

Preparation of wood apple (*Feronia limonia* L.) seed protein concentrate and evaluation of its nutritional and functional characteristics

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Abstract: Wood apple (*Feronia limonia* L.) seed protein concentrate (WSPC) was prepared and its properties were compared with the wood apple seed meal (WSM). The protein content was found to be 33.79 and 77 g/100 g in WSM and WSPC respectively. WSPC was good source of essential amino acids leucine, phenyl alanine, valine, iso-leucine and threonine. Protein extractability of WSPC showed optimum WSPC to water ratio of 1:50 (w/v), over 60 min. Maximum protein extractability (95 g/100 g) was observed at pH 12 and minimum (11 g/100 g) at pH 6. Protein precipitability was maximum (91 g/100 g) at pH 5.5. A higher buffer capacity of WSPC was observed in the pH range 6 - 2. SDS-PAGE of the WSM and WSPC showed 9 protein bands ranging from 205 kDa to 12 kDa. Higher water absorption, lower oil absorption capacity, stable foam and presence of essential minerals of WSPC favour its industrial application.

Keywords: Amino acid composition, functional properties, minerals, protein extractability, proximate composition, wood apple seed protein concentrate

Introduction

Plant seeds are important unconventional sources of proteins which when incorporated in food products would improve the functional properties such as absorption of water or oil and formation of stable foam. They are also good nutritional supplements. Numerous studies were reported earlier on screening various seed meals. Most of the studies were related to determination of proximate composition, mineral content, physico-chemical and functional characteristics and protein extractability. They were based on soy flour (Surekha and Jamuna, 2006), pumpkin seed (Lazos, 1992), cashew nut (Fagbemi, 2008), erythrina seed flour (Jyothirmayi *et al.*, 2006) and beach pea protein isolate (Chavan *et al.*, 2001).

One important plant material which has not been widely studied is the wood apple (*Feronia limonia* L.) seed. The wood apple plant belongs to the family *Rutaceae*. The fruit is rich in pectin (3-5%), and is an excellent material for preparation of wood apple jelly. The fruit is also rich in calcium and phosphorous (Wealth of India, 1952). Most of the published works are related to studies on fruit pulp, with the nutritional quality and related aspects reported (Paul and Shaha, 2004). Interestingly, though the seed is a rich source of protein and oil, very little work was reported on these aspects. The seed composition and fatty acid profile were reported as 28% protein and 34% oil (Ramakrishna *et al.*, 1979).

The literature on utilization of this seed protein is very limited and hence, the present investigation was undertaken to study wood apple seed meal and

seed protein concentrate of the wood apple. The proximate composition, mineral content and amino acid composition of the seed meal and functional properties of the seed protein were evaluated.

Materials and Methods

Materials

Wood apple fruits were purchased in one lot from a local fruit market in Hyderabad, India. Refined sunflower oil was purchased from a local market in Hyderabad. Solvents such as hexane and methanol were of laboratory grade (98-99% purity) and chemicals used were of analytical grade (99.5-99.9% purity). The solvents and chemicals were procured from Sd. Fine Chemicals Company limited, Mumbai and Merck (I) Ltd., Mumbai, India.

Preparation of wood apple seed meal (WSM) and wood apple seed protein concentrate (WSPC)

The outer hard shell of wood apple fruit was broken and the pulp was separated from the shell by scooping. From the pulp, the seeds were manually separated by washing under running water. The seeds were dried in a cabinet tray drier at $45 \pm 2^\circ\text{C}$ for 4 h. A part of the dried seeds was ground in a laboratory mixer/grinder (Sumeet make, Nasik, India) to obtain the wood apple seed meal (WSM). The other part the seeds was subjected to coarse grinding in a porcelain mortar to separate the hulls and they were manually removed to obtain the cotyledon (dehulled seed). The de-hulled seed was ground in a laboratory mixer/grinder. The dehulled seed flour was soaked in

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hexane at room temperature with occasional stirring for a period of 6 h to remove the fat. The solvent was separated and extractions were carried out thrice with fresh solvent. After each extraction, the solvent was decanted. Later the fat free meal was air-dried at room temperature and ground to pass through a BS 60 (240 μm) mesh sieve. The final product was wood apple seed protein concentrate (WSPC) and it was packed in a polyethylene pouch to store at room temperature for further investigations.

Proximate composition

Moisture, total ash, crude fat and crude fibre of WSM and WSPC were assayed by standard methods of Association of the Official Analytical Chemists (AOAC, 1995). The nitrogen content was determined by micro Kjeldahl method (Ranganna, 1986) and protein content was calculated as $\text{N} \times 6.25$. The carbohydrate content was estimated by difference. Bulk density of WSM and WSPC was computed by measuring the volume occupied by known weight of samples in a measuring cylinder.

Mineral content

The mineral content in the sample was determined from the total ash of the sample. Total ash was prepared by igniting 5 g sample in silica dish and further combusting in a muffle furnace at 550°C for 6 h. The ash was digested with 25 mL of 6M hydrochloric acid on water bath for 30 min and made up to 50 mL in a standard flask with acid. The above ash solution was taken for estimation of certain minerals such as cadmium, chromium, copper, iron, lead, magnesium, potassium and zinc by atomic absorption spectroscopy (Shimadzu AA 6701 F, Atomic Absorption Flame Emission Spectrophotometer, Shimadzu Ltd, Kyoto, Japan) equipped with hollow cathode lamp. A 1000 $\mu\text{g/mL}$ of standard metal solution (Shimadzu, Kyoto, Japan) was diluted successively to 2, 1, 0.5, 0.4, 0.2, 0.1 $\mu\text{g/mL}$ and aspirated before analysis of test samples (AOAC, 1995). The calcium content was estimated by titrimetric method (Ranganna, 1986). Phosphorous content was analyzed by using Fiske-Subbarow reagent (Raghuramulu *et al.*, 2003). A standard graph was drawn using potassium dihydrogen phosphate (0.1-0.6 mg/mL). The blue colour developed was read at 650 nm in UV-Visible spectrophotometer (Shimadzu, UV-160A model).

Total polyphenol content

The total polyphenol content was determined by extracting 1 g of sample with 80% ethanol in water (v/v) and treating with Folin-Ciocalteu reagent (phenol reagent). A standard calibration curve was

drawn by using pure gallic acid 19-76 $\mu\text{g/mL}$ and the optical density was recorded at 675 nm. The total polyphenol content was expressed as mg of gallic acid equivalents /100 g sample (Sadasivam and Manickam, 1997).

Functional properties

Water absorption capacity (WAC) and Oil absorption capacity (OAC)

The water absorption capacity and oil absorption capacity of WSM and WSPC were determined by dispersing 1 g of sample in about 10 mL of distilled water or refined sun flower oil. The contents were mixed thoroughly in a vortex mixer for 2 min. and then centrifuged at $3000 \times g$ for 30 min. The supernatant water was decanted carefully and residue weighed (Johnson, 1970). The WAC of the sample was expressed as grams of water absorbed per 100 g, and the OAC as grams of oil absorbed.

Foam measurements

Foaming capacity (FC) and foam stability (FS) of WSM and WSPC were determined (Lawhon *et al.*, 1972) by dispersing 2 g of sample in 100 mL distilled water and stirred for 5 min using a mechanical stirrer (Eltek Motor type 1, Mumbai, India). The contents along with foam were immediately transferred into a 250 mL graduated cylinder. Volume of initial foam was recorded and expressed as foam capacity in milliliters. The foam stability was measured by noting down the volume of the stable foam with time intervals of 30, 45 and 75 min.

Protein extractability

The protein was extracted from WSPC in water and sodium chloride solutions (0.1, 0.5 molar) for 1 h at room temperature ($28 \pm 2^\circ\text{C}$). The ratio of meal to water was studied in the range of 1:10 to 1:70 (w/v). Subsequently, the extraction times were varied from 10 to 80 min, and the pH was varied from 2 to 12. These studies were similar to those reported in the literature (Dev *et al.*, 1986). The required pH was adjusted using 0.5 M hydrochloric acid or sodium hydroxide solutions. The unextracted material was separated by centrifugation using Remi C-30 BL refrigerated Centrifuge (Remi Instruments Division, Mumbai, India) using a rotor 28 cm in diameter with 12×50 mL capacity tubes at $3000 \times g$ for 30 min. The supernatants were analysed for protein content and the results were expressed as the protein/100 g of WSPC.

Protein precipitability

Protein precipitability of WSPC was carried out (Taher *et al.*, 1981) by dispersing 7 g of WSPC in 350 mL distilled water and the pH of the suspension was adjusted to 12. The mixture was stirred for 60 min, and the suspension was centrifuged using a rotor 28 cm in diameter with 8 × 100 mL capacity tubes at 3000 × g for 30 min. The supernatant liquid of 20 mL each was taken in a number of graduated centrifuge tubes. The pH was adjusted (2 – 6.5) and the suspensions were vortexed for 10 min and centrifuged using a rotor 28 cm in diameter with 12 × 50 mL capacity tubes at 3000 × g for 30 min. The volumes of supernatant liquid were noted and protein contents were analysed. Protein precipitability was calculated by the following equation in 100 g of soluble protein:

$$\text{Protein precipitability (g/100 g soluble protein)} = \frac{(V_1 \times P_1 - V_2 \times P_2)}{V_1 \times P_1} \times 100$$

where V_1 and V_2 are volumes of aliquots in mL before and after precipitation and P_1 , P_2 are mg of protein in 1 mL of V_1 and V_2 , respectively.

Buffer capacity

WSPC (1 g) was dispersed in 50 mL distilled water and a measured volume of 0.1 M sodium hydroxide solution was added in small increments and the corresponding changes in pH were recorded. To another portion, hydrochloric acid was added and the corresponding pH changes were measured. The quantity of NaOH or HCl added was plotted against pH and the buffer capacity was expressed in terms of mmol of NaOH or HCl required bringing about a change of one pH unit per gram of WSPC.

Amino acid composition

The amino acid profile was determined by analysing the hydrolysed samples on Biochrom Automatic amino acid analyzer (Biochrom 30, England). The vacuum tubes were purged with nitrogen to displace air. WSPC equivalent to 5 mg protein was placed in 20 mL vacuum hydrolysis tube, to which 6N HCl (10 mL) was added. The tubes were evacuated and sealed under vacuum. The sample was allowed to hydrolyse in a Lab model oven (Dalal, Mumbai, India) at $110 \pm 2^\circ\text{C}$ for 24 h. The hydrolysed reaction mass was cooled, volume was made up to 25 mL and filtered. An aliquot of 5 mL was evaporated under vacuum to dryness and the residue was mixed with 2.5 mL of loading buffer (pH 2.2).

Aliquot of 20 μL was injected into automatic amino acid analyzer (Biochrom 30, England). All the amino acids were detected after post column

derivatization with Ninhydrin reagent. Elutes were spectrophotometrically monitored at 570 nm and the concentrations of the unknown samples were determined by comparing with standard peak areas (Agilent amino acid standard kit, USA).

Cysteine and methionine content in WSPC was determined after converting into cysteic acid and methionine sulphone respectively, by oxidizing the sample with performic acid (9:1 mixture of 80% formic acid and 30% hydrogen peroxide) for 18 h at room temperature by the method of Moore (1963). The excess of oxidizing reagent was removed by evaporation and the mass was hydrolysed with 6N HCl. Later it was derivatized and eluted as described above. All analyses were conducted in duplicate and mean values were presented.

Electrophoresis (SDS-PAGE)

WSM and WSPC were examined for relative molecular weights using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). Protein samples (0.5 g) were dispersed in 25 mL SDS (2 g/100 g), vortexed and centrifuged at 3000 × g for 30 min. An aliquot (400 μL) of the supernatant was treated with 100 μL sample loading buffer containing 60 mmol Tris-HCl buffer (pH 6.8), 25 mL glycerol, 2 g SDS, 2.5 mL -mercaptoethanol and 0.1 mL bromophenol blue. The samples were heated in a water bath maintained at 95°C for 5 min. SDS-PAGE was performed using stacking gel (4 g/100 g) and separating gel (10 g/100 g) polyacrylamide gels on Mini Slab Gel Electrophoresis Unit (Balaji Scientific Services, Chennai, India) with loading of 25 μL in each well. Electrophoresis was carried out at 10 mA and 25°C . Protein bands were stained with 0.1 g Coomassie brilliant blue R-250, 20 mL acetic acid, 30 mL methanol and 50 mL water, and de-staining was carried out in a solution containing glacial acetic acid, methanol and water (2:3:5, v/v/v). Relative molecular masses were compared using a wide range MW marker (Sigma Chemicals Co., St. Louis, USA) consisting of myosin (205 kDa), galactosidase (116 kDa), phosphorylase B (97 kDa), Bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa). Cytochrome C (12 kDa) (Sigma Chemicals Co.) was also mixed in the marker solution.

Moisture sorption isotherm

Moisture sorption isotherm of WSPC was plotted at room temperature. WSPC (5 g), each in glass petri plates were exposed to the different relative humidity (RH) conditions of 10, 30, 50, 70, 90 and 100% were maintained by using appropriate concentrations of

sulphuric acid (39, 29.6, 23, 16.6, 7.8 and 0 M) to determine the equilibrium moisture content (EMC) and relative humidity (Landrock and Procter, 1951). The sample was observed visually for change in colour, lump formation and mold growth during the study. The samples which were exposed to different RHs were weighed at regular intervals on electronic monopan balance (Shimadzu, Model Libror AGE 220, Asia pacific Singapore) till they reached a constant weight (no gain or loss). A graph was plotted for EMC against RH to obtain sorption isotherm of WSPC.

Statistical analysis

The data was collected at room temperature ($28 \pm 2^\circ\text{C}$) in triplicate and mean values \pm standard deviation (SD) were reported. The statistical analysis was conducted using SPSS 11.5 for windows (SPSS Inc., Chicago, IL). The statistical analysis differences were calculated using T-test. Differences between means were evaluated as significant at $P \leq 0.05$.

Results and Discussions

Proximate composition

The kernel and hull portions in wood apple whole seed were 60.6 and 39.4 g/100 g respectively. Proximate composition of WSM and WSPC were presented in Table 1. The protein content was found to be 77 g/100 g of WSPC as compared to 33.8 g/100 g of WSM. Similarly the carbohydrate accounted for 19.74 of WSM and 6.9 g/100 g of WSPC on sample basis. The energy values were calculated to be 487 and 340 kcal/100 g for the wood apple seed meal and wood apple seed protein concentrate respectively. The higher protein content in WSPC would suggest its potentiality for incorporation in foods. The WSPC product was a free flowing white powder with a bland taste possessing a bulk density of 0.33 g/mL when compared to 0.42 g/mL for WSM. Similar trends were observed for melon seed flour by Akubor (2005).

Essential nutrients and polyphenols

The mineral content of WSM and WSPC are presented in Table 2. The analysis showed that higher concentrations of calcium and phosphorus were found (105 and 1448 mg/100 g) in WSPC than in WSM (50 and 612 mg/100 g). Similarly, iron content in WSM was 3.0 mg/100 g while that in WSPC was 4.2 mg/100 g. Toxic minerals such as cadmium, chromium and lead were found to be lower than the detectable limits of the method employed.

Polyphenols are important antioxidant components present in most legume seeds. Hence they were also

measured in the wood apple seeds (Table 2). The results showed that WSPC possess higher polyphenol content than WSP. This may be due to the release of bound phenols during processing. Comparison could be made with very high polyphenol contents observed in certain legume seeds (Amarowicz *et al.*, 2004).

Table 1. Proximate composition of wood apple seed^a

Parameter (g/100 g)	WSM	WSPC
Moisture	5.00 \pm 0.12 a	7.23 \pm 0.17 b
Total ash	3.04 \pm 0.11 a	6.79 \pm 0.36 b
Crude fat	30.40 \pm 1.00	NM
Crude protein	33.79 \pm 0.55 a	77.07 \pm 0.40 b
Crude fibre	8.00 \pm 0.40 a	1.99 \pm 0.09 a
Carbohydrates by difference	19.77 \pm 0.54 a	6.92 \pm 0.93 a
Energy kcal /100 g	487.00 \pm 0.20 a	336.00 \pm 0.17 b
Bulk density, g / ml	0.42 \pm 0.01a	0.33 \pm 0.01 b

^aValues are means of triplicate with \pm SD.

WSM: Wood apple seed meal.

WSPC: Wood apple seed protein concentrate.

a, b different letters with in the same raw indicate significant difference at $P < 0.05$.

NM: Not measured.

Table 2. Mineral content of wood apple seed^a

Minerals (mg/100g)	WSM	WSPC
Cadmium	VLOD	VLOD
Calcium	50.00 \pm 0.83 a	105.00 \pm 0.8 b
Chromium	VLOD	VLOD
Copper	0.74 \pm 0.04 a	1.50 \pm 0.22 a
Iron	3.00 \pm 0.20 a	4.20 \pm 0.53 a
Lead	VLOD	VLOD
Magnesium	1.50 \pm 0.26 a	2.00 \pm 0.28 b
Phosphorus	612.00 \pm 1.32 a	1448.00 \pm 1.80 b
Potassium	12.00 \pm 0.40 a	15.00 \pm 0.72 b
Zinc	1.80 \pm 0.48 a	3.20 \pm 0.26 b
Total polyphenols	235.00 \pm 0.34 a	358.00 \pm 0.42 b

^aValues are means of triplicate with \pm SD.

WSM: Wood apple seed meal.

WSPC: Wood apple seed protein concentrate.

a, b different letters with in the same raw indicate significant difference at $P < 0.05$.

VLOD Values lower than limit of detection, (LOD values in ppm Cadmium 0.007;

Chromium 0.024; Pb, 0.015).

Functional properties of WSPC

Water holding capacities of 158 and 97 g/100 g were observed for WSM and WSPC respectively. Higher values of water absorption capacity and oil absorption capacities are desirable attributes of any seed protein. Higher water absorption values of proteins indicate the swelling ability and property of dissociation for exposing additional binding sites. Water absorption is influenced by several factors such as the number of hydration positions, physical environment, pH, solvent, presence of lipids and carbohydrates (Kinsella, 1982). The hulls would contribute for higher water absorption. Hence the WSM containing a higher content of hulls (39 g/100 g) had a higher water absorption capacity.

WSM and WSPC exhibited oil holding capacity of 119 and 111 g/100 g and the presence of fixed oil might be causing WSM to absorb less oil. These values are comparable to that of raw horse gram meal reported by Pavithra *et al.* (2006) and in peanut flour (Surekha and Jamuna, 2006). The lower water absorption and higher oil absorption in WSPC might also be due to the presence of polar amino acids (Lazos, 1992).

The WSPC, because of its higher protein content, possessed good amount of foaming capacity (58 mL) with greater stability (50 mL after 75 min.) when

compared with WSM which showed only 4 mL foam volume and the stability was also poor (2 mL after 75 min.).

Amino acid composition

Amino acid analysis of the WSPC showed Alanine (3.84 g/100 g), Arginine (11.84 g/100 g), Aspartic acid (Aspartate + Asparagine, 8.18 g/100 g), Cysteine 1.44 g/100 g), Glutamic acid (Gltamate + Glutamine, 18.54 g/100 g), Glycine 5.15 g/100 g), Histidine (2.05 g/100 g), Isoleucine (3.30 g/100 g), Leucine (6.71 g/100 g), Lysine (2.42 g/100 g), Methionine (0.84 g/100 g), Phenylalanine (4.19 g/100 g), Proline (4.87 g/100 g), Serine (3.64 g/100 g), Threonine (2.59 g/100 g), Tyrosine (3.60 g/100 g), and Valine (3.94 g/100 g), which was comparable with the reported results for cereals, legumes and oil seeds. The protein content was found to be 77% in WSPC which was also comparable with soy protein concentrate (Obulesu and Bhagya, 2006).

The ratio of essential to non essential amino acids in WSPC was 0.40 which was almost comparable to that reported (0.42) for defatted sesame seed meal (Johnson *et al.*, 1979).

Effect of extraction parameters on protein extractability

Initially, studies were made to examine the effect of ratio of water to WSPC for maximum protein extractability by taking different ratios of WSPC to water in the range of 1:10 to 1:70 (w/v). The optimum WSPC to water ratio for maximum protein extractability was found to be 1:50 as shown in Fig 1 (A). To find the optimum extraction time, it was increased from 10 to 80 min, and the optimum time was noted to be 60 min. Protein extractability increased from 4.2 g/100 g in the first 10 min to 8.6 g/100 g in 60 min and later it remained practically constant. The data are presented in Fig 1 (B). The solubility of protein in water was much less, which might be explained on the basis of hydrophobic nature of the proteins. Similar studies with defatted flaxseed flour were reported by Oomah (1994) and the optimum meal to water ratio was reported to be 1:40 (w/v).

The effects of pH and sodium chloride concentration on protein extractability were presented in Fig 1 (C). The highest and lowest protein extractability values (95 g/100 g and 11 g/100 g) were observed at pH 12 and 6, respectively. The protein extractability increased on either side of pH 6. It increased from 11 g/100 g (pH 6) to 58 g/100 g (pH 2) and to 95 g/100 g (pH 12). As is evident, the protein extractability increased rapidly in alkaline pH

as compared to that in acidic pH. The data obtained indicated that the isoelectric point was at pH 6. The addition of salt enhanced the protein solubility at isoelectric point. At pH 2 and 12, the protein extractability was decreased by the addition of 0.1 and 0.5 M sodium chloride solutions. Similar studies on protein extractability of neem seed (*Azadiracta indica*) were reported by Usman *et al.* (2005) which showed similar trend for pH of 8-10 in 0.1 to 0.5 M sodium chloride solutions.

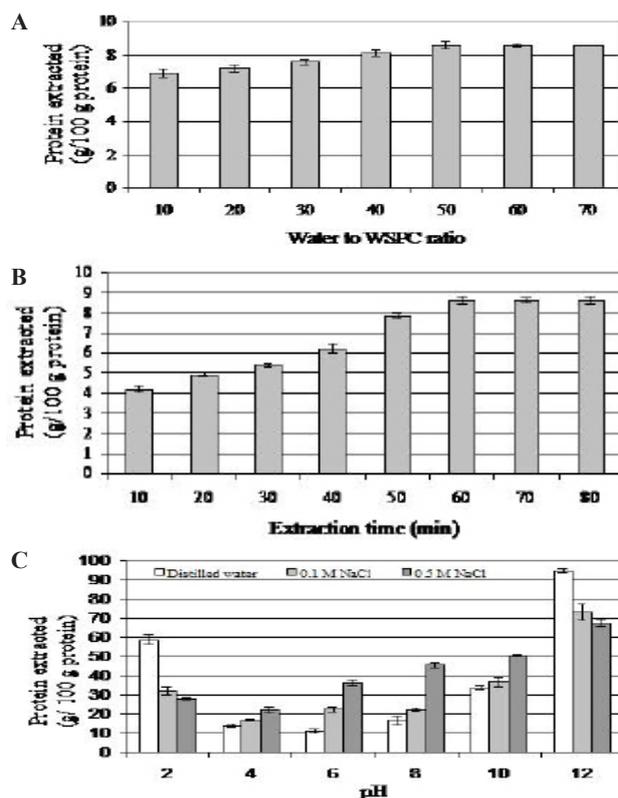


Figure 1. Effect of water to WSPC ratio, time, pH and sodium chloride on protein extractability of wood apple seed protein concentrate: (A) effect of water to WSPC on protein extractability; (B) influence of extraction time; (C) effect of pH and sodium chloride concentration

Maximum amount of extractable protein was noted at pH 5.5 (91 g/100 g) as shown in Fig 2 (A). The remaining 9 g/100 g of the protein was present in solution. On either side of pH 5.5 the precipitability of soluble protein decreased, indicating that pH 5.5 was the optimum for precipitating the extractable protein. Simth *et al.* (1946) also observed maximum precipitable of 79 g/100 g of soluble protein in linseed meal at pH 5.1.

Buffer capacity of WSPC was reported in Fig 2 (B). At acidic pH (6 - 2), an average of 0.426 mmol of hydrochloric acid was required per gram of WSPC to change the pH by one unit. Similarly, to bring a change of one pH unit in the alkaline pH range (6 - 10) 0.073 mmol of sodium hydroxide was required per gram of WSPC. Higher buffer capacity of the seed protein concentrate was observed in acidic medium

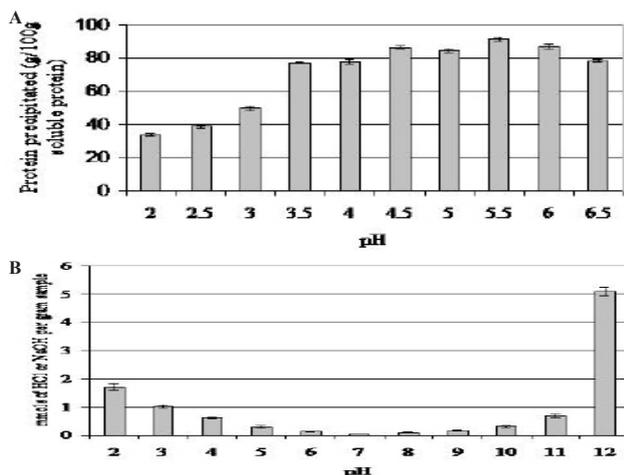


Figure 2. (A) Influence of pH on protein precipitability of wood apple seed protein concentrate; (B) buffer capacities of wood apple seed protein concentrate

SDS-PAGE

Fig 3 shows the SDS-PAGE profile of water and SDS soluble fractions of WSM and WSPC. In total nine protein bands were observed, whereas six of them were found to match with the molecular marker at 205, 116, 97, 45, 29 and 12 kDa in both WSM and WSPC. Apart from the above 150, 10 kDa bands were also observed. The SDS-PAGE profile indicated that solvent extraction had very little effect on the peptide linkage as seen from the protein band densities. A similar trend in SDS-PAGE separation of hemp seed was observed where defatting process did not affect the protein bands (Tang *et al.*, 2006). Proteins of molecular weights lower than 12 kDa were also observed in both WSM and WSPC. The protein bands were similar in both water and SDS soluble fractions.

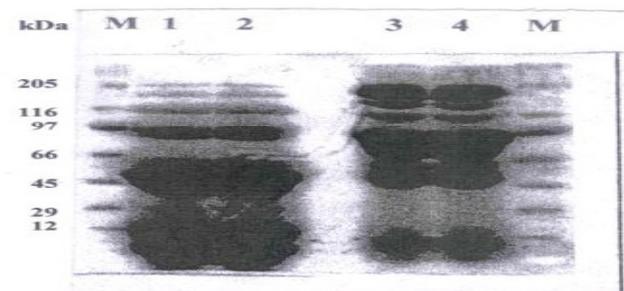


Figure 3. SDS-PAGE patterns of wood apple seed: (1) Water soluble WSM (2) SDS soluble WSM (3) Water soluble WSPC (4) SDS soluble WSPC (M) marker: Broad band standard molecular weight protein markers (myosin 205 kDa, galactosidase 116 kDa, phosphorylase B 97 kDa, bovine serum albumin 66 kDa, ovalbumin 45 kDa, carbonic anhydrase 29 kDa and cytochrome C 12 kDa).

Experimental moisture sorption isotherms

The WSPC had an initial moisture content (IMC) of 7.2%, which equilibrated at 51% RH at room temperature. The critical moisture content (CMC) was found to be 9.9%, which equilibrated at 69% RH. The moisture content increased sharply at higher RHs beyond 70% indicating that the WSPC is non-hygroscopic in nature and can be stable at room

temperature in polyethylene (PE) pouches.

Conclusion

Wood apple seed protein concentrate was rich in protein (77 g/100 g), and possessed essential amino acids. It exhibited considerable protein extractability (95 g/100 g) at alkaline pH 12 and a low solubility (11 g/100 g) at pH 6. 91 g/100 g of extractable protein can be precipitated at pH 5.5. The WSPC had considerable amounts of essential minerals calcium, iron and phosphorous. The SDS-PAGE profile indicated that the solvent extraction had very little effect on the peptide linkage as seen from the protein bands. The functional characteristics of the seed protein concentrate were comparable to those of legume seed meals. Hence, the application of the protein rich material of WSPC may be explored further in various processed foods.

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