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Characterization of hyacinth bean (*Lablab purpureus* (L.) sweet) seeds from Indonesia and their protein isolate

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Abstract

Hyacinth bean (*Lablab purpureus* (L.) sweet) seeds from Indonesia were characterized for the purposes of usage as a protein source. Protein isolate was prepared from the seeds using an isoelectric method, which was also used to characterize the physicochemical and functional properties. Hyacinth bean seeds have a moderate concentration of protein (17.1 ± 1.5%), and low concentration of HCN (1.1 ± 0.1 mg/100 g). However, before using the seeds as food, some treatments are needed to reduce their antinutritional factors, since the contents of trypsin inhibitor and phytate are 0.15 ± 0.02 TIU/mg and 18.9 ± 0.2 mg/g, respectively. Using the isoelectric preparation, the yield of protein isolate was low (7.38 ± 0.2 g per 100 g of the seeds), but the protein isolate had good colour, neutral odour, high protein content (89.8 ± 0.82%), and low ash (2.97 ± 0.36%). The protein isolate also had good functional properties, such as solubility, foaming capacity, and emulsifying activity. However, the foaming and emulsifying stabilities were low.

Keywords: Functional properties; Hyacinth bean; Protein isolate

1. Introduction

The properties of protein that determine their uses in foods are collectively called functional properties. Functional properties denote those physicochemical properties of food proteins that determine their behaviour in foods during processing, storage and consumption. These properties, and the manner in which protein interacts with other components, directly and indirectly affect processing applications, food quality, and acceptance. Water binding, solubility, swelling, viscosity, gelation and surface activity are important properties, determining usefulness and final product quality in a food system (Kinsella, Damodaran, & German, 1985). As is obvious in soybean, that is the source most widely used as a plant functional protein, the legume protein could be used as a food ingredient, such as emulsifier, flavour enhancer, gel former, stabilizer and supplement with high nutritional value.

Indonesia is rich in non-oilseed legumes, such as hyacinth bean, which commonly is planted in marginal land with little attention. According to Stephens (1994), hyacinth bean has many local names, such as lablab, bonavist, Chinese flowering, Egyptian, Pharao, shink, val, wild field, and Indian bean. It is generally considered to have originated in southeast Asia. However, some authorities place its origin in Africa where it has been known since the eighth century. Presently, it is widely cultivated throughout the tropics and subtropics. According to Indonesian farmers, productivity of hyacinth beans in dry land can reach 1000–1200 kg of dry seeds/ha. Unfortunately, no data are available for the total production of the seeds in Indonesia. In some

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areas, which produce hyacinth beans, the young pods are boiled as vegetable, or together with corn are used for soup. The dry seeds are cooked, together with rice, for a protein supply after soaking in water over night. Moreover, the seeds are used as raw materials for tempeh, an Indonesian traditional fermented food, which is commonly made from soybeans.

Hyacinth bean is a non-oilseed legume, which generally contain a moderate amount of protein (18–25% of the seeds). These seeds may be considered as a suitable source of functional protein, due to the good balance of amino acids, their high bioavailability and their relatively low levels of anti-nutritional factors (Roberts, 1985). However, the use of plant proteins in both *modern* and *conventional* foods has been a focus of much research in recent years (Chavan, Mckenzie, & Shahidi, 2001). For these reasons, this work deals with the development of protein from hyacinth bean as a food ingredient. In this paper, the characterizations of the seed of hyacinth bean and its protein isolate are reported.

2. Materials and methods

2.1. Materials

One batch of hyacinth beans used for this study was collected from a farm in Bondowoso City, East Java, Indonesia. After arrival in the laboratory, the seeds were sorted to remove immature and defective beans. The sorted beans were divided into portions for the various analyses, and stored in a cold room (4–6 °C) until used. The chemicals and solvents used were of guaranteed grade.

2.2. Fractionation by protein solubility

Extraction of the proteins of ether-defatted seed flour was conducted at 20 °C according to the method of Hamada (1997) with modification. The method was based on the classical Osborne protein fractionation procedure. Three solvents were used, consecutively, to extract the proteins of ether-defatted seed flour: 2% NaCl, 70% ethanol, and 0.1 M NaOH. Defatted seed flour (2.0 g) was extracted with 100 ml of solvent by shaking for 1 h, followed by centrifugation at 5000*g* for 15 min at 20 °C. The extraction was repeated on the precipitated seed flour with 50 ml of the same solvent. The corresponding supernatants of the two extractions were combined. The NaCl combined extracts were fractionated into albumins and globulins by dialysis (MW cut-off = 12 kDa) for three days at 4 °C against distilled water. Recovered supernatants and precipitates, after centrifugation at 5000*g* for 15 min at 20 °C, were considered as the albumin and globulin fractions, respectively. After sampling for later protein analysis, the fractions for each extraction were combined, dialyzed, and freeze-dried.

2.3. Preparation of the protein isolate

To find the optimal pH for precipitation, hyacinth bean seeds (100 g) were soaked in aqueous solution, pH 10.0 (300 ml), at room temperature for 20 h. Then, the seeds were peeled manually, and were milled with 0.01 M NaOH solution (500 ml), and filtered through a double-layer of cheese cloth. The residue was extracted twice with the same solution. The filtrates obtained were combined, and centrifuged at 4000*g* for 10 min to remove starches. From the filtrate, 50 ml of each were taken into a glass beaker, and then the pH was adjusted to various values from 10 to 2.0. After centrifugation (8000*g* for 10 min), protein concentration in the filtrate was analyzed using Lowry methods (Bollag & Edelstein, 1991). Relationship of the pH with protein concentration in the filtrate is shown in Fig. 1. As is obvious in the Figure, the optimal pH for precipitation was pH between 4.0 and 4.5.

In the extraction of protein isolate, a similar method to the isoelectric one above was also used. After centrifugation and filtration, the pH was adjusted to 4.5 by 1 M HCL, and, then, the protein isolate was recovered by centrifugation at 8000*g* for 10 min. To remove sugars and phenols, the protein was washed with 200 ml of 70% ethanol, twice, before being freeze-dried.

2.4. Physical measurements

The seed length, width, and thickness were measured with a vernier calliper. The seed weight was measured by analytical balance. To evaluate the colour of the seeds and the protein isolate, a Chromameter CR-100 (Minolta) was used; illuminant C for daylight, and an averaging mode with eight replications per sample. The instrument was calibrated externally with a standard

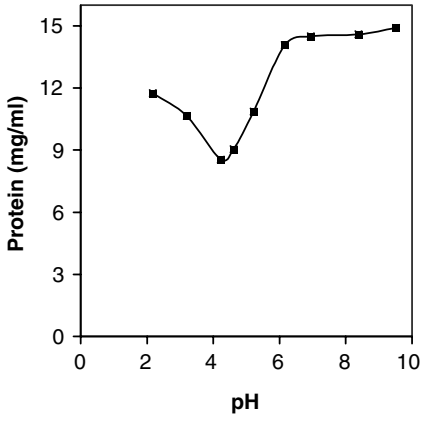

Fig. 1. Effects of pH on protein solubility in milk of hyacinth beans.

white tile, and the tristimulus coordinates L^*, a^*, and b^* (CIE Lab. colour scale) system was applied to express the colour. Values of L^* indicate lightness, and range from black = 0 to white = 100. The colour at the grid origin ($a^* = 0$, $b^* = 0$) is achromatic (gray). On the horizontal axis, positive a^* indicates a hue of red–purple, whereas negative a^* is a hue of bluish-green. On the vertical axis, positive b^* indicates yellow, and negative b^* blue. Furthermore, values of hue angle ($H^* = \tan^{-1} b^*/a^*$) indicate sample colour where hue angle 0° coincides with red colour; 90°, yellow colour; 180°, green colour; and 270°, blue colour (McGuire, 1992; Voss, 1992), whereas C^* is for metric chroma, the correlate of saturation ($C^* = (a^{*2} + b^{*2})^{0.5}$), as described by Gonnet (1999). Degree of whiteness was calculated by the formula, $W(\%) = 100 - ((100 - L)^2 + ((a^*)^2 + (b^*)^2))^{0.5}$.

2.5. Chemical analysis

Protein, fat, ash, hydrogen cyanide (HCN), phytate and moisture contents of the seeds and the protein isolates were analyzed using AOAC methods (1990), and the carbohydrate content was calculated by difference method. Trypsin inhibitor fraction was extracted from the hyacinth bean seeds according to Akpapunam and Sefa-Dedeh (1997), and then the fraction was used to determine the trypsin inhibitor activities using casein as substrate.

2.6. Functional properties of protein isolate

Solubility of the protein isolate was analyzed at various pH values. Sample (5 g) was dissolved in 0.1 N NaOH (150 ml) by stirring for 2 h at room temperature; 10 ml of the solution were taken, and adjusted to desired pHs by 1 N HCl. Then, the solutions were centrifuged at 5000*g* for 30 min. Protein content of the supernatants were analyzed using the Lowry method (Bollag & Edelstein, 1991).

Emulsifying properties of the protein isolate were analyzed by the absorbance method. Sample (0.1 g) was dissolved in phosphate buffer, pH 7, (50 mM, 100 ml) with stirring for 15 min. Palm oil (25 ml) was added, and then homogenized for 3 min. The emulsion (1 ml) was taken immediately, and mixed with 5 ml of 0.1% sodium dodecyl sulfate by gently stirring with a glass rod. Absorbance was read at 500 nm. The emulsifying activity index (EAI) (unit: m^2/g) and emulsifying stability index (ESI) were calculated according to the Pearce and Kinsella method (Webb, Naeem, & Schmidt, 2002).

The test on foaming property was based on the Were, Hettiarachchy, and Kalapathy (1997), wherein foam capacity was the foam volume (ml) immediately after aeration, and foam stability was the initial foam volume divided by the decrement of foam volume after standing for 2 min and was multiplied by 2 min. Oil holding capacity (OHC) of the protein isolate was determined by mixing the isolate (0.5 g) with palm oil (7 ml) for 1 h, then centrifuging at 2000*g* for 5 min. After decantation, the sample was weighed and OHC was calculated as the percentage of oil trapped by the protein isolate. Water holding capacity (WHC) of the protein isolate was determined similarly to OHC but replacing oil by water.

3. Results and discussion

Seeds of hyacinth bean from Indonesia used in this study were very homogeneous as shown by their physical properties (Table 1). The seed size was small with a volume of about 0.19 ml/seed. Their shape was flat and mostly round. There was a white hilum along one third of the seed, and slightly stuck out from the seed surface. The seed coat was very thin (0.10 ± 0.01 mm) and it was very difficult to remove it from the seeds mechanically. The seed colour was brownish yellow ($H = 67.3° \pm 4.6$), and not very vivid, as shown by the small value of c^* (Table 1).

According to the chemical analysis (Table 2), carbohydrate was the dominant component of the hyacinth bean seed, accounting for about 67.9 ± 4.2% on a wet basis, followed by protein and ash with 17.1 ± 1.5%, and 3.6 ± 0.1%, respectively. The protein content of the hyacinth bean seed studied was lower than that of other non-oilseed legumes, such as jack bean (23–30%) (Akpapunam & Sefa-Dedeh, 1997), pigeonpea (22.6%) and cowpea (29.3%) (Mwasaru, Muhammad, Bakar, & Che Man, 1999). Moreover, the seed contained HCN (1.1 ± 0.1 mg/100 g), which was at lower concentration than the maximal allowed amount of 20 mg/100 g (Akpapunam & Sefa-Dedeh, 1997), suggesting that this seeds could be consumed without any concern of HCN poisoning. Furthermore, since the hyacinth bean seed was easily infected by bugs, this suggested that the seed contained no significant poison. However, the phytate

Table 1
Physical properties of seeds of hyacinth bean (*L. purpureuos* (L.) sweet)

Physical properties	Value
Seed thickness (cm)	0.40 ± 0.03
Seed width (cm)	0.74 ± 0.05
Seed length (cm)	1.05 ± 0.10
10 Seeds volume (ml)	1.91 ± 0.399
Seed weight (g)	0.2334 ± 0.0287
Seed longitudinal surface area (cm^2)	0.8466 ± 0.1253
Seed husk thickness (mm)	0.10 ± 0.01
Percentage after de-coating (%)	83.2128 ± 1.1077
Seed colour	$L = 73.4 \pm 3.4$; $a^* = 3.9 \pm 1.4$; $b^* = 9.3 \pm 2.3$; $c^* = 10.1 \pm 1.2$; $H = 67.3° \pm 4.6°$

Table 2
Chemical composition of hyacinth bean seeds[a]

Components	Amount
Moisture (%)	9.3 ± 0.5
Protein (%)	17.1 ± 1.5
Lipid (%)	1.1 ± 0.4
Ash (%)	3.6 ± 0.1
Carbohydrate (%) [b]	67.9 ± 4.2
HCN (mg/100g)	1.1 ± 0.1
Phytate (mg/g)	18.9 ± 0.2
Trypsin inhibitor (TIU/mg)	0.15 ± 0.02

[a] Calculated on wet basis.
[b] Calculated using by difference from moisture, protein, lipid and ash.

and trypsin inhibitor contents were high, suggesting that treatment should be applied to reduce these anti-nutritional factors before the seeds are consumed, such as by soaking, germination and cooking (Akpapunam & Sefa-Dedeh, 1997).

By using the Osborne sequence method, the protein of hyacinth bean seed was fractionated based on solubility in some solvents, as shown in Table 3. The globulin fraction was dominant in the protein of the seeds (about 55%), followed by the NaOH soluble fraction and albumin, 27% and 18%, respectively. This result supported the report that in the legume seed, the storage protein is predominantly globulin (Marcone, 1999).

Further analysis showed that the amount of 11S globulin in the hyacinth bean seed was very low, only 9.44% of the total protein, while that of 7S globulin was very high (20.5%). Commonly, dicotyledonous seeds have been found to favour the presence of the 11S globulin form (Marcone, 1999). In soybeans, the content of 11S globulin equals that of 7S globulin (Nielsen, 1985), or the ratio of 11S–7S is about 0.5–1.7 (Utsumi, Matsumura, & Mori, 1997). However, the total amounts of 7S and 11S globulins detected in this study were only about 30% of total proteins, suggesting that this amount was much lower than that of total globulin (55%). It may be that the amounts of other globulins, such as 2S and 15S, which were not analyzed, were high, and/or the method used in this study could not analyze 7S and 11S globulins of hyacinth bean seeds optimally.

Table 3
Protein fraction in hyacinth bean seeds[a]

Protein	Amount (%)
Albumin	18.2
Globulin	55.2
7S Globulin	20.5
11S Globulin	9.44
Prolamine	ND[b]
NaOH-soluble fraction	26.6

[a] Calculated as percentage of total protein.
[b] Not detected.

The lower amount of 11S globulin suggests that the protein of the seeds could not form a strong gel, since there were not enough sulfhydryl groups, which are needed to form the disulfide bond in the gel. Accordingly, this seed protein is not recommended as a raw material for gel-like products, such as tofu (Utsumi & Kinsella, 1985).

3.1. Characteristics of protein isolate

Protein isolate of hyacinth beans prepared by the method described in the Section 2 had 89.8 ± 0.82% protein (Table 4), while the yield of protein isolate was 7.38 ± 0.2 g per 100 g of the seeds. Since the protein content in the seeds was 17.1 ± 1.5%, the total protein recovery ranged from 37% to 40%. Compared with other studies on protein isolate from other bean seeds, this value was very low. Chavan et al. (2001) showed that, by NaOH extraction, the yield of total protein from beach pea ranged from 67.9% to 77.3% while, in pigeon pea, the range was 49.7–63.6% (Ant'Anna & Vilela, 1985) and 35–58% (Mwasaru et al., 1999). Incomplete recovery of protein may, in part, be due to complexation with other components (Chavan et al., 2001), so that it is difficult to extract. Furthermore, the precipitation pH used also could not recover all extracted proteins, as shown in Fig. 1, because only about 50% of extracted protein could be precipitated at pH 4–4.5. This may be caused by the soaking treatment with aqueous solution, pH 10, for 20 h before extraction. This treatment may drive the hydrolysis of protein to small polypeptides, which could not be precipitated at pH 4. Unfortunately, this soaking treatment is necessary to remove the hard hull of the seeds, which gives unpleasant colour and taste to the isolate. However, this result also indicated that soaking of hyacinth bean seed gave protein having good solubility at low pH, suggesting its high functionality as a food ingredient. Therefore, future, studies should address extraction to achieve high protein yields and to produce isolates that show high solubility in water.

Table 4 shows the chemical composition of the protein isolate. As shown in this Table, the protein isolate had 89.8 ± 0.82% protein on a dry basis, suggesting that the preparation method used was good enough to re-

Table 4
Chemical composition of protein isolate from hyacinth beans

Components	Wet basis (%)	Dry basis (%)
Moisture	8.58 ± 0.18	–
Protein	82.1 ± 0.80	89.8 ± 0.82
Ash	2.73 ± 0.35	2.97 ± 0.36
Starch	4.10 ± 0.04	4.60 ± 0.04
Lipid	1.96 ± 0.05	2.15 ± 0.07
Total sugar	ND	ND

Table 5
Colour of protein isolate from hyacinth bean seeds

Colour parameters	Value
L	90.32 ± 0.13
*a**	1.76 ± 0.23
*b**	3.32 ± 0.13
*c**	3.76 ± 0.12
H (°)	62.09 ± 3.61
W (%)	89.62 ± 0.17

Table 6
Functional properties of protein isolate from hyacinth bean seeds

Properties	Value
Foaming capacity (ml/g)	232 ± 12.2
Foaming stability (min)	2.3 ± 0.2
OHC (%)	254 ± 0.2
WHC (%)	321 ± 12.2
EAI (m^2/g)	534 ± 4.5
ESI (h)	2.7 ± 0.1

move the other components, such as lipid (2.15%) and sugar (not detected). Furthermore, ash content of the isolate (2.97 ± 0.36%) was much lower than that of beach pea (5.99%) (Chavan et al., 2001), and chick pea (4.23%) (Clemente et al., 1999), which were prepared by the same isoelectric point methods. These results suggested that washing treatment (using 70% ethanol in the final step of isolate preparation) might remove the other impurities of the protein isolate from hyacinth bean seeds. The colour of the isolate also was good, as shown by invivid colour (c^* = 3.76 ± 0.12) and a high degree of whiteness (W = 89.62 ± 0.17%) (Table 5), suggesting that: (a) the pigments in the seeds, which may disturb the whiteness of the isolate, could be eliminated during preparation, and/or (b) the sugars, which may lead to Maillard reaction, forming brown colour, could be reduced. However, there was no evidence of effects of the ethanol washing treatment on other properties, especially functional properties, of the isolate.

Solubility of the protein isolate was very low in the pH range 3–5.5, but the solubility increased significantly when the pH was adjusted to a value lower than 3 or higher than 5.5 (Fig. 2). Solubility profile of protein isolate was typical. However, the high protein solubility at pH 5.7–6 (about 60%) was interesting, since common protein isolates from beans have high solubility at pH above 6.5 (Chavan et al., 2001).

The foaming stability and capacity of the protein isolate are shown in Table 6. As shown in the Table, the isolate had a forming capacity of 232 ± 12.2 ml/g. This

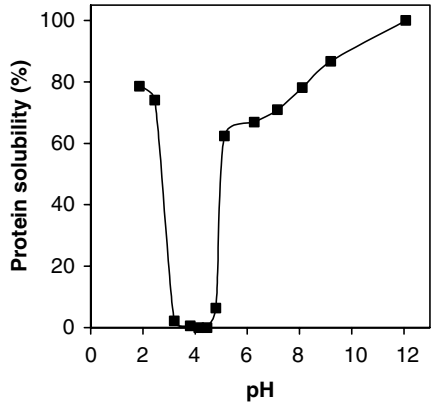

Fig. 2. Solubility of protein isolate from hyacinth bean.

foam capacity was markedly higher than that of other isolates, such as greenpea, grasspea and beachpea, which were below 200% (Chavan et al., 2001). Increase in foam capacity in certain protein isolates might be due to increased solubility, rapid unfolding at the air–water interface, limited intermolecular cohesion and flexibility of the protein surfactant molecules (Kinsella et al., 1985). However, the foaming stability of the protein isolate from hyacinth bean seed was very low, only 2.3 min (Table 6). This means that the 232 ml of foam would disappear after 2.3 min of standing.

OHC of the protein isolate from hyacinth bean seed was high (254 ± 0.2%), as shown in Table 6. This value was, above those of beach pea (64–82%), Woodstone pea (90.1–94.5%) and fieldpea (90–127%) (Chavan et al., 2001). The high OHC suggested the presence of a large proportion of hydrophobic as compared to hydrophilic groups on the surface of protein molecules. Interestingly, WHC of the isolate was also high, at 321 ± 12.2% (Table 6).

Emulsifying properties of protein isolate from Indonesian hyacinth bean seed were analyzed by EAI and ESI, as shown in Table 6. The EAI reflects the ability of the protein to rapidly adsorb at the water/oil interface during the formation of the emulsion, preventing flocculation and coalescence. The ESI reflects the ability of the protein to maintain a stable emulsion over a period by preventing the flocculation and coalescence of the oil globules. As shown in Table 6, EAI of the protein isolate from hyacinth bean (534 ± 4.5 m^2/g) was much higher than other isolates such as soybean protein isolate (116 ± 6.4 m^2/g) (Webb et al., 2002). A high value of EAI might due to the high hydrophobic capacity of the isolate, as is also shown by the high OHC (Table 6). However, the ESI was very low (2.7 ± 0.1 h), compared with that of soybean protein isolate (52.0 ± 3.8 h). The reason for this phenomenon is still unknown.

4. Conclusion

Hyacinth bean seeds have a moderate concentration of protein, and low concentration of HCN. However, some treatments are needed to reduce their trypsin inhibitor and phytic acid contents before using the seeds as food. Using an isoelectric preparation, the yield of protein iso-

late was low, but the protein isolate had good colour, neutral odour, high protein content and low ash. The protein isolate also had good functional properties, such as solubility, foaming capacity and emulsifying activity. However, the foaming and emulsifying stabilities were low.

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