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Some chemical investigations of *Amoora rohituka* seed proteins

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ABSTRACT

The inadequate supply of protein food, especially in the context of increasing population, has been an important factor in malnutrition in third world countries. Proteins were extracted from de-oiled seeds of *Amoora rohituka*, a potential source of non-conventional seed, in aqueous solutions of various pHs or by different concentrations of NaCl, KCl, $CaCl_2$ and $MgSO_4$ at pH 7.0. The nitrogen content of seeds and de-oiled seeds showed good protein content. Fractionation of protein was done to separate albumin, globulin, prolamine and glutelin. Gel-permeation chromatography on Sephadex G-200 revealed the presence of six components. PAGE identified different polypeptide bands in the range of 15.5–67.6 kDa in the total protein isolate (TPI) as well as in protein fractions. Amino acid analysis of TPI and fractions isolated identified 16 amino acids, most of which are essential. Studies on surface topographies of proteins and seed flour by scanning electron microscope are also incorporated.

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1. Introduction

Absence or inadequacy of protein diet causes malnutrition, which is perhaps the most acute problem of the developing countries where a population explosion is prevailing. The generation as a whole is under the grief and rigid clutches of this problem. A time has come to assess and explore the best possible measure to combat such an important issue. This can only be done with a scientific and methodical approach to the whole issue and to choose for an alternative substitution, over the traditional matters for proteins. In developing countries, the main dietary food is from the plant kingdom, which infers that the plant proteins are exploited for the use of livelihood of human beings. It is worth a close look at this issue from this particular angle. Endeavour should, therefore be made to exploit the plant seeds in the best possible way, in order to overcome the hurdle of malnutrition.

One such plant, *Aphanamixis polystachya* syn. *Amoora rohituka* (Pittaraj), which belongs to the family Meliaceae has been chosen for the present study. This plant is widely distributed in many parts of India, especially in Uttarpradesh, West Bengal, Assam, Sikim, Chota Nagpur region, and Western Ghats. This plant has several folk medicinal uses in Indian villages. Its bark is astringent and used also in spleen and liver diseases, tumours and abdominal complaints. Seed oil is used as liniment in rheumatism (Chopra, Nayar, & Chopra, 1956). A number of compounds have been isolated from the plant, some of which are also active. The leaves contain a diterpene named aphanamixol (Editorial Committee, CSIR, 1985), and the stem bark of the plant contains a triterpenoid, amooranin, with cytotoxic activity (Rabi, Karunagaran, Nair, & Bhattathiri, 2002). Aphanamixin lactone, aphanamixolide, rohitukin (Editorial Committee, CSIR, 1985), some guaiane derive sesquiterpenoids (Chowdhury, Hasan, & Rashid, 2003) were also isolated from this plant. A flavone glycoside isolated from the root of the plant (Jain & Srivastava, 1985), and a keto fatty acid was also isolated from the seed oil of this plant (Daulatabad & Jamkhandi, 1997). Some more compounds like triterpenoids, limonoids, alkaloids and saponin were also isolated from this plant (Bhatt, Saxena, & Nigam, 1981; Chatterjee, Kundu, Chakraborty, & Chandrasekharan, 1970; Harmon, Weiss, & Silverton, 1979; Kundu, Roy, & Chatterjee, 1985; Mulholand & Naidoo, 1999). Amooranin, a triterpenoid isolated from stem bark of *A. rohituka* showed an anticancer effect against a colon carcinoma cell line *in vitro* (Ramchandran et al., 2006). Seed extracts of the plant were also evaluated as a source of repellents, antifeedant, toxicants and protectants in storage against *Tribolium castaneum* (Herbst) (Talukder & Howse, 1995). But the seeds of *A. rohituka* are still unutilised and previously no attempt has been made to isolate the protein from the seed of this plant, in spite of the fact that a good amount of nitrogen and storage protein is present therein. So the seed is chosen for the present study to investigate chemically the extent up to which this seed protein can be utilised.

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2. Materials and methods

2.1. Materials

Mature fruits of *A. rohituka* were collected from Burdwan Forest Department, Burdwan, West Bengal, India, and authenticated by Professor A. Mukherjee, Department of Botany, Burdwan University, West Bengal, India. A voucher specimen *Burdwan, Moumita 245* has been deposited at the herbarium of the Botany Department, Burdwan University, Burdwan, bearing the acronym BURD. Fruits were de-coated, and collected seeds were then air dried. The air dried seeds were then used for analysis and protein extraction.

All chemical reagents used in this study were of analytical grade. Reagents for SDS–polyacrylamide gel electrophoresis, Sephadex G-200 (for gel filtration) and proteins used for standard calibration of gel filtration (BSA, Ovalbumin, Pepsin and Lysozyme) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Protein standard kit for PAGE was purchased from GENEI, Bangalore, India.

2.2. Sample preparation

The air dried seeds were crushed to powder by a hand crusher and the seed flour (550 g) was then defatted by extracting with petroleum ether (3 l) at room temperature (18 °C) for 21 days and the solvent was recovered by filtration. The seed flour was then washed thrice with chloroform:methanol (3:1), the seed flour was then air dried and stored in airtight sample bottles in the refrigerator at 4 °C until further use.

2.3. Protein content

Nitrogen content of whole seeds and de-oiled seeds were determined by the micro-kjeldahl technique (AOAC, 1990), and protein content of each sample was estimated (N × 6.25), following the method of AOAC (1990).

2.4. Protein solubility

The protein solubility profile of the de-oiled seed flour was determined under two different conditions (Basak, Bhattacharya, Sinhababu, & Laskar, 1994; Guerra & Park, 1975; Laskar, Ghosh Majumder, & Basak, 1985; Laskar, Sinhababu, Thakur, & Basak, 1998; Laskar, Thakur, & Basak, 1988). In case I, extraction was carried out with an electrical stirrer at room temperature for 30 min using deionised distilled water to de-oiled seed flour (20:1, w/v) at pHs 2–12. The pH of extraction was adjusted throughout the experiment to 2–6 by adding 0.5 M hydrochloric acid or to pH 7–12 by adding 0.5 M sodium hydroxide. In case II, the solubility profile of the seed protein was determined at a fixed pH (7.0) using various concentrations (0.1–1.0 M) of NaCl, KCl, $CaCl_2$ and $MgSO_4$ (Basak et al., 1994; Guerra & Park, 1975; Laskar et al., 1985; Laskar, Sinhababu, et al., 1988; Laskar, Thakur, et al., 1998). Respective pHs in both the cases were maintained throughout the experiment. Both bi-bivalent, bi-univalent and uni-univalent salts were used to study the effects of salts on the solubility profile. The nitrogen content of each extract was determined by the micro-kjeldahl method (AOAC, 1990).

2.5. Preparation of total protein isolate (TPI)

The de-oiled seed flour was extracted by stirring with distilled water using a flour to solvent ratio of 1:20 for 40 min and the pH of the solution was adjusted to 7.0 by 0.5 M HCl or 0.5 M NaOH. It is known that a maximum extraction of proteins from the seed is possible at pHs ranging from 6.0 to 9.0, and most of the proteins have their highest stability at a pH near neutrality, thus the extraction procedure was carried out at pH 7.0. The extraction was carried out successively three times. The suspension obtained was first filtered through cotton and finally centrifuged at 10,000*g* for 20 min. All recovered supernatant were combined and saved. The pH of the supernatant was lowered to the pH of minimum solubility (3.0) of the seed protein by 10% trichloroacetic acid. This pH is probably near the isoelectric point of the protein. The precipitate thus formed was recovered by centrifuge at 10,000*g* for 20 min. The precipitate was redissolved with deionised distilled water at pH 7.0, dialysed against distilled water for 72 h at 4 °C, freeze-dried and finally stored in a refrigerator at 4 °C for further investigation.

2.6. Proximate chemical analysis

Nitrogen, moisture and ash contents of de-oiled seeds and the total protein isolate (TPI) were analysed using AOAC methods (AOAC, 1990). The percentage of nitrogen was converted to crude protein by multiplying with 6.25.

2.7. Fractionation of seed protein

Protein fractions were isolated by successive extraction of defatted seed flour with different solvent according to a modified Osborne method (Osborne, 1924) as described by Chavan, Mckenzie, and Shahidi (2001). Defatted *A. rohituka* seed flour (20 g) was stirred in 200 ml distilled water with a magnetic stirrer for 30 min at room temperature (18 °C). It was then centrifuged at 5000 rpm for 15 min and the resultant supernatant was recovered by filtration through Whatmann filter paper no. 41. The residue thus obtained was again extracted two more times with the same solvent. The recovered filtrates were combined with the previous filtrate and designated as the 'water soluble fraction' (albumin). The residue was then successively extracted with 0.5 M NaCl solution of pH 7.0 (globulin), 70% (v/v) ethanol at 70 °C in a shaking water bath (prolamine), and finally with 0.1 M NaOH solution (glutelin) to separate the total seed proteins. The precipitates were then washed and re-dispersed in distilled water. Finally protein fractions were dialysed against distilled water for 72 h at 4 °C, and separately lyophilised. The protein content of each fraction was determined by the micro-kjeldahl procedure (AOAC, 1990). These freeze-dried protein fractions were used for amino acid analysis, SDS–PAGE, and SEM.

2.8. Amino acid analysis

Amino acid composition was determined using a Pico-Tag amino acid analyser according to the Pico-Tag operation manual (Waters, USA). Each dialysed and dried protein (20 μg) was hydrolysed by 6 N HCl containing 1% phenol for 24 h at 105 °C in the presence of nitrogen gas at a Pico-Tag work station. Hydrolysed samples and standard amino acid mixture, 'Standard H' (0.005 ml) were taken in respective tubes, introduced into the reaction vial and were dried completely. These were then separately derivatised in a solution mixture (ethanol:triethylamine:water:phenylisothiocyanate, 7:1:1:1, by volume) for 20 min at 25 °C in a nitrogen atmosphere. The vials were then dried and samples were reconstituted in diluent solution (Na_2HPO_4, 0.071% w/v in distilled water, pH 7.4 was adjusted by 10% H_3PO_4, containing 5% v/v acetonitrile) (Ghosh, Naskar, & Sengupta, 1997). The samples were analysed at 38 °C as per the Pico-Tag manual, using a Pico-Tag C_{18} hydrophobic column (5 μm 3.9 × 150 mm, Waters). The detector setting was at 254 nm, chart speed was 2 cm/min and run time was 32 min. Amino acids pres-

Table 1
Nitrogen solubility of *A. rohituka* seed protein in aqueous solution at various pHs and in various concentration of different salt solution at pH 7.0[a].

pH of aqueous solution	Soluble protein (%)	Molar concentration of different salts	Soluble protein (%) in presence of NaCl	Soluble protein (%) in presence of KCl	Soluble protein (%) in presence of $CaCl_2$, $2H_2O$	Soluble protein (%) in presence of $MgSO_4$, $7H_2O$
2	42.87 ± 0.03	0.1	62.85 ± 0.20	52.38 ± 0.05	72.80 ± 0.02	78.77 ± 0.10
3	41.21 ± 0.10	0.2	65.15 ± 0.04	53.52 ± 0.03	74.62 ± 0.06	79.08 ± 0.05
4	43.36 ± 0.06	0.3	67.34 ± 0.07	55.89 ± 0.07	78.16 ± 0.13	81.79 ± 0.12
5	46.84 ± 0.53	0.4	69.16 ± 0.07	57.37 ± 0.14	68.68 ± 0.18	83.93 ± 0.06
6	49.62 ± 0.22	0.5	63.48 ± 0.07	59.37 ± 0.06	65.29 ± 0.16	86.68 ± 0.08
7	55.88 ± 0.03	0.6	60.65 ± 0.06	59.25 ± 0.07	61.49 ± 0.21	81.28 ± 0.90
8	54.04 ± 0.13	0.7	57.70 ± 0.25	58.46 ± 0.05	58.34 ± 0.04	79.45 ± 0.04
9	50.95 ± 0.22	0.8	59.89 ± 0.06	56.18 ± 0.06	63.49 ± 0.07	76.33 ± 0.07
10	50.51 ± 0.38	0.9	60.92 ± 0.04	55.60 ± 0.09	62.22 ± 0.07	75.5 ± 0.09
11	59.78 ± 0.10	1.0	62.57 ± 0.13	55.30 ± 0.05	60.42 ± 0.10	75.27 ± 0.10
12	61.54 ± 0.38	–	–	–	–	–

[a] Values are mean ± SD, $n = 3$.

ent in the unknown samples were determined quantitatively by comparing the peak areas (745 B data module print out) of amino acids present in the 'Standard H' (Pierce, Rockford, IL, USA). A ratio of essential to total amino acid was reported as E/T (%).

2.9. Gel filtration

The method of Whitaker (1963), was used with slight modifications for gel filtration chromatography. The protein extract at pH 7.0 was dialysed against distilled water for 72 h at 4 °C. It was then freeze-dried. The proteins obtained were again dissolved in 0.01 M phosphate buffer (pH 7.0) containing 0.2 M NaCl, resulting in a protein sample of 4 mg/ml concentration. This was carried out at 20 °C in a 2.0 cm i.d. × 45 cm Sephadex G-200 column. Void volume of the column was measured (42 ml) using Blue dextran. A protein sample (2 ml) was then applied. The eluting buffer was 0.01 M phosphate buffer (pH 7.0) containing 0.2 M NaCl. Fractions of 2 ml were collected at a rate of 0.4 ml/min and monitored at 280 nm with a Shimadzu UV–visible spectrophotometer (Model no. UV1601 PC; Shimadzu Corporation, Kyoto, Japan), which was calibrated with reference protein standards (BSA, Ovalbumin, Pepsin, and Lysozyme). The molecular weight of each of the components (A–G) was also calculated by the following equation as described by Leach and Óshea (1965).

$$\log \text{molecular weight} = -0.959\,(V/V_0 - 1) + 5.7$$

where V and V_0 were the elution volume and the void volume, respectively.

2.10. Polyacrylamide gel electrophoresis (PAGE)

Gel electrophoresis studies were performed with the TPI and the freeze-dried protein fractions, albumin, globulin, prolamine and glutelin. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the method of Laemmli (1970), on Bio-Rad gels composed of stacking gel (5%, w/v) using 1.0 M Tris–glycin buffer containing 0.4% SDS at pH 6.8 and resolving gel (12%, w/v) using 1.5 M Tris–glycin buffer containing 0.4% SDS at pH 8.8. After electrophoresis, gels were stained with 0.2% (w/v) $AgNO_3$ solution after treatment with fixing solution (methanol–acetic acid–H_2O–*p*-formaldehyde) and sodium thiosulphate solution. It was then treated with developer (Na_2CO_3–sodium thiosulphate-37% *p*-formaldehyde) until the bands developed. The gels were soaked with stop solution and stored in 30% methanol (v/v) at 4 °C.

The molecular weight markers (GENEI, India) used were Phosphorylase b (97.4 kDa), bovine serum albumin (66.0 kDa), Ovalbumin (43.0 kDa) and Carbonic Anhydrase (29.0 kDa).

2.11. Scanning electron microscopy

Structural morphologies of protein fractions were studied using scanning electron microscopy. Lyophilised TPI and protein fractions, along with seed flour, were mounted on circular aluminium stubs with double sticky tape, and coated with 20 nm of gold using a IB_2 ion coater. The samples were then examined and photographed in a Hitachi scanning electron microscope (Hitachi S-530 Scanning Electron Microscope, Hitachi Ltd., Tokyo, Japan) at an accelerating potential of 20 kV.

3. Results and discussion

Nitrogen content (1.23%) and protein content (7.69%) of the seeds were found to be lower than those of de-oiled seeds (2.20% and 13.77%, respectively). The protein content is comparable with the 15.81% protein reported for seed protein from *Ailanthus excelsa* and hyacinth bean (Kundu & Laskar, 2008; Subagio, 2006). Moisture content of seeds and de-oiled seeds were 20.41% and 14.76%. The ash content of seed was 2.93% and that of the de-oiled seed was 2.07%.

Solubility profiles of protein in water at different pHs and in presence of different salts at different concentrations are given in Table 1. The highest nitrogen solubility was found to be 61.54% at pH 12.0 and the lowest to be 41.21% at pH 3.0 in case of only water. Electrostatic, hydrophobic, and nitrogen bonding are three major forces involved in the solubility of proteins. At the isoelectric state a protein molecule possesses zero net charge, as there is no electrostatic repulsion between the molecules. As a result at this point the solubility reaches to a minima. As TPI is a mixture of proteins and therefore does not indicate a particular p*I*-value, but can show a minimum solubility at certain pH (here it is pH 3.0).

It is also known that proteins keep their native properties and can be extracted more in inorganic salt solutions than in salt free conditions. For these reason some common bi-bivalent, bi-univalent and uni-univalent salts are used to study the solubility profile. In the presence of salts, nitrogen solubility with KCl and $MgSO_4$ showed a similar pattern, whereas the solubility pattern for NaCl and $CaCl_2$ are more or less similar. For NaCl and $CaCl_2$ the maximum nitrogen solubility is 69.16%, and 78.16% at 0.4 M and 0.3 M, respectively. For KCl and $MgSO_4$ the value is 59.37% and 86.68% at 0.5 M. The above results revealed that the protein solubility is more in case of bivalent salts, due to the higher tendency of the bivalent cations to bind with –COOH groups than the monovalent species (Gilberg & Toernell, 1976). This pattern of solubility was also observed for the seeds of *Mimusops elengi* and *Ailnathus excelsa* (Hazra & Laskar, 2005; Kundu & Laskar, 2008). Another important trend can be seen from the above study that nitrogen solubility decreased with an increase in salt concentrations. This

Table 2
Amino acids composition of *A. rohituka* seed proteins[d].

Name of amino acids	TPI (g/16 g N)	Albumin (g/16 g N)	Globulin (g/16 g N)	Prolamine (g/16 g N)	Glutelin (g/16 g N)	Soybean protein[c] (g/16 g N)
Aspartic acid + asparagine	7.94 ± 0.03	45.24 ± 0.04	53.70 ± 0.14	6.48 ± 0.11	40.64 ± 0.18	11.60
Glutamic acid + glutamine	36.40 ± 0.23	1.23 ± 0.12	4.53 ± 0.05	15.93 ± 0.23	0.94 ± 0.01	19.10
Serine	4.33 ± 0.19	7.03 ± 0.28	1.80 ± 0.19	20.36 ± 0.01	5.71 ± 0.08	5.60
Glycine	4.61 ± 0.11	4.74 ± 0.06	4.62 ± 0.01	0.09 ± 0.08	6.24 ± 0.06	4.20
Histidine[a]	0.54 ± 0.02	–	1.06 ± 0.11	6.31 ± 0.14	–	2.80
Arginine[a]	14.09 ± 0.30	1.42 ± 0.22	2.08 ± 0.07	42.43 ± 0.21	4.46 ± 0.12	7.70
Threonine[a]	4.32 ± 0.17	6.06 ± 0.13	0.81 ± 0.03	–	5.71 ± 0.03	4.10
Alanine	2.48 ± 0.15	3.05 ± 0.10	4.72 ± 0.17	–	4.98 ± 0.09	4.30
Proline	3.18 ± 0.08	–	–	0.24 ± 0.13	–	–
Tyrosine	2.09 ± 0.24	2.93 ± 0.25	2.17 ± 0.18	–	3.77 ± 0.15	3.80
Valine[a]	3.79 ± 0.10	5.37 ± 0.02	4.81 ± 0.02	5.56 ± 0.17	6.37 ± 0.22	5.00
Methionine[a]	1.84 ± 0.07	2.32 ± 0.09	2.55 ± 0.09	–	1.43 ± 0.04	1.40
Cysteine	0.64 ± 0.01	0.72 ± 0.01	0.62 ± 0.02	0.23 ± 0.05	0.39 ± 0.07	1.80
Isoleucine[a]	3.20 ± 0.17	3.36 ± 0.33	2.69 ± 0.12	–	0.21 ± 0.05	4.00
Leucine[a]	5.69 ± 0.09	7.58 ± 0.18	6.73 ± 0.26	–	10.90 ± 0.18	7.80
Phenylalanine[a]	3.13 ± 0.12	4.10 ± 0.04	3.11 ± 0.12	0.24 ± 0.01	4.75 ± 0.10	5.20
Lysine[a]	1.73 ± 0.06	4.85 ± 0.12	4.00 ± 0.06	2.13 ± 0.12	3.50 ± 0.02	6.40
E/*T* (%)[b]	38.33	35.06	27.84	56.67	37.33	46.83

[a] Essential amino acids.
[b] Ratio of total essential amino acids to total amino acids.
[c] Brul'e and Savoie (1998).
[d] Values are mean ± SD, $n = 3$.

Table 3
Determination of molecular weights of *A. rohituka* seed protein by gel filtration procedure.

Proteins	Elution volume/void volume (V/V_0)	Molecular weight determined from the standard curve (Da)[a]	Molecular weight determined by equation (Da)	Literature molecular weight (Da)[b]
BSA[c]	1.88	–	–	66,000
Ovalbumin	2.08	–	–	45,000
Pepsin	2.20	–	–	34,700
Lysozyme	2.62	–	–	14,300
Amoora rohituka seed proteins				
Component A	1.50	144,500	166,000	–
Component B	1.90	63,100	68,700	–
Component C	2.0	51,300	55,000	–
Component D	2.30	27,500	28,400	–
Component E	2.45	20,400	20,400	–
Component F	2.55	16,600	16,350	–

[a] Whitaker (1963).
[b] Literature of molecular weights of standard proteins from Sigma Chemical Co., St. Louis, MO.
[c] Bovine serum albumin.

may be due to the low ionic strength of these salts, which allows dissociation and consequent interaction with the proteins, therefore increasing solubility ('Salting in' effect). But at higher concentrations, these salts produce a dehydrating effect on the protein, which tends to aggregate, resulting in the decrease of solubility ('Salting out' effect) (Kundu & Laskar, 2008; Padilla, Alvarez, & Alfero, 1996).

Protein fractions were isolated from *A. rohituka* seed flour as water soluble (albumin), alkali soluble (glutelin), salt soluble (globulin) and alcohol soluble (prolamine) fractions. Albumin was the most dominant fraction (53.19%), followed by glutelin (20.82%), globulin (15.35%) and prolamine (10.63%). From the data above we can conclude that albumin is the major storage protein of seeds of *A. rohituka*.

The protein content of TPI was also quite high at 71.33%, and the percentage of protein that can be extracted as TPI is 85.85%. Amino acid compositions of the total protein isolate (TPI) and four protein fractions prepared from *A. rohituka* are given in Table 2 with the data of soybean protein as a reference (Brul'e & Savoie, 1998). The globulin fraction contained the highest amount of sulphur containing amino acid, followed by albumin, glutelin and prolamin in *A. rohituka* seed protein. A characteristic amino acid profile showed a high aspartic acid and asparagine content in albumin, glutelin and globulin fractions, but in the case of the prolamine fraction glutamic acid and glutamine content is highest. Among the four fractions, the essential amino acid, histidine is absent in albumin, glutelin fraction but threonine is absent in the prolamine fraction only. The total protein isolate contained a higher content (38.33%) of essential amino acids. The amino acid composition of *A. rohituka* showed that the prolamine fraction has the highest content of essential amino acids than the other three fractions. The amino acid composition and *E*/*T* (%) of the total protein isolate (TPI) is almost comparable with the reference soybean protein. Based on the *E*/*T* (%) ratio, the present study showed that the prolamine and globulin fractions may be considered the most and least nutritive, respectively.

Gel filtration chromatography of *A. rohituka* seed protein revealed six fractions (A–F). The two different methods used for the molecular weight determination of six components obtained were very close to each other (Table 3). So we may conclude that *A. rohituka* seed protein is a mixture of at least six polypeptides (Fig. 1).

SDS–PAGE patterns of TPI and protein fractions are shown in Fig. 2. Most of the polypeptides were seen to bind to SDS in a constant ratio, such that they had essentially the same charge densities and migrated in the gel according to their molecular weights.

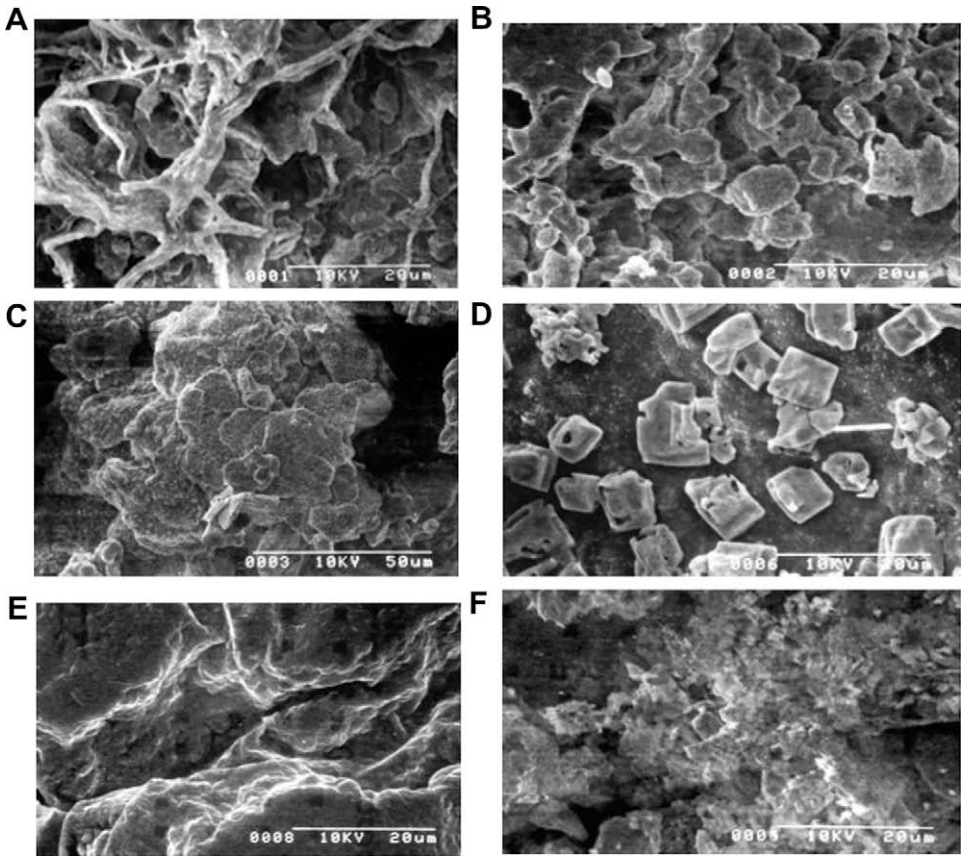

Fig. 1. Gel filtration chromatography of *A. rohituka* seed protein in Sephadex G-200.

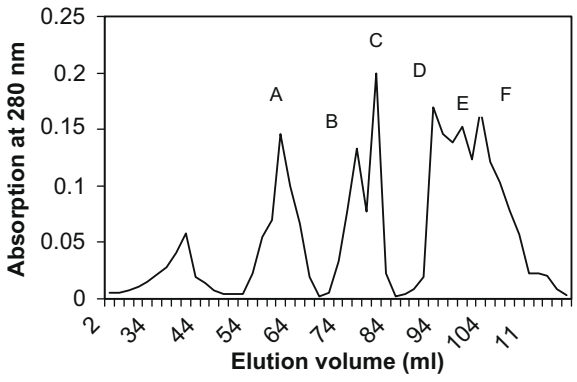

Fig. 2. Scanning electron micrograph of *A. rohituka* (A) seed flour, (B) total protein isolate (TPI) and protein fractions (C, albumin; D, globulin; E, prolamine; and F, glutelin).

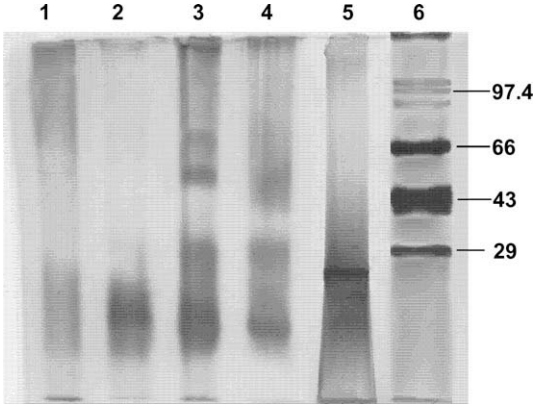

Fig. 3. SDS–PAGE of *A. rohituka* seed proteins (1, glutelin fraction; 2, globulin fraction; 3, total protein isolate; 4, albumin fraction; 5, prolamine fraction; and 6, molecular weight marker). Twenty-five micrograms of protein were loaded in each lane.

TPI (lane 3) showed five bands ranging from 67.6 to 15.5 kDa. The molecular weight distribution pattern of total protein isolate in this method was very close to the molecular weight determined from gel filtration study (Table 3). Glutelin (lane 1) and prolamine (lane 5) showed only one band at 50.1 and 24.5 kDa, respectively. Globulin (lane 2) showed two major bands at 29.5 and 17.8 kDa. Finally albumin (lane 4) showed three distinct bands at 50.1, 29.5 and 15.5 kDa. The results show that the protein is simple in nature and characterised by the presence of all the types of storage protein fractions generally associated with food legumes.

Structural morphology of the protein fractions was studied with the aid of scanning electron microscope (SEM). SEM pictures of seed flours, total protein isolate and different fractions of protein (Fig. 3) showed distinct surface structures, which was also seen for *Erythrina variegata* Linn. seed proteins (Datta Samanta & Laskar, 2009). The TPI showed flaky plate like structures; where as the albumin fraction appeared as a small flaky structure. A distinct different crystal like structure was observed in the case of globulin. A cloudy fibre like array was seen in the case of prolamine, and glutelin appeared as a flaky structure. As seed flour consists of these entire protein fractions, their morphological structure showed the mixture of fibre and flaky plate shape with rough surfaces and large pores. The different characteristics of various protein fractions along with TPI may contribute to the overall physiochemical and functional properties of *A. rohituka* protein.

4. Conclusion

From the above investigation it is found that TPI isolated from the seeds of *A. rohituka* and its different fractions contains most of the essential amino acids in considerable amount. The seed protein has good solubility in aqueous solution. The protein can be easily extracted from seeds, and also contains a high percentage of protein. The seed protein contains the highest amount of the albumin fraction and molecular weight of the different fractions are not very high. Considering all these facts, and also its high availability in India, it may be concluded that the *A. rohituka* seed protein may be used as an edible protein after its proper toxicological screening, and overall may serve as an important source of unexploited protein from legumes. As the *A. rohituka* seed contains a good amount of protein and are widely found in India, there may be scope to utilise it in food.

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