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*Research Article*

## **Biochemical and functional properties of proteins from red kidney, navy and adzuki beans**

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### **Abstract**

Chemical composition and functional properties of proteins extracted from adzuki, navy and red kidney beans were investigated. Protein contents were 17.33, 18.15 and 19.91% for red kidney, navy and adzuki beans, respectively. Red kidney bean exhibited the highest carbohydrates content, while the highest fat, ash and crude fibre content was found in navy bean. The highest trypsin inhibitory activity was found in adzuki bean. Globulin polypeptides (molecular weight ranges 45-55 kDa) were the major polypeptides in both whole protein and protein isolate. Protein isolate from all seeds contained at least one major glycopeptide, while more than two major glycopeptides were observed in the whole proteins. The minimum protein solubility of isolated proteins from all seeds was at around pH of 4-5. High foamability and foam stability were obtained in red kidney and navy beans when compared with those of adzuki bean. The highest oil absorption was found in adzuki bean, while no significant differences for water absorption between adzuki and red kidney beans was observed ( $p > 0.05$ ). Red kidney bean showed lower emulsifying activity as well as stability when compared with the other two beans.

**Keywords:** adzuki bean, composition, functional property, legume seed, navy bean, protein isolate, red kidney bean, Thailand.

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## Introduction

Legumes are an important food source and play a significant role in traditional diets all over the world. Legumes are widely grown and consumed in various regions, are excellent sources of proteins, complex carbohydrates and fairly good sources of minerals, vitamins and polyunsaturated fatty acids [1]. Proteins in legume seeds represent about 20% (dry weight) in peas and beans, up to 38–40% in soybean and lupin [2]. Thus legume seeds are among the richest food sources of proteins and amino acids for human and animal nutrition.

The importance of legume seeds as food and functional ingredients has stimulated much attention to their utilization. They have been used by food industries as ingredients and supplements in food products for many years. For successful use in food applications, they should possess several desirable characteristics, referred to as functional properties. These properties of legume seed storage proteins have been studied as purified 11S or 7S proteins or as isolates, both in their original and chemically modified forms [3]. These properties affect the behaviour of proteins in food systems during processing, manufacturing, storage and preparation.

The Royal Project Foundation of Thailand has funded legume breeding programs for more than two decades. It is home to a large variety of legumes, including red kidney, navy, pinto, adzuki and soybean. Adzuki, red kidney and navy beans are highly promising raw materials because of their high consumption and excellent agricultural yields. However, biochemical characteristics and functional properties of some legumes from The Royal Project Foundation have not been published.

The objective of this study was to determine the biochemical properties of red kidney, navy and adzuki beans. Preparation of protein isolate and functional properties determination of these legumes were also investigated.

## Materials and Methods

### *Chemicals and raw materials*

*N*- $\alpha$ -benzoyl-*DL*-arginine-*p*-nitroanilide (BAPNA), trypsin from bovine pancreas,  $\beta$ -mercaptoethanol ( $\beta$ ME) and *N,N,N',N'*-tetramethyl ethylene diamine (TEMED) were purchased from Sigma Chemical Co., (St. Louis, MO). Bovine serum albumin (BSA), sodium dodecyl sulphate (SDS) and Coomassie brilliant blue R-250 were procured from Fluka Chemical-Biochemika (Buchs, Switzerland). GelCode® Glycoprotein Staining Kit was purchased from Pierce Biotechnology Perbio (Rockford, IL).

Three varieties of bean sample namely, red kidney bean (*Phaseolus vulgaris L.*), navy bean (*Phaseolus vulgaris*) and adzuki bean (*Vigna angularis*) were obtained from the Royal Project Shop, Chiang Rai, Thailand.

### *Sample preparation*

The dried seeds were ground by using a hammer mill and then sieved with a 600  $\mu$ m screen. The flour was defatted by extraction with cold acetone for 1 h at 4°C (flour/solvent ratio of 1:2.4

w/v). The slurry was then filtered through Whatman filter paper no. 4. Defatted flour was then air-dried, ground and kept in an airtight plastic container for further use.

### **Chemical properties analysis**

**Proximate composition.** Samples were determined for their moisture, ash, fat, fibre and protein ( $6.25 \times N$ ) content according to the standard methods of analysis of AOAC [4].

**Trypsin inhibitor activity assay.** Trypsin inhibitory activity was measured according to the method of Benjakul, *et al.* [5] by using BAPNA as a substrate. A solution containing 100  $\mu$ L of the sample, 200  $\mu$ L (20 $\mu$ g/mL) trypsin and 100  $\mu$ L of distilled water was pre-incubated at 37°C for 10 min. Then 500  $\mu$ L (0.4 mg/mL) of BAPNA was added to start the reaction. After incubation at 37°C for 10 min, 1000  $\mu$ L of 30% (v/v) acetic acid was added to terminate the reaction. Activity of the trypsin was determined by measurement of the absorbance at 410 nm due to *p*-nitroaniline released. One unit of trypsin inhibitor was defined as 0.01 decrease in absorbance at 410 nm under assay conditions compared with the control sample (without inhibitor).

**Protein determination.** Protein content in the sample was determined by the Biuret method [6] using BSA as a standard.

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).** SDS-PAGE was carried out following the method of Laemmli, [7] by using 10% separating and 4% stacking gels. The samples were mixed with the sample buffer (0.5 M Tris-HCl; pH 6.8, 0.5% bromophenol blue, 10% glycerol, and 10% SDS) at the ratio of 1:1 in the absence of  $\beta$ ME and without heating. Twenty micrograms of protein were loaded and then subjected to separation at 15 mA/gel using Mini Protean Tetra Cell unit (Bio-Rad Laboratory, Inc, Richmond, CA, USA). After separation, gel was stained with Coomassie brilliant blue R-250 and de-stained with a mixer of method-acetic acid solution.

**Glycoprotein staining.** Glycoprotein staining was conducted using GelCode® Glycoprotein Staining Kit. The separated protein was fixed by immersing the gel in 50% methanol for 30 min. The gel was then washed with 3% acetic acid for 10 min (repeating this step once). The gel was transferred to the oxidizing solution and gently agitated for 15 minutes. The gel was then washed with 3% acetic acid for 5 min (repeating this step two additional times) before transferring to the GelCode® Glycoprotein Staining Reagent. The gel was incubated for 5 min with the reducing solution before being washed with 3% acetic acid and then with distilled water. Glycoproteins appear as magenta bands.

### **Protein isolate preparation**

Protein isolate from legume seeds was prepared using the method described by El-Adawy, [8]. Dispersions of defatted bean flours (5% w/v) in distilled water were adjusted to pH 8 with 0.1 N NaOH, shaken for 1 h and then centrifuged at 8000 $\times$ g for 15 min. The pH of the extract was adjusted to 4.5 with 1 N HCl to precipitate the target proteins. The proteins were recovered by centrifugation at 8000 $\times$ g for 15 min, followed by removal of the supernatant by decantation.

Protein curd was washed twice with distilled water and centrifuged at 8000xg for 10 min. The washed precipitate was then freeze-dried and referred to as “protein isolate”.

#### ***Functional properties of protein isolate determination***

**Protein solubility.** Protein solubility was tested in the pH ranges of 2.0–10. Sample (1000 mg) was suspended in 20 ml distilled water and the pH of the suspensions was adjusted to a specific value using 0.1 N HCl or NaOH solutions. These suspensions were agitated over a shaker for 1 h at room temperature; the pH was checked and re-adjusted, then centrifuged at 8000xg for 15 min. The protein content of the supernatant was determined by the method of Lowry, *et al.* [9] using BSA as standard. Protein solubility was expressed as the percentage of the total protein of the original sample that was present in the soluble fraction.

**Foaming capacity and foam stability.** The capacity and stability of foams were determined by the method of Lin, *et al.* [10]. The 50 ml of 3% (w/v) of protein in distilled water were whipped using homogenizer (10,000 rpm) for 2 min. The homogenous foam was immediately transferred to a graduated cylinder. The volume was recorded before and after whipping. FC was expressed as the volume (%) increase due to whipping. For the determination of FS, foam volume changes in the graduated cylinder were recorded at 60 min of storage at room temperature.

**Water and oil absorption.** Water absorption was measured by the centrifugation method [11]. The sample (1.0 g) was dispersed in 25 ml of distilled water and placed in pre-weighed centrifuge tubes. The dispersions were stirred after intervals of 5 min, held for 30 min, followed by centrifugation for 25 min at 3000xg. The supernatant was decanted, excess moisture was removed by draining for 25 min at 50°C, and then the sample was reweighed.

Fat absorption was determined by using the method of Lin, *et al.* [10]. Samples (0.5 g) were mixed with 6 ml of corn oil in pre-weighed centrifuge tubes. The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. After a holding period of 30 min, the tubes were centrifuged for 25 min at 3000xg. The separated oil was then removed with a pipette and the tubes were inverted for 25 min to drain the oil prior to reweighing. The water and oil absorption capacities were expressed as grams of water or oil bound per gram of the sample on a dry basis.

**Emulsion capacity and stability.** Oil in water emulsions were prepared by adding 2.5 ml corn oil into 50 ml of protein isolate solution while mixing with the aid of a mechanical stirrer. The resulting crude emulsion was then homogenized for 1 min using a homogenizer operating at 20,000 rpm (IKA Labortechnik homogenizer, Selangor, Malaysia). Emulsion capacity was expressed as the volume increase after being centrifuged at 5,000xg for 15 min. Emulsion stability was expressed as the volume change after incubation at 90°C for 60 min and centrifuged at 5,000xg for 15 min.

#### ***Statistical analysis***

All experiments in this study are reported as means of three replicate analyses. One-way analysis of variance (ANOVA) was carried out to compare the mean values of different bean species. Differences in the mean values were determined using Duncan’s multiple range tests (SAS, 1990).

## Results and Discussion

### *Biochemical properties of three legume seeds*

**Chemical compositions and trypsin inhibitor.** The proximate composition of red kidney bean, navy bean and adzuki beans is shown in Table 1. The major component of all legumes was carbohydrate with the amount of 54 to 59%. Protein (17-19%), water (10-11%), fibre (4-8%) and ash content (3-4%) were found in the following quantity in these three legumes. The adzuki bean contained the highest moisture, protein and carbohydrate content when compared with others ( $p < 0.05$ ). The highest lipid, ash and fibre content were found in navy bean ( $p < 0.05$ ). In general, grain legume seeds are an important source of protein, energy, vitamins and minerals for human and animal consumption [1]. Alonso, *et al.* [12] reported that crude protein in raw kidney seed meals was 23.2 g/100g sample. Kereliuk and Kozub [13] stated that the protein content of small white (navy) beans was 25.3 g/100g of dry weight. Total insoluble and soluble fibre in kidney bean was 22% and 18% respectively [14]. Fibre, especially which is found in whole grains, is helpful in the treatment and prevention of constipation, hemorrhoids and diverticulosis [15]. The different amount of any component in these legumes possibly depends on the difference in spice of cultivar and growth conditions.

Trypsin inhibitory activity from red kidney, navy and adzuki beans was 94.30, 265.98 and 509.53 unit/mg protein, respectively. Adzuki bean showed the highest amount of trypsin inhibitor compared to those in red kidney and navy beans for 2-5 folds ( $p < 0.05$ ). Trypsin inhibitors strongly inhibited trypsin activity which reduces the digestion and absorption of dietary protein in animal and human consumption. From that statement, the protease inhibitors in legume seed can have a major impact on nutritional value. Different research has shown that the positive or negative effect on the nutritional value of these inhibitors depends on the quantity in each legume and on the dose and time of consumption. The trypsin inhibitor activity of fababeans and its variation among cultivars, earlier studied by many authors, was found in the range 0.7-12 units/mg dry matter [3].

**Table 1. Proximate composition and trypsin inhibitor of three legumes (g/100g sample)\***

Constituent	Red kidney bean	Navy bean	Adzuki bean
Moisture**	10.42 ± 0.01 <sup>c***</sup>	11.35 ± 0.01 <sup>b</sup>	11.57 ± 0.01 <sup>a</sup>
Protein	17.37 ± 0.07 <sup>c</sup>	18.15 ± 0.05 <sup>b</sup>	19.91 ± 0.46 <sup>a</sup>
Lipid	1.46 ± 0.01 <sup>b</sup>	2.63 ± 0.01 <sup>a</sup>	1.47 ± 0.01 <sup>b</sup>
Ash	4.14 ± 0.02 <sup>b</sup>	4.71 ± 0.02 <sup>a</sup>	3.26 ± 0.04 <sup>c</sup>
Fiber	7.86 ± 0.16 <sup>b</sup>	8.87 ± 0.77 <sup>a</sup>	4.71 ± 0.54 <sup>c</sup>
Carbohydrates	58.75 ± 0.41 <sup>a</sup>	54.30 ± 0.20 <sup>b</sup>	59.08 ± 0.18 <sup>a</sup>
Trypsin inhibitor (unit/mg protein)	94.30 ± 22.14 <sup>c</sup>	265.98 ± 26.69 <sup>b</sup>	509.53 ± 5.93 <sup>a</sup>

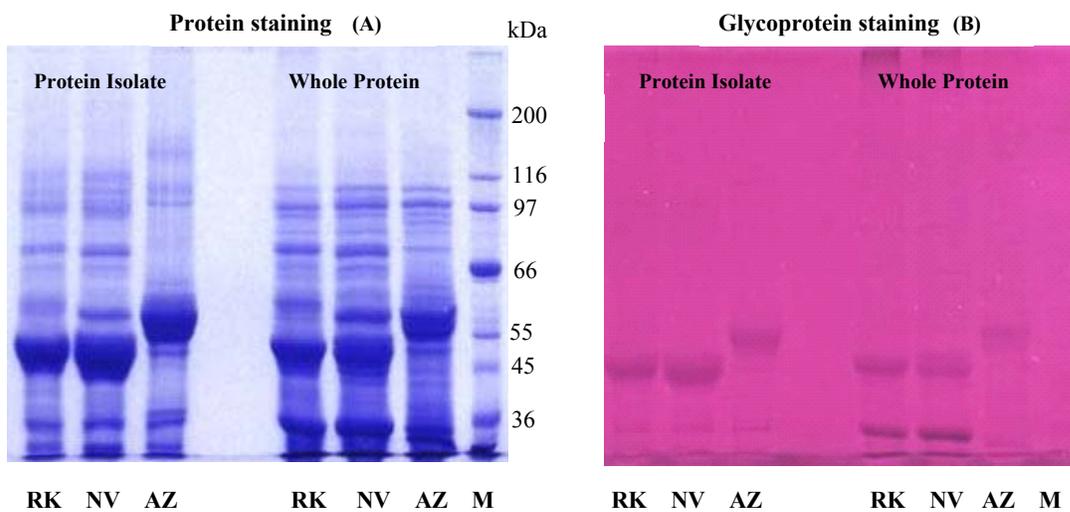
\*Values are given as means ± SD from triplicate determinations.

\*\* Each values expressed on wet weight basis.

\*\*\* Different superscripts in the same row indicate significant differences ( $p < 0.05$ ).

**Molecular weight distribution.** Protein pattern and glycoprotein staining of whole legume and protein isolated from the three legumes are shown in Figure 1. The protein staining indicated a broad range of protein molecules approximately 36 to above 97 kDa with major polypeptides (36, 45 or 55 kDa) were observed in the whole protein (Figure 1A). However, when the protein was isolated, some minor proteins were removed. The major component in protein isolated from red kidney and navy beans was about 50 kDa, while the molecular weight of 55 kDa was the major protein found in the adzuki bean. Globular proteins were the major constituent in all legume seeds [8]. Protein band with the molecular weight of 97 kDa became low band intensity caused by isolation process. Whilst the protein band of 50 kDa for red kidney and navy beans and 55 kDa for adzuki bean were increased by the same process, it should be noted that low molecular weight proteins (below 36 kDa) were observed in all legume proteins as shown in Figure 1A.

The result of glycoprotein staining of the whole protein and protein isolate are also shown in Figure 1B. Two major bands with the molecular weight of 50, 55 kDa and below 36 kDa are glycoprotein for red kidney and navy beans whole protein. Only one band with the molecular weight of 55 kDa was found in adzuki bean as the glycoprotein. However, when the legume protein was isolated, the minor glycoprotein with the molecular weight of 36 kDa in both red kidney and navy beans was removed. Glycoprotein is protein that contains oligosaccharide chains (glycans) covalently attached to their polypeptide side-chain. The carbohydrate is attached to the protein in a co-translational or posttranslational modification. This process is known as glycosylation. Glycoprotein is often an important integral membrane protein, where it plays a role in cell-cell interactions [16].

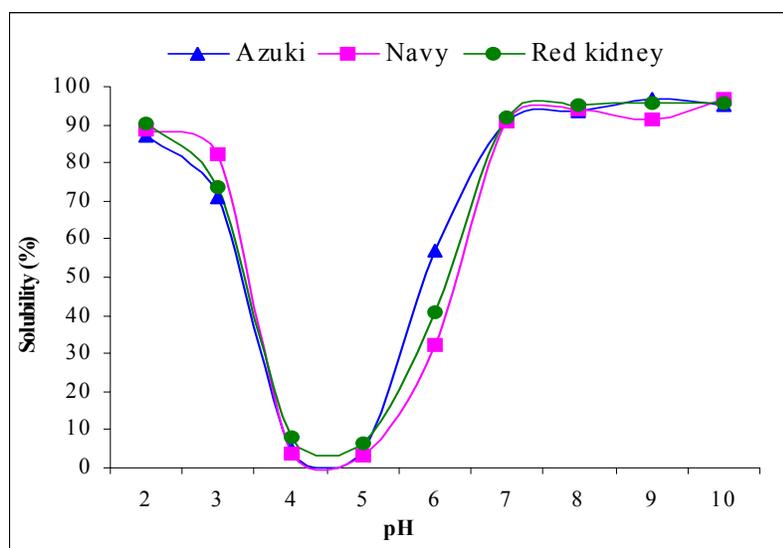


**Figure 1. Protein (A) and glycoprotein staining (B) of the sample from three legume seeds.** RK: Red kidney bean; NV: Navy bean; AZ: Adzuki bean; M: standard protein marker.

### ***Functional properties of protein isolated from three legume seeds***

**Protein solubility.** Protein solubility at different pH may serve as a useful indicator of the performance of protein isolates in the food system and also the extent of protein denaturation because of chemical treatment [17]. The solubility profiles of protein isolates from red kidney bean, navy bean and adzuki bean are shown in Figure 2. The pH solubility curves obtained for the protein isolated from all legumes was a deep V profile with a minimum in the range of 4 to 5 and two maxima, above pH 7 and below pH 3. The whole protein solubility is clearly dominated by the behaviour of the globulins. In the acid range, however, the protein becomes significantly less soluble than the globulins.

Lowest protein solubility of all sample caused by at that region pH is the isoelectric point (pI) of legume proteins. The highest solubility of the isolated protein was found at the acid or alkaline conditions. Vani and Zayas, [18] reported that most of the plant proteins have isoelectric pH at 4.0–5.0. At the isoelectric point, there is no net charge on the protein; resulting in no repulsive interactions and the protein–protein interactions disfavoring solubility [19]. At low pH, positive net charges of proteins are induced, while the proteins at high pH are conducted to the negative net charge. Of these, repulsive forces are increased, resulting in more solubility of those proteins. At pH above 6.5, all proteins had solubility greater than 70%. These observations are in agreement with those reported earlier for chickpea, lentil and soy protein [20, 21, 22]. Solubility in water of protein concentrates prepared by alkaline extraction was minimal at pH 4.0 but was higher at pH 5.0 [3].

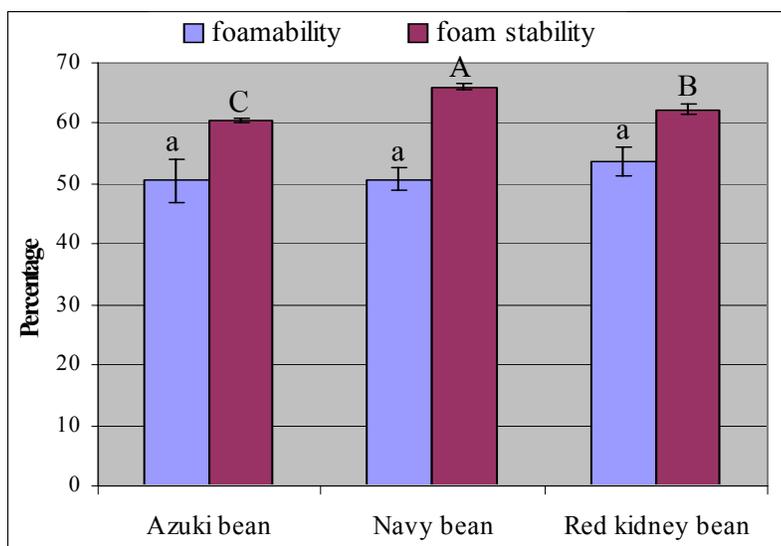


**Figure 2.** The solubility of protein isolated from red kidney, navy, and adzuki beans.

**Foaming properties.** Foaming capacity and foaming stability of all proteins isolated from three legumes are presented in Figure 3. Foam is produced when air is injected into a liquid and entrapment in the form of bubbles occurs. No significant differences of foaming capacity (50–55%) was observed in the three protein isolates ( $p > 0.05$ ). The differences in the foaming properties of the legume proteins may be due to the different composition and nature of the

protein fractions. Graham and Phillips, [23] reported that the protein with good foamability can reduce surface tension, while a globular protein which is relatively difficult to surface denature gives low foamability. The major proteins of legumes are also globular in nature which may be difficult to surface denature, hence resulting in lower foaming properties. The basic requirements for a protein to be a good foaming agent are the ability to: (a) adsorb rapidly at the air/water interface during bubbling, (b) undergo rapid conformational change and rearrangement at the interface and (c) form a cohesive viscoelastic film via intermolecular interactions [20, 21].

Navy bean showed the highest foaming stability, followed by red kidney and adzuki beans, respectively. Foam stability requires the specific properties of protein films as formation of cohesive, viscous, elastic, continuous, air-impermeable film around each gas bubble. The foam stability is influenced by film thickness, mechanical strength, protein-protein interactions and environmental factors such as pH and temperature. High foam stability found in protein isolate from navy bean might be due to the high surface viscosity, high molecular weight globular proteins and also high lipid content in this legume. Some of the proteins such as BSA can enhance foam stability in the system containing lipids [23].



**Figure 3. Foaming properties of the protein isolate from three legume seeds.**

Values are means of triplicate determinations  $\pm$  SD.

**Water and oil absorption capacity.** Water and oil absorption capacities are crucial factors in protein functionality, as they influence emulsion and other properties. These properties of isolated proteins from three legume seeds are shown in Table 2. Navy bean showed the highest water absorption (3 times higher), while no significant differences were observed in the other two isolated proteins ( $p > 0.05$ ). The water retention capacities of pea isolates were estimated to be 2.7-2.8g of water/g isolate [3]. Lower water absorption of protein isolate might be due to the fact that the protein isolates have low ability to swell, low dissociation and unfolding to expose additional binding sites and low solubility. In addition, lower water absorption of protein isolated could be due to the low availability of polar amino acids which have been shown to be primary sites for water interaction of proteins [24].

Oil absorption capacity is of great importance from an industrial viewpoint, since it reflects the emulsifying capacity, a highly desirable characteristic in products such as mayonnaise [25]. Oil absorption of the three protein isolates ranged between 3.42 and 4.26 g oil /g isolate (Table 2). Protein isolate from adzuki bean exhibited significantly higher oil absorption than those of navy bean and red kidney bean, suggesting the presence of more hydrophobic amino acids in adzuki bean. The existence of several non polar side chains may bind the hydrocarbon chains of fats, thereby resulting in higher absorption of oil [23]. Oil absorption capacity of three legumes protein isolates in the present study was comparable to commercial soy isolate (3.29 g/g) and winged bean protein concentrate (4.01 g/g) [26]. High oil absorption properties of the protein are required in ground meal formulation, meat replacers and extenders, doughnuts, baked goods and soups.

**Table 2. Water and oil absorption and emulsifying properties of isolated proteins.**

Sample	Absorption (g solution/g)*		Emulsifying (%)*	
	Water	Oil	Capacity	Stability
Azuki bean	0.47 ± 0.12 <sup>b**</sup>	4.26 ± 0.68 <sup>a</sup>	52.18 ± 0.78 <sup>a</sup>	46.18 ± 4.63 <sup>ab</sup>
Navy bean	1.20 ± 0.20 <sup>a</sup>	3.78 ± 0.18 <sup>b</sup>	54.08 ± 1.46 <sup>a</sup>	56.95 ± 9.92 <sup>a</sup>
Red kidney bean	0.40 ± 0.20 <sup>b</sup>	3.42 ± 0.18 <sup>c</sup>	48.80 ± 1.19 <sup>b</sup>	36.33 ± 1.16 <sup>b</sup>

\* Values are given as means ± SD from triplicate determinations.

\*\* Different superscripts in the same row indicate significant differences ( $p < 0.05$ ).

**Emulsifying properties of protein isolate.** Emulsifying properties of the protein isolates from three legume seeds are also shown in Table 2. Adzuki bean and navy bean protein isolates had higher emulsification capacity than red kidney bean ( $p < 0.05$ ). From the result, navy bean showed the highest, both of emulsifying capacity and stability, when compared with other legume protein isolates ( $p < 0.05$ ). At the same time, the lowest emulsifying capacity and stability was found in the protein isolate from red kidney bean. Emulsification of proteins is influenced by solubility and surface hydrophobicity [27]. The influence of structural changes on the availability of the apolar sites may explain the great variations of the surface behaviour of these proteins depending on their conformation [3]. The emulsifying properties of the protein isolates were examined as functions of their globulin composition. The effect of the globulins ratio in the sample was probably masked by the presence of phospholipids, which can act as surface active components, as well as by the partial denaturation of the proteins [3].

## Conclusion

Three legume seeds were investigated and found to be a good source of proteins, carbohydrates and fibre. Glycopeptides were the major composition in both whole protein and protein isolates. Navy bean had the highest foaming stability, while foamability between seeds was comparable. Azuki and navy beans had the higher oil and water absorption and emulsifying properties than those of red kidney bean. This information can attract consumers and processors for value-based food products by using these legume seeds.

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## References

- [1] Chung H., Liu Q., Peter P., Ming Z. and Rickey Y. (2008). In vitro starch digestibility, expected glycemic index and some physicochemical properties of starch and flour from common bean (*Phaseolus vulgaris* L.) varieties grown in Canada. **Food Research International**, 111, (2), 316-321.
- [2] Duranti M. (2006). Grain legume proteins and nutraceutical properties. **Fitoterapia**, 77, 67-82.
- [3] Hudson B.J.F (1994). New and Developing source of food proteins. Chapman & Hall. London.
- [4] AOAC. (1995). Official Methods of Analysis of the Association of Official Analytical Chemists, 16th ed. Virginia. U.S.A.
- [5] Benjakul S., Wonnop V. and Paiboon T. (1999). Isolation and characterization of trypsin inhibitor from some Thai legumes. **Journal of Food Biochemistry**. 24, 107-127.
- [6] Gornall A. G., Bardawill C. J. and David M. M. (1948). Determination of serum proteins by means of the biuret reaction. **The Journal of Biological Chemistry**. 177, 751-766.
- [7] Laemmli V. K. (1970). Cleavage of structural proteins during the assembly of the heads of bacteriophage T4. **Nature**, 227, 680–685
- [8] El-Adawy T.A. (1996). Chemical, nutritional and functional properties of mung bean protein isolate and concentrate. **Menofiya Journal of Agricultural Research**, 21, 657–672.
- [9] Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the folin reagent. **Journal of Biological Chemistry**, 193, 265.
- [10] Lin M.J.Y., Humbert E.S. and Sosulski F. W. (1974). Certain functional properties of sunflower meal products. **Journal of Food Science**, 39, 368–370.
- [11] Sosulski F.W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. **Cereal Chemistry**, 39, 344–350.
- [12] Alonso R., Rubio L.A., Muzquiz M. and Marzo F. (2001). The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. **Animal Feed Science and Technology**. 94, 1-13.

- [13] Kereliuk G.R. and Kozub G.C. (1994). Chemical composition of small white (*navy*) beans. **Academic Press Limited**, 28, 272-278.
- [14] Kahlon T.S., Smith G.E. and Shao Q. (2005) In vitro binding of bile acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), bengal gram (*Cicer arietinum*) and moth bean (*Phaseolus aconitifolius*). **Food Chemistry**, 90, 241–246.
- [15] Slavin J. (1990). Dietary fiber: mechanisms or magic in disease prevention? **Nutrition Today**. 25(6), 6-10.
- [16] Funakoshi Y, and Suzuki T (1790). "Glycobiology in the cytosol: The bitter side of a sweet world". **Biochemistry Biophysical Acta**. 2, 81-94
- [17] Horax R., Hettiarachchy N.S., Chen P. and Jalaluddin M. (2004). Functional properties of protein isolate from cowpea (*Vigna unguiculata L. Walp.*). **Journal of Food Science**, 69, 119–121.
- [18] Vani B. and Zayas J.F. (1995). Wheat germ protein flour solubility and water retention. **Journal of Food Science**, 60, 845–848.
- [19] Singh N., Kaur M. and Sandhu, K.S. (2005). Physicochemical and functional properties of freeze-dried and oven dried corn gluten meals. **Drying Technology**, 23, 1–14.
- [20] Sanchez-Vioque R., Climente A., Vioque J., Bautista J. and Millan F. (1999). Protein isolate from chickpea (*Cicer areitinum L.*): chemical composition, functional properties and protein characterization. **Food Chemistry**, 64, 237–243.
- [21] Bora P.S. (2002). Functional properties of native and succinylated lentil (*Lens culinaris*) globulins. **Food Chemistry**, 77, 171–176.
- [22] Achouri A., Zhang W. and Shying, X. U. (1998). Enzymatic hydrolysis of soy protein isolate and effect of succinylation on functional properties of resulting protein hydrolysates. **Food Research International**, 31, 617–623.
- [23] Graham D.E. and Phillips M.C. (1976). The conformation of proteins at the air–water interface and their role in stabilizing of foam. In R. J. Akers (Ed.). *Foams* (pp. 237). London: Academic Press.
- [24] Kuntz I.D. (1971). Hydration of macro-molecules. III: hydration of polypeptides. **Journal of American Oil Chemists Society**, 93, 514–516.
- [25] Escamilla-Silva E.M., Guzman-Maldonado S.H., Cano-Medinal A. and Gonzalez-Alatorre G. (2003). Simplified process for the production of sesame protein concentrate. Differential scanning calorimetry and nutritional, physicochemical and functional properties. **Journal of the Science of Food and Agriculture**, 83, 972–979.

- [26] Mwasaru A.M., Muhammad K., Bakar J. and Cheman Y.B. (1999). Effect of isolation technique and conditions on the extractability, physiochemical and functional properties of pigeon pea (*Cajanus cajan*) and cow pea (*Vigna unguiculata*) protein isolates. II. **Functional properties.** *Food Chemistry*, 67, 445–452.
- [27] Voutsinas L. P. and Nakai S. (1983). A simple turbidimetric method for determining fat binding capacity of proteins. *Journal of Agricultural and Food Chemistry*. 31, 58–63.