 0.3 Hz (Tamm & Drusch, 2017). The end of the linear viscoelastic region (critical point) was denoted by the change in magnitude of G' of more than 3% (Shan, Chen, Federici, Jones, & Campanella, 2022). The crossover points where the G' value becomes lower than the G'' value is known as the gel breaking point, where the gel acts more liquid-like than solid-like as it starts to break and flow.

2.6.7. Heated and non-heated water holding capacity and oil holding capacity

The water holding capacity (WHC) and oil holding capacity (OHC) of the samples were measured in accordance with Zhao et al. (2023) with slight modifications. 1% (w/w) MBPI₁ solutions were prepared by dissolving T₁ and Th₁ samples in DI water or canola oil in pre-weighed centrifuge tubes. For non-heated conditions, the solution was left at room temperature for 1 hour and centrifuged at 4000 × g for 25 minutes. For heated conditions, the solution was heated at 95 °C for 1 hour and cooled under running water for 1 minute. The solution was then centrifuged at 4000 × g for 25 minutes. The supernatant was discarded and the resulting tube with residue was weighed. The WHC and OHC of the samples was calculated as shown in equation (2).

$$WHC \text{ or } OHC(g/g) = \frac{\text{Weight of residue (g)} - \text{weight of dry protein (g)}}{\text{Weight of dry protein (g)}} \quad (2)$$

2.6.8. Foaming capacity and stability

The foaming capacity and stability of the samples were measured using the method described by Liu et al. (2022) with some changes. 1% (w/w) MBPI₁ solutions were prepared by dissolving the extracted MBPI (T₁ and Th₁) in DI water. The solution was homogenized with an IKA homogenizer (IKA ULTRA-TURRAX T18 digital, Germany) at 15,000 rpm for 1 min. The volume of the foam was recorded at 0, 15, 30, 45 and 60 minutes. The foaming capacity was calculated using equation (3).

$$\text{Foaming capacity (\%)} = \frac{V_1 - V_0}{V_0} \times 100 \quad (3)$$

where V₀ is the total volume of protein solution before homogenization and V₁ is the total volume of the protein solution after homogenization.

243 The foaming stability was calculated using equation (4).

$$244 \text{ Foaming stability (\%)} = \frac{V_t}{V_1} \times 100 \quad (4)$$

245 where V_t is the foam volume after t mins.

246

247 2.7. Statistical analysis

248 The measurements in this paper were performed in triplicates and the results were
249 presented as the mean and standard error of mean. The data were processed using Minitab
250 statistical software, version 19.2020.2.0 (Minitab Inc., State College, PA, USA). To reveal
251 statistically significant differences between multiple samples, one-way analysis of variance
252 (ANOVA) was conducted, followed by Tukey's pairwise comparison module ($P < 0.05$) for normal
253 data distribution.

254

255 3. Results and discussion

256 3.1. Protein profiles of mung bean extracts

257 3.1.1. SDS-PAGE analysis

258 Figure 1A shows the SDS-PAGE profiles of all generated mung bean protein samples. The
259 most prominent bands were observed at around 50 kDa, consisting of 8s and 7s globulin based on
260 the molecular weight (Mendoza, Adachi, Bernardo, & Utsumi, 2001), in all extracts except S. Only
261 little differences in band intensities were visible between T and Th samples. MBPI sample T₂
262 appeared to contain more globulins as compared to corresponding Th₂ while PPR T samples
263 contained less globulins compared to Th. In S, a band at around 30 kDa, consisting of seed albumin
264 based on the molecular weight (Misra et al., 2011), was much more abundant than in any other

extract. Notably Th but not T S exhibited a 25 kDa band, which is presumably a globulin fragment and found also in all other extracts.

3.1.2. Mass spectrometry analysis and allergen abundances

Over 7,000 different peptides were detected after tryptic digestion, corresponding to 1,385–2,664 identified proteins in the mung bean extracts. Proteins were grouped based on properties and sequence identities, and their relative abundance in each extract was determined, with a focus on globulins and seed albumin (Figure 1B). The mung bean allergen Vig r 2 (8s globulin) was the most abundant protein in all extracts (19–45%) except S (2–3%) with a higher content in Th as compared to T samples in flour, MPBI T₁ and PPR, and similar content in MPBI T₂ (40%) and Th₂ (39%). 7s globulin was most abundant in Th S (12%) - much higher than in the corresponding T sample (3%) - and least abundant in flour (2–3%). Th MBPI samples contained more 7s globulin as compared to respective T samples with the biggest difference between Th₂ (9%) and T₂ (5%). All vicilins, including 7s and 8s globulin and not further defined conglycinin constituted 6–15% of all proteins in S, 39–42% in PPR, 56–63% in flour, and 71–73% in MBPI with the higher abundance for Th₁ as compared to T₁ samples as also suggested by SDS-PAGE. In contrast, Th₂ contained 66% vicilins while it was 67% in T₂. 11s globulin (glycinin G4), which is commonly described as legumin, made up less than 1% of proteins in S, 3% in PPR, 5% in flour, 6% in MBPI₂ and 7% in MBPI₁. Mung bean allergen seed albumin (Vig r 4) was most abundant in S (12–18%) and least abundant in MBPI₁ (less than 1%). In T₂, seed albumin was 3-fold more abundant (3%) as compared to Th₂ (1%) as also suggested by SDS-PAGE analyses. Flour and MBPI samples contained 65–81%, and S and PPR 25–50% seed albumin and cupin superfamily members vicilins and legumins, which are all commonly reported legumine allergens. However, neither legumins

and nor vicilins other than 8s globulin have been reported as mung bean allergens, which is likely a reflection of lower abundance and lack of molecular allergy research in this field (Verma, Kumar, Das, & Dwivedi, 2013). All T samples except T₂ contained less legumine allergens compared to corresponding Th samples. MBPI₁ exhibited highest legumine allergen content followed by MBPI₂, flour, PPR and S. Extensive processing, including hydrolysis and germination, was shown to reduce mung bean allergenicity (Budseekoad, Takahashi Yupanqui, Alashi, Aluko, & Youravong, 2019). No cytokinin-specific binding protein (Vig r 6) and only very little (0–0.2%) pathogenesis-related protein (Vig r 1) were detected in the extract. Both these mung bean allergens are frequently recognised by IgE antibody from birch pollen allergic patients (birch pollen-related food allergy) and are predominantly found in seedlings/sprouts but not in seeds, the major eatable plant part investigated in this study (Guhs et al., 2014). In contrast, seedlings/sprouts were shown to contain less vicilin (Gupta, Sathe, Su, & Liu, 2021). Trypsin inhibitors made up 15-90% of detected enzyme inhibitors and were 6- to 32-fold more abundant in T S as compared to other corresponding T samples, which was 6- to 13-fold for Th samples. Soaking of the mung bean flour have been known to reduce anti nutritional factor due to their water solubility, the reduction in trypsin inhibitor could have been due to the soaking in DI water during the extraction process (Samtiya, Aluko & Dhewa, 2020).

3.2. *Techno-functional properties characterisation*

3.2.1. *Solubility*

As depicted in Figure 2A, the trends of the solubility as a function of pH of all extracted MBPI was similar amongst all samples, with the lowest solubility (< 4%) at the pH range of 4.5 to 5.5 which corresponds to the isoelectric point of MBPI (Yi-Shen et al., 2018). The solubility then

increased by 5–6% at pHs of 4 and 6. The solubility of all samples was higher by 50–90% at pHs of 3.5 and lower, and pHs of 6.5 and above. Th₁ exhibited lower solubility at lower pHs and higher solubility at higher pH as compared to T₁. This phenomenon could be attributed to be due to different protein compositions. Th₁ contained more vicilin and legumin as compared to T₁, Chang et al. (2022) reported for green peas and chickpeas that a higher vicilin content contributed to the highest solubility at higher pHs due to the lower molecular weight of vicilin. According to Yang et al. (2023), a high legumin content generally decreases solubility, as legumin consists of an acidic and a basic subunit connected by a disulphide bond which are more likely to form aggregates.

3.2.2. Zeta potential

According to Figure 2B, the zeta potential of all the extracted MBPI increased at a lower pH and decreased as the pH increased. The isoelectric point for all the samples was between pH 4.5 and 5, which aligned with the solubility results in which it was at the lowest. When comparing the different samples, the isoelectric points of Th₁ and Th₂ samples were higher than of T₁ and T₂ samples. This result agrees with the past findings (Helmick, Hartanto, Bhunia, Liceaga, & Kokini, 2021; Shanthakumar et al., 2022), where the isoelectric point of pea protein legumin fraction was found to be higher than the vicilin fraction (5-6 vs 4-6), which could explain why the Th₁ and Th₂ samples with the higher legumin content had slightly higher isoelectric points.

3.2.3. FTIR analysis

According to FTIR analyses (Figure 3), β -sheets made up the majority of the secondary structures. The β -sheets content for T₁ and Th₁ samples was 47–48%, which was lower than for T₂ and Th₂ samples with 50–52%. α -helix content for T₁ and Th₁ samples was 34%, which was then

reduced in T₂ and Th₂ samples to 9–10%. The amount of turns in T₁ and Th₁ samples were 17% and increased to 38–40% for T₂ and Th₂ samples. There were no random coils observed in any MBPI. When comparing the first and second extraction (T₁ and Th₁ vs T₂ and Th₂), the percentage of α -helix decreased while the percentage of turns and β -sheets increased. In this study, a cause for the change in secondary structure was the increased use of chemicals during the second round of extraction to adjust the pH of the protein solutions. Chen et al. (2019) and Li et al. (2020) conducted experiments to study the effect of pH shifting on the secondary structure of different protein isolates. Both studies reported a decrease in α -helix at pHs of 10 to 12, and a slight increase in β -sheets and turns. It was stated that chemical treatments caused the unfolding and rearrangement of the protein molecules which led to the changes in secondary structures. In addition, the extra stirring and centrifugation performed during the second round of protein extraction could be a source of the mechanical stress which led to the changes in secondary structures. Mechanical stress has been known to affect the secondary structure of proteins (Hu et al., 2013; Jiang et al., 2014), which was reported to decrease α -helix content and increase the percentage of β -sheets and turns. It was mentioned that mechanical stress could have disrupted the molecular interactions between the structure of the proteins, which led to the change in the secondary structures.

3.2.4. Least gelation concentration

According to Figure S.3, the least gelation concentration of both T₁ and Th₁ was found to be at 10% (w/w) concentration with no observable differences between the two samples. In order to ascertain the differences in properties of the formed gel between the two samples, rheological property measurements were conducted on these samples with the same concentration used.

Rheological property measurements can be used to reveal the viscoelastic behaviour of a samples, structural networks as well as strength and stability of a gel formed. It can also provide information on the sample during the gel formation process.

3.2.5. Rheological properties

3.2.5.1. Temperature sweep

Figures 4A and 4B show the temperature sweep of T₁ and Th₁ 10% (w/w) sample solutions, respectively. At the beginning of the temperature ramp from 25 °C to 90 °C, the storage modulus (G') was lower than the loss modulus (G'') and the Tan δ value was higher than 1, which indicated that the samples was more liquid-like. As the temperature increased, the difference between G' and G'' gradually decreased and then increased until they reached a crossover point at around 44 °C. As shown in Table 1, the gelation temperatures obtained from these two samples were in the range of 48–52 °C with no significant differences ($p < 0.05$). As the temperature continued to increase to 90 °C, both G' and G'' continued to increase and Tan δ decreased below 1 as the gel became more solid-like. The increase in the G' and G'' was also observed as both samples were cooled to 4 °C, with the final G' of Th₁ and T₁ at the end of the temperature sweep at around 184 Pa and 41.5 Pa, respectively. The higher G' value at the end of the temperature sweep indicated that the gel formed with Th₁ sample was stronger as compared to the gel formed with T₁ sample (Mession, Chihi, Sok, & Saurel, 2015).

Guldiken, Stobbs, & Nickerson (2021) and Tang (2008) tested various legume proteins which resulted in comparable trends during the temperature sweeps. The G' and G'' values increased gradually during the heating and cooling phase until they reached the maximum at the end. Similarly, the samples with the higher vicilin- content formed gels with the higher end G'

value which suggested that vicilin-rich samples have a higher gelling capability and are able to form stronger gels. In addition, as reported in past studies, pea legumins were unable to form gels while pea vicilin were able to form gels (Bora, Brekke, & Powers, 1994). This can explain the results obtained in this study as Th₁ samples had a higher vicilin content as compared to T₁ samples, therefore Th₁ solution were able to form stronger gels with higher end G' values.

3.2.5.2. Frequency sweep

Figure 4C depicts the G', G'' and Tan δ of Th₁ and T₁ gels from the frequency of 0.1 to 10 Hz. The effect of increasing frequency on both Th₁ and T₁ gels was similar, with the G' of both Th₁ and T₁ gels increasing slightly from 160 Pa to 282 Pa and 36 Pa to 66 Pa respectively as the frequency increased (Table 1). However, the slope of the G' curve of Th₁ gel was slightly lower at 0.12 as compared to the slope of G' of T₁ gel at 0.13. Katzav, Chirug, Okun, Davidovich-Pinhas, & Shpigelman (2020) stated that the lower slope of G' in the frequency sweep indicated a stronger gel structure. Therefore, the gel formed with Th₁ was stronger than the gel formed with T₁ in the results of this study. Katzav et al. (2020) stated the weaker gel had less time to relax at higher frequencies, therefore the gels were more easily affected by the increase in frequency and led to a larger increase in G'. In addition, as compared to the temperature sweep results, the higher overall G' values of Th₁ gel as compared to T₁ also showed that the Th₁ gel formed was stronger. The trend of the increasing G' with frequency was also present in past studies (Shrestha, Hag, Haritos, & Dhital, 2023), where frequency sweeps were run on various legumes protein gels. It was observed that samples with higher vicilin content formed firmer gels and resulted in a lower slope of G' in the frequency sweep. It was also mentioned that the larger slope of G' in the frequency sweep indicated a less structured gel matrix.

3.2.5.3. Amplitude sweep

Figure 4D shows the amplitude sweep results of T₁ and Th₁ gels from a shear strain of 0.01 to 100%. The trends of the curve for both T₁ and Th₁ were similar, with an initial linear viscoelastic (LVE) region of $G' > G''$. The critical point, which denotes the end of the LVE region and the start of gel deformation (Shan, Chen, Federici, Jones, & Campanella, 2022), was similar at 1.32% shear strain for both T₁ and Th₁ samples (Table 1). The critical G' was 39.1 Pa for T₁ gels and 175 Pa for Th₁ gels. After the start of the non-linear region, the G' and G'' of both T₁ and Th₁ gels continued to decrease until they reached a crossover point where G'' started to be higher than G' . The crossover G' and shear stress for the Th₁ gel was higher at 12.5 Pa and 5.99 Pa as compared to the T₁ gel at 3.33 Pa and 1.59 Pa respectively, which indicated that Th₁ had the stronger gel network (Rafe, Mousavi, & Shahidi, 2014). The gel breaking strain for both samples were not significantly different at around 34–42%. In addition to the effect of a high vicilin content on the gelling ability, the higher protein solubility of Th₁ at more neutral pHs as shown in *Section 3.3.1.* could also have contributed to the better gelling properties. This is supported by a previous study (Rodriguez & Beyrer, 2023), where the gel formed by more soluble protein fractions had a higher crossover strain and G' during the amplitude sweep test, which indicated a more stable macrostructure was formed.

3.2.6. Protein flexibility

Figure 5A depicts the protein flexibility of T₁ and Th₁ samples. T₁ samples had higher structural flexibility as compared to Th₁ samples, which showed that the proteins in T₁ were more susceptible to proteolytic hydrolysis by trypsin than the more rigid proteins in Th₁ (Zhu et al., 2020). Sun et al. (2022) mentioned that vicilin content and protein secondary structure content

were likely to influence the flexibility of the protein. Legumin globulins contain tightly folded disulphide bonds which increase the rigidity of the protein. More α -helix represents a higher ordered structure which was less susceptible to unfolding and β -sheets contributes hydrogen bonds which decreases protein aggregation (Chen et al., 2017). However, the hypothesis was not true in this study as T₁ samples had the lower vicilin content as compared to Th₁ samples. In addition, the secondary structure content of both T₁ and Th₁ samples were not significantly different. This suggests that the protein flexibility of T₁ and Th₁ samples were influenced by other factors such as amino acid composition (Zhu et al., 2020).

3.2.7. Heated and non-heated WHC and OHC

The non-heated and heated WHC and OHC of T₁ and Th₁ samples are shown in Figure 5B. The non-heated WHC was similar at 3.3–3.4 g/g for both T₁ and Th₁ samples. Heating increased WHC to 5.2 g/g for both samples. Farrokhi, Badii, Ehsani, & Hashemi, (2019) mentioned that the increase in heated WHC testing could be due to the protein becoming more charged as it denatures, therefore increasing water interactions. In another study about the heat treatment of quinoa protein samples (Yang et al., 2022), it was suggested that the increase in WHC was due to the increase in hydrophilic groups on the protein surfaces caused by the structural changes during heating. Bühler et al. (2020) presented another theory on why there was an increase in WHC after heat treatment of the samples: Solubility tests after heat treatment showed decreased solubility of the protein sample, which was credited to the aggregation of proteins which led to the increased WHC.

Similar to the WHC, the heated OHC was also increased from 3.8 and 4.1 g/g to 4.5 and 4.6 g/g for T₁ and Th₁ samples, respectively. The increase in OHC could be attributed to the denaturation of proteins with exposed hydrophobic peptide bonds that were able to bind effectively

to oil (Farrokhi et al., 2019). Under both heated and non-heated conditions, the WHC and OHC for both samples were not significantly different with the exception of non-heated OHC which was slightly increased for Th₁ samples.

3.2.8. Foaming capacity and stability

T₁ demonstrated a significantly higher foaming capacity at 53% as compared to Th₁ at 37% (Figure 5C). These results contradicted with past studies (Chang et al., 2022; Zeng et al., 2022) where samples with higher vicilin content resulted in better foaming capacities. Foams formed by T₁ samples had a gradual decrease in overrun from 100% to 71.8% after 60 minutes, while foam formed by Th₁ samples dropped more intensively from 100% to 45.8% after 60 minutes (Figure 5D). These results indicated higher foaming capacity and stability for T₁ as compared to Th₁ samples. Protein flexibility and particle size composition influenced the foaming capacity and stability of the samples. T₁ samples with the higher flexibility were able to form foams more effectively. The increased flexibility exposed more hydrophobic groups which in turn allowed the protein molecules to diffuse more efficiently between the air-liquid interface, thus forming foams with higher overrun and stability (Wierenga & Gruppen, 2010). As depicted in Table S.2, the particle size for T₁ samples at pH 7 was lower at 162.2 nm as compared to Th₁ samples at 196.3 nm. Morales, Martínez, Pizones, & Pilosof (2015) tested on soy protein samples of varying particle sizes and concluded that the foaming capacity of samples were dependent on the particle size. Similar to this study, the samples with lower particle size formed foams with higher overrun, however, the effect of particle size on the stability of the foams were found to be less prominent.

4. Conclusion

Imara and KPS2 mung beans were chosen and underwent protein extraction to study their difference in protein composition and its subsequent effect on the allergenicity and techno-functional properties. KPS2 samples' higher solubility, ability to form stronger and more stable gels was due to higher vicilin and legumin content as compared to Imara mung beans. In contrast, Imara's higher protein flexibility and lower particle size contributed to the increased foaming capacity and stability. Nevertheless, both water and oil holding capacities were comparable. FTIR analysis revealed the effect of an additional cycle of protein extraction on the secondary structure of the samples, with the decrease in α -helix and increase in β -sheets and turns. This additional extraction of proteins decreased the abundance of allergenic globulins but increased the abundance of allergenic seed albumin. The effect of physical and chemical treatment on techno-functional properties and allergenicity should be further investigated in future studies. Overall, this study provides molecular insight into two mung bean varieties and how their protein composition, allergenicity and techno-functional properties differ, guiding possible applications in food product formation as established for some other food ingredients.

CRedit authorship contribution statement

Tak Gun Jeremy Chin: Methodology, Investigation, Software, Visualization, Writing-original draft, Writing-review & editing. **Thimo Ruethers:** Methodology, Investigation, Software, Visualization, Writing-original draft, Writing-review & editing. **Chan Bing Aleo:** Investigation, Software, Visualization. **Andreas Ludwig Lopata:** Conceptualization, Methodology, Investigation, Supervision, Writing-review & editing. **Juan Du:** Funding acquisition, Conceptualization, Methodology, Project administration, Supervision, Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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Figure Captions

Figure 1. SDS-PAGE profiles of generated mung bean proteins extracts (A) and relative abundance of legumine allergen (B).

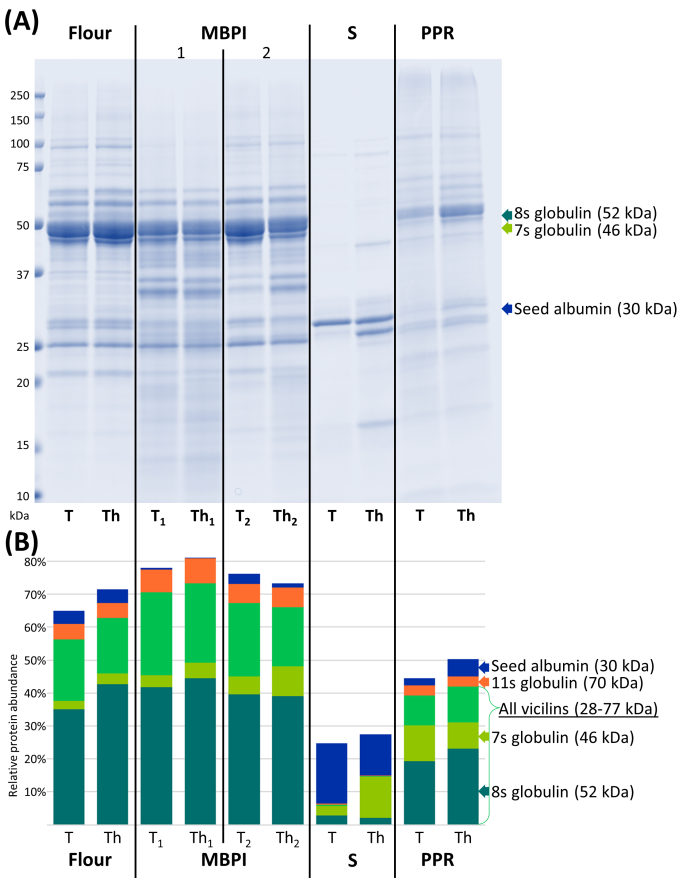
Figure 2. Solubility (A) and zeta potential (B) of the extracted MBPI. The data are shown as average values $\pm$ standard deviation. For both graphs, average values within the same pH by different proteins represented by different lowercase letters are significantly different ($p < 0.05$).

Figure 3. Percentage of secondary structure of extracted MBPI. The data are shown as average values $\pm$ standard deviation. Average values within the same component by different samples represented by different lowercase letters are significantly different ($p < 0.05$).

Figure 4. Rheometer analysis of extracted MBPI. Temperature sweep of T_1 (A) and Th_1 MBPI (B). The modulus and $\tan \delta$ of the extracted MBPI₁ as a function of frequency (C). The modulus and $\tan \delta$ of the extracted MBPI₁ as a function of shear strain (D).

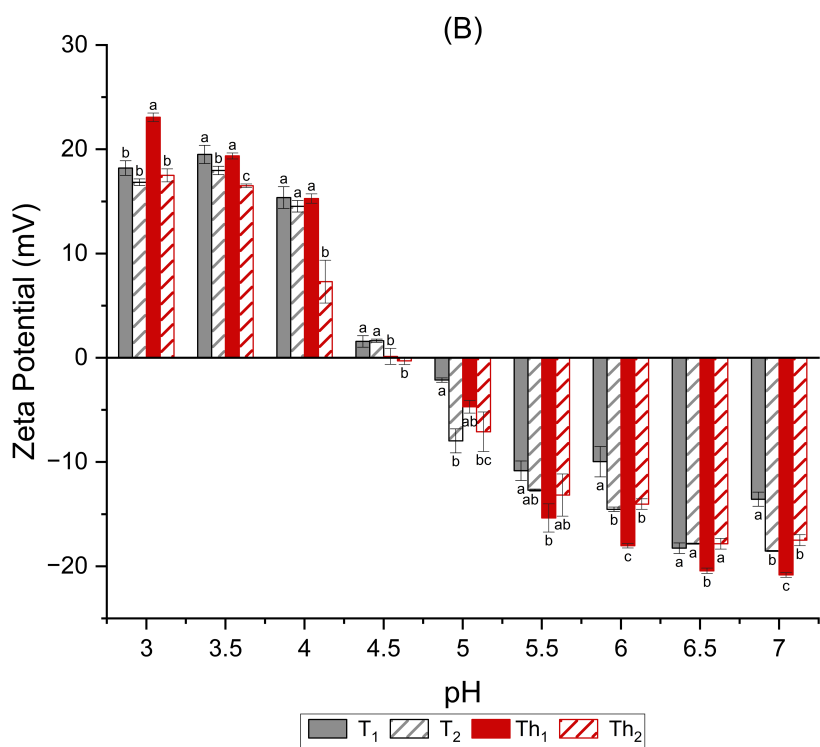
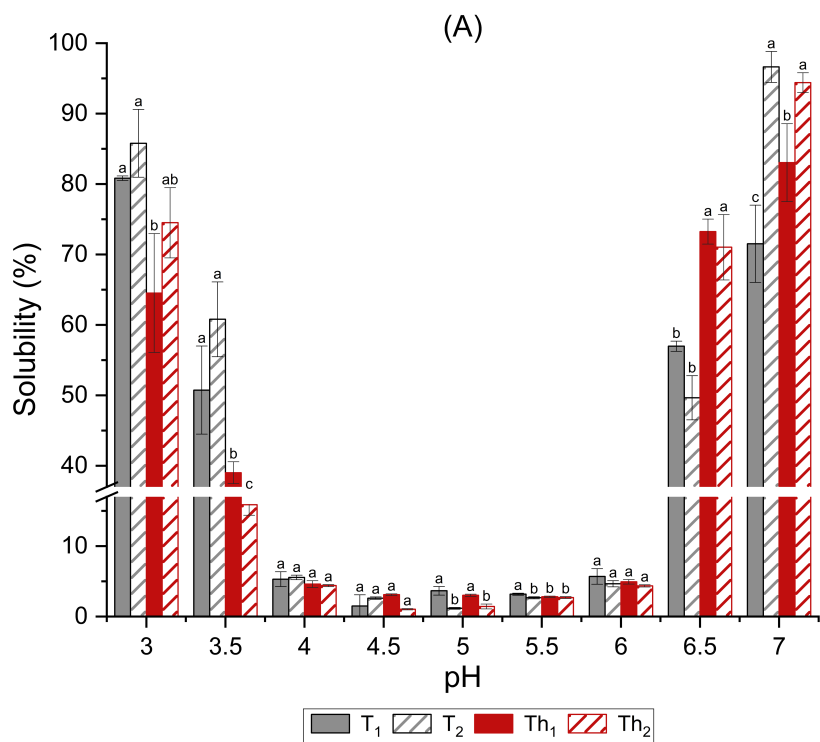
Figure 5. Protein flexibility (A), heat and non-heated water and oil holding capacity (B), foaming capacity (C) and stability (D) of T_1 and Th_1 samples. The data are shown as average values $\pm$ standard deviation. Average values within the same test/protein by different proteins/parameters/time elapsed with different lowercase letters are significantly different ($p < 0.05$). Average values within the same parameter/time elapsed by different proteins with different uppercase letters are significantly different ($p < 0.05$).

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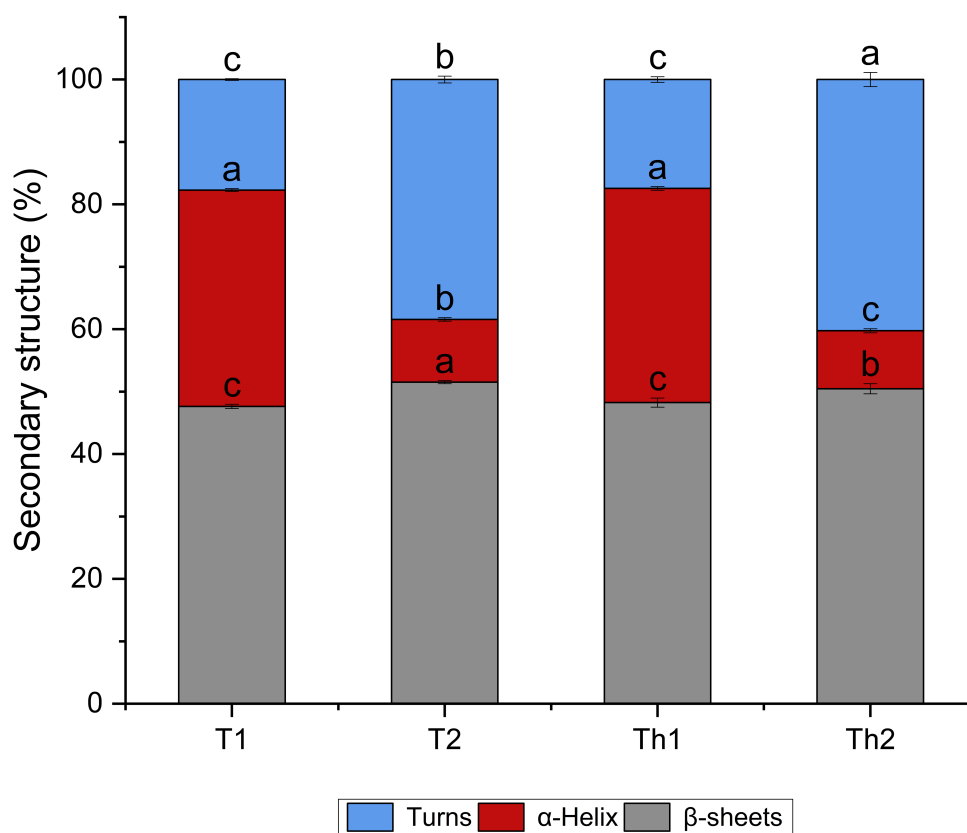


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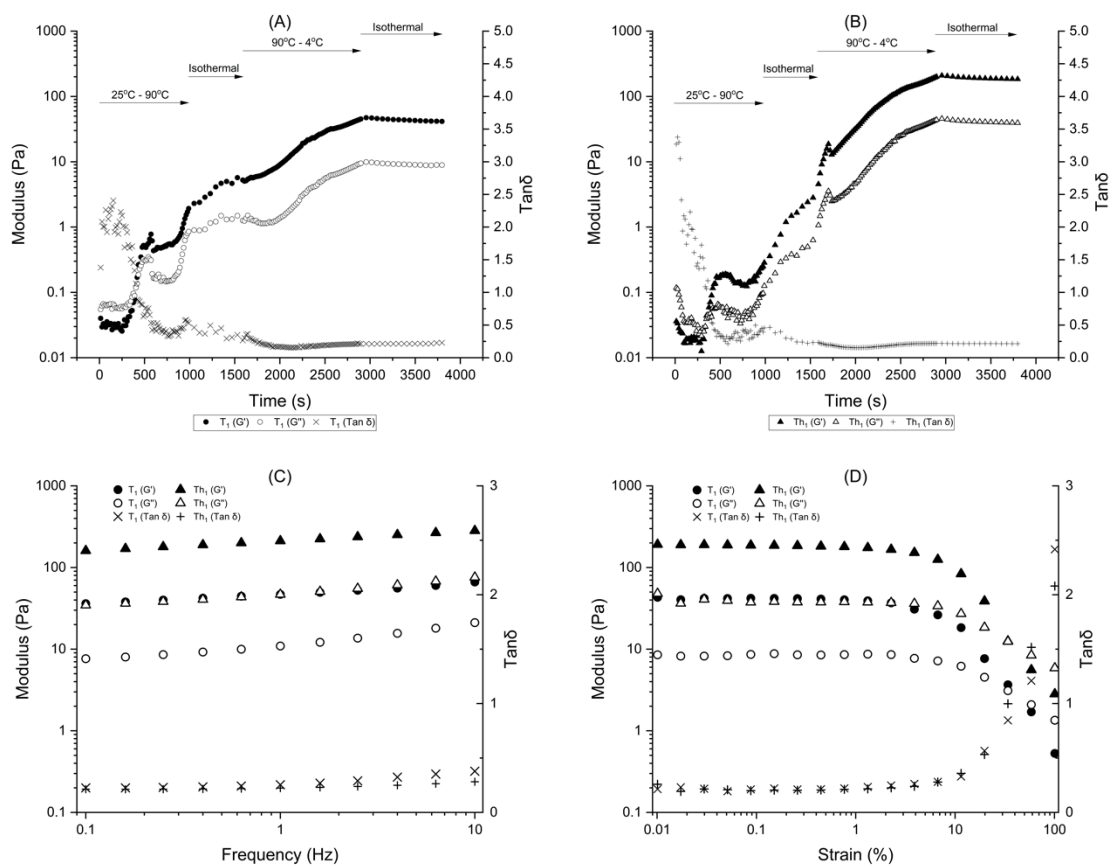
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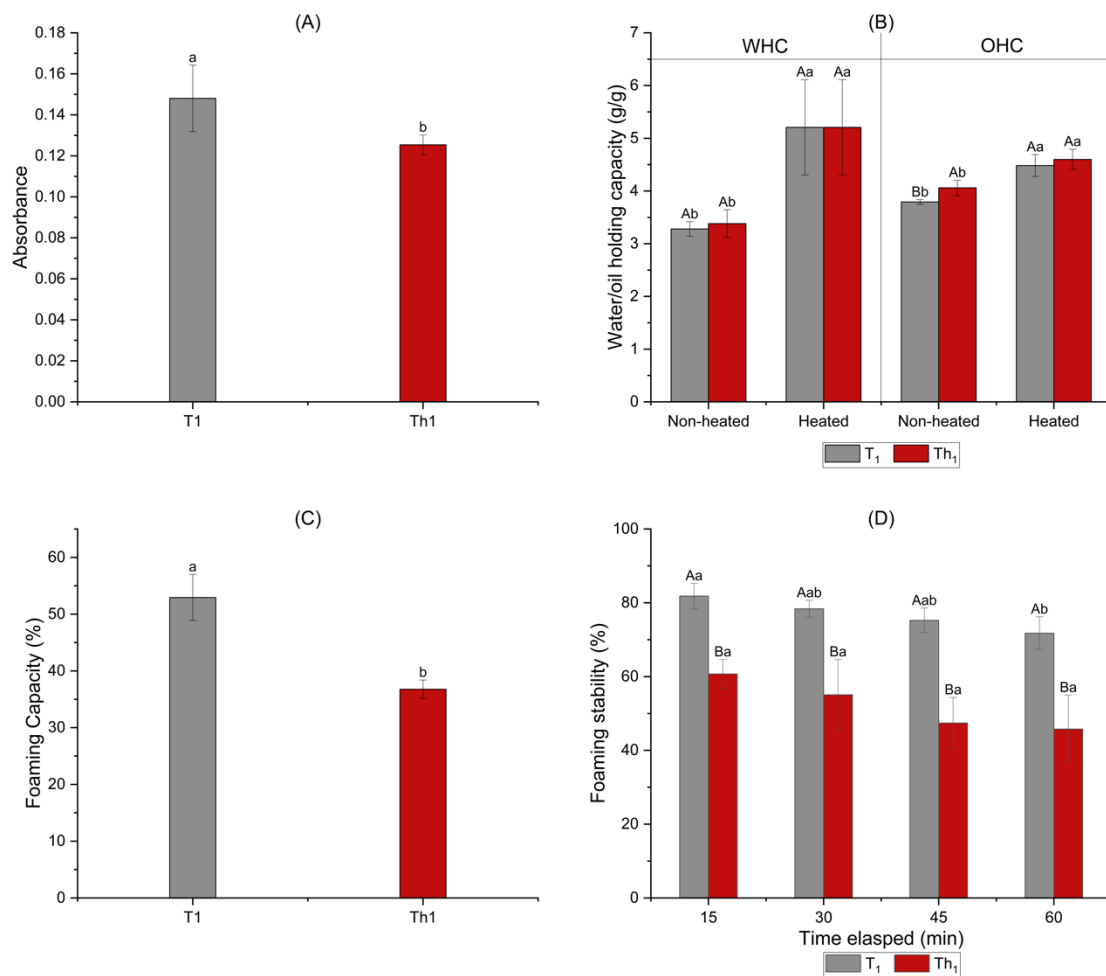
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Supplementary:

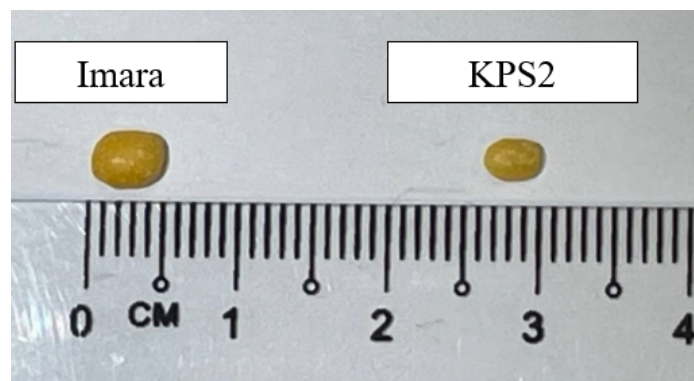


Figure S.1 Dehulled mung bean from two varieties.

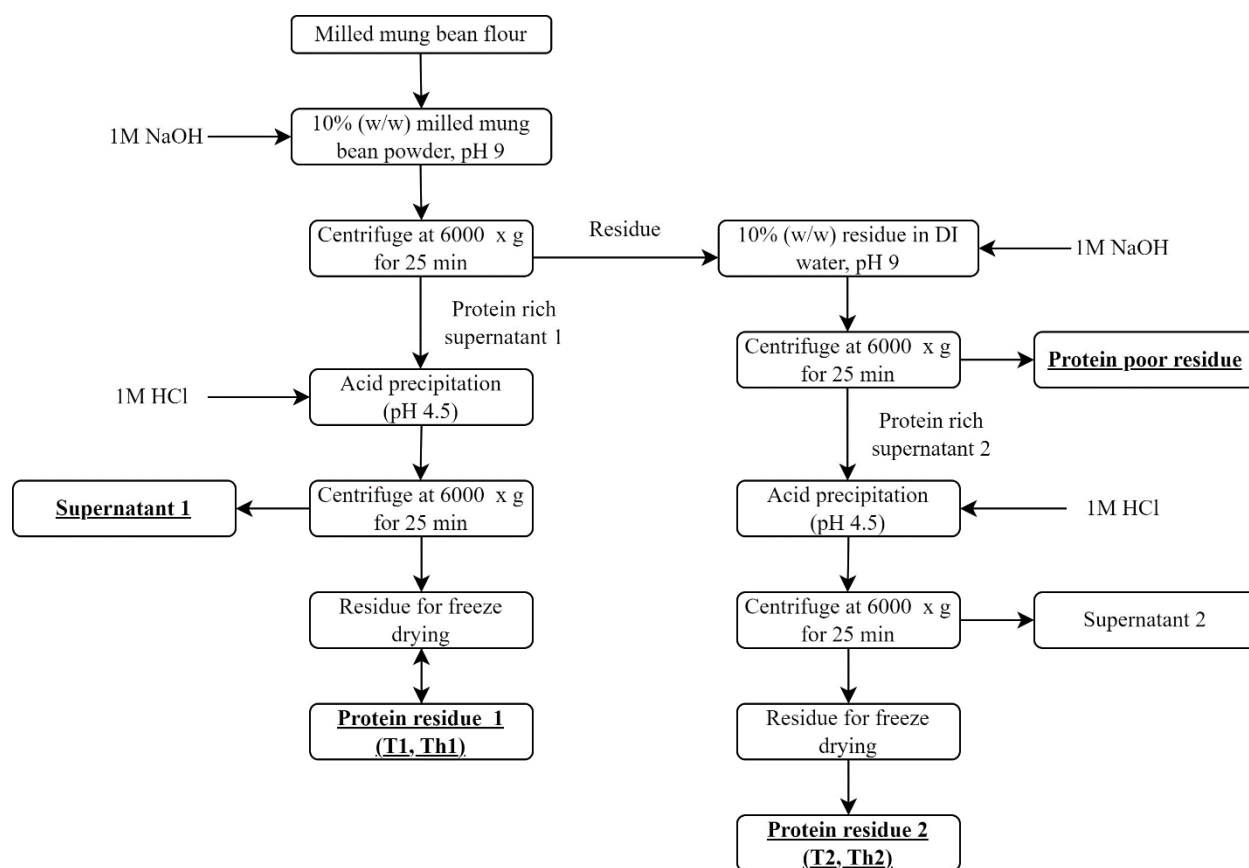


Figure S.2 Workflow of extraction process of dehulled mung beans.

Table S.1 Protein content of flour and extracted protein isolates from Imara and KPS2 as determined by Dumas combustion method. Average values within the same variety by different sample types represented by different lowercase letters are significantly different ($P < 0.05$). Average values within the same sample type by different varieties represented by different uppercase letters are significantly different ($P < 0.05$).

Sample		Protein content (%)
Imara	T flour	25.57 ± 0.33^A
	T ₁	88.14 ± 0.25^{Aa}
	T ₂	83.42 ± 0.58^{Ab}
KPS2	Th flour	25.48 ± 0.55^A
	Th ₁	87.75 ± 0.29^{Aa}
	Th ₂	76.45 ± 0.06^{Bb}

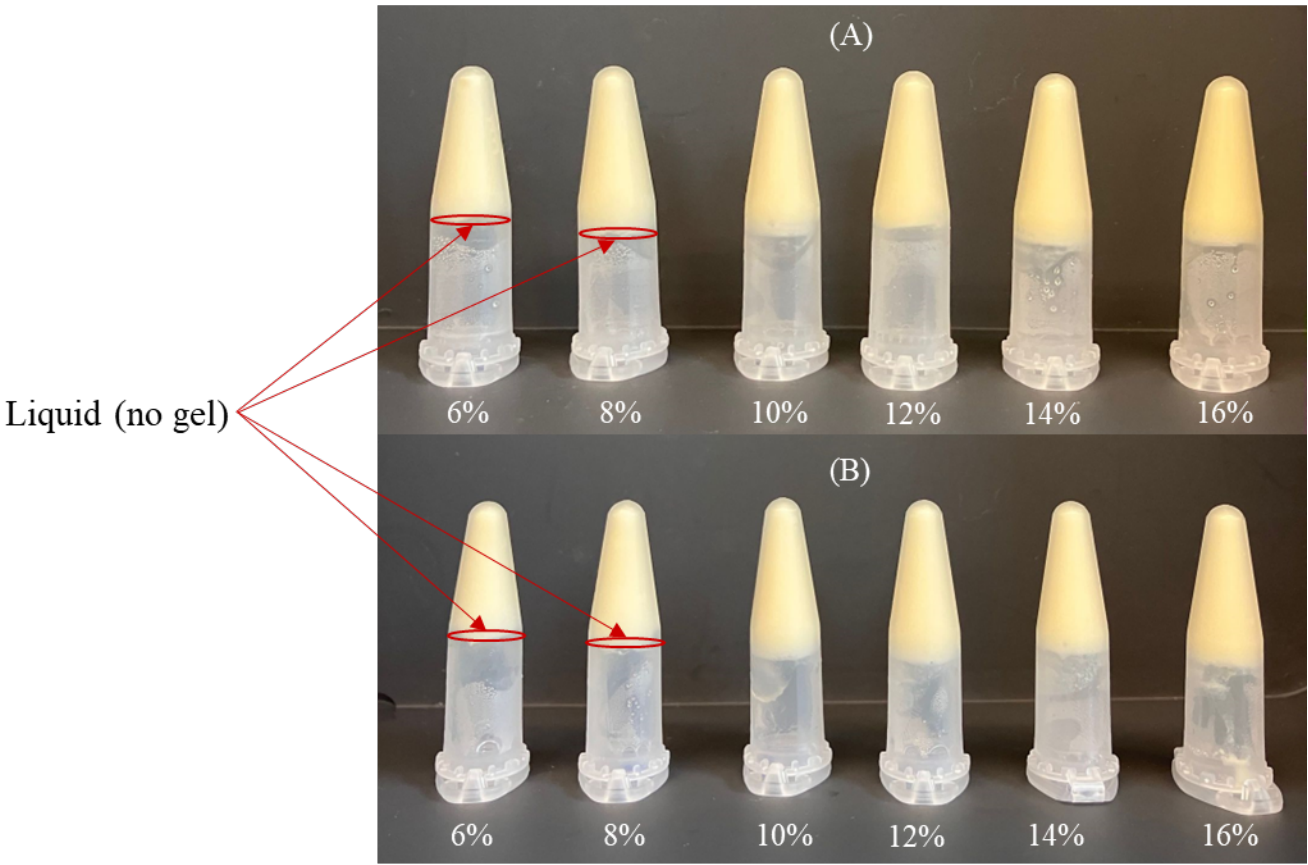


Figure S.3 Least gelation concentration of the extracted mung bean protein. Note: A: T₁, B: Th₁

Table S.2 DLS particle size of T₁ and Th₁ samples. Average values within the same sample by different pH represented by different uppercase letters are significantly different, average values within the same pH by different samples represented by different lowercase letters are significantly different (P < 0.05).

	Z-average (nm)		
	pH 3	pH 5	pH 7
T ₁	149.9 ± 0.40 ^{Ba}	1109 ± 176 ^{Aa}	162.2 ± 2.22 ^{Bb}
Th ₁	146.8 ± 0.27 ^{Cb}	1089 ± 18.3 ^{Aa}	196.3 ± 1.27 ^{Ba}