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Fractionation and Some Chemical Studies on *Ailanthus excelsa* Roxb. Seed Protein

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Abstract The unavailability of protein foods, particularly in the context of population growth, has been an important factor in the protein malnutrition encountered in developing countries. The fractionation, gel filtration and polyacrylamide gel electrophoresis (PAGE) of *Ailanthus excelsa* seed (a nontraditional source containing 15.81% protein) proteins were carried out in the present study, and their solubility profiles, surface topographies and amino acid compositions were evaluated. The globulin fraction dominated the seed protein composition, accounting for 51.31% (w/w) of the total soluble proteins in the seeds. Protein isolate and protein fractions of *A. excelsa* seeds showed similar topographical structures to those of other plant seed proteins. Analysis of the isolated proteins identified 17 amino acids, of which nine were essential. Gel filtration on Sephadex G-200 revealed the presence of seven components. PAGE detected different polypeptide bands in the range of 28.8−154.9 kDa in the protein isolate as well as in protein fractions for *A. excelsa*. The amino acid compositions, the solubility patterns and the high abundances of low molecular weight proteins indicate that the isolated seed protein of *A. excelsa* may be a potential food protein.

Keywords Seed protein · *Ailanthus excelsa* · Amino acids · Nitrogen solubility · Gel filtration · SDS–PAGE

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Introduction

The search for alternative or new protein sources has become an important research trend over the past few decades, especially in developing countries, where the average protein intake is less than that required. Therefore, industrial interest in the production of plant protein isolates is growing because of the increasing use of plant proteins in food and non-food markets. These applications in food are almost limited to proteins from soybean seeds; other more unconventional plant proteins are only infrequently used as protein sources. The objective of the present study was to characterize an unconventional seed material as an alternative source of protein with the potential to combat the increasing levels of malnutrition that are occurring in India due to inadequate protein intake.

In the near future, many parts of the world will have to depend on nontraditional food materials. Seeds from plants will be one of the major alternative food protein sources. One such plant, *Ailanthus excelsa* Roxb. (Mahanimba), which belongs to the family Simaroubacae, can be found throughout the tropical and subtropical parts of India, especially the dry tracts [1]. It is also widely cultivated along roadsides, in gardens, and in some Indian forests [2].

Many parts of this plant, i.e., its leaves, bark, etc., have folk medicinal uses in Indian villages [1]. Phytochemical studies on *A. excelsa* have identified the presence of quassinoids, alkaloids and terpenoids [3–5]. Some bioactive constituents have also been found in the bark, leaves, roots, etc., of this plant, which have significant antibacterial, antifungal and antifertility activities [6–8]. It has been reported that leaves of this plant are a good source of digestible leaf proteins and calcium [9, 10]. However, seeds of *A. excelsa* are still not used and are considered a

nontraditional source. Next to no attempts have been made to isolate protein from the seeds, in spite of the presence of appreciable amounts of nitrogen and storage protein in them. Therefore, these seeds were chosen for the present study. The object of the study was to chemically characterize the isolated seed protein from *A. excelsa*.

Experimental Procedures

Materials

Fresh, mature fruits of *A. excelsa* were collected from Burdwan Forest Department, Burdwan, West Bengal, India, and identified by Prof. A. Mukherjee, Department of Botany, University of Burdwan, West Bengal, India. A voucher specimen (Burdwan, Kundu SS2) has been deposited at the herbarium of the Botany Department, University of Burdwan, Burdwan, bearing the acronym BURD. Fruits were initially decoated and the seeds were dried in air. The air-dried seeds were ground to a fine powder and kept in a refrigerator at 4 °C until further use. 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 the standard calibration of gel filtration (BSA, ovalbumin, pepsin and lysozyme) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The protein standard kit for PAGE was purchased from GENEI (Bangalore, India).

Extraction and Nitrogen Solubility of Protein

Finely powdered *A. excelsa* seeds were de-oiled with petroleum ether (40–60 °C) in a Soxhlet apparatus for 60 h, twice washed well with chloroform:methanol mixture (3:1), and air-dried. The nitrogen solubilities of proteins extracted from de-oiled seeds were evaluated under two different conditions [11–15]. In case I, extraction was carried out at various pHs between 2 and 12. The solubility at each pH value was determined by stirring de-oiled seeds with distilled water (1:20, w/v) at room temperature (25 °C) for 30 min. The pH of each extractant was maintained throughout the experiment by adding 0.5 M HCl or 0.5 M NaOH. In case II, the nitrogen solubility profile of the protein was determined by a similar process in the presence of sodium chloride (NaCl), sodium sulfite (Na_2SO_3), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) and magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$) at different molar concentrations (0.1–1.0 M) under a fixed pH of 7.0. The nitrogen content of each extract was monitored by the micro-Kjeldahl method [16].

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 45 min, and the pH was adjusted to 7.0 by 0.5 M HCl or 0.5 M NaOH. Maximum extraction of proteins from the seed is possible at pH values of between 6.0 and 9.0, and it is well known that the greatest stability for most proteins would be expected near neutrality; thus a pH of 7.0 was used for extraction. The suspension thus obtained was first filtered through cotton and then centrifuged at $10{,}000{\times}g$ for 15 min. The supernatant was recovered and saved. The residues were re-extracted twice more with the same solvent, and recovered supernatants were combined. The pH of the supernatants was adjusted with 0.5 M HCl to the pH of the minimum solubility (pH 4.0) of the seed protein (probably near the isoelectric point of the protein). The precipitate thus formed was recovered by centrifugation at $10{,}000{\times}g$ for 15 min. The precipitate was dissolved with distilled water adjusted to pH 7.0 and dialyzed against distilled water for 48 h at 4 °C. It was then freeze-dried and stored in a refrigerator for further investigation.

Proximate Chemical Analysis

The nitrogen, ash and moisture contents of the de-oiled seeds and TPI were analyzed using AOAC methods [16]. The percentage of nitrogen was converted to crude protein by multiplying with 6.25.

Protein Fractionation

Protein fractions were isolated by sequentially extracting defatted seed flour with different solvents (the defatted seed flour to solvent ratio was 1:10, w/v, in each case) according to the modified Osborne scheme [17], as described by Chavan et al. [18]. Defatted and dried samples (2 g) were dispersed in 20 ml distilled water by stirring with a magnetic stirrer and extracted over 45 min periods at room temperature (25 °C). The suspension was then centrifuged at $5{,}000{\times}g$ for 15 min, and the resultant supernatant was filtered (Whatman filter paper no. 41). The residues were re-extracted twice more with the same solvent and the recovered filtrates were combined and designated the “water-soluble fraction.” The residue was then extracted successively with 0.5 M NaCl solution (pH 7.0), 70% (v/v) ethanol at 65 °C in a shaking water bath, and 0.1 M sodium hydroxide in order to separate the total seed proteins into albumin, globulin, prolamin and glutelin fractions, respectively. Filtrates containing the desired protein fractions were dialyzed against distilled water for 48 h at 4 °C and separately lyophilized. The protein content of each fraction was determined by the micro-Kjeldahl

procedure [16]. All lyophilized protein fractions were then stored in the refrigerator in airtight plastic bottles until further use.

Amino Acid Composition

Amino acid composition was determined using a Pico-Tag amino acid analyzer according to the Pico-Tag operation manual (Waters, Milford, MA, USA). Each dialyzed and dried protein (20 μg) was hydrolyzed 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 [19]. Hydrolyzed samples and the standard amino acid mixture, "Standard H" (0.005 ml), were placed in tubes, introduced into the reaction vial, and dried completely. These were then derivatized 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 the samples were reconstituted in diluent solution (Na_2HPO_4, 0.071% w/v in distilled water, pH 7.4; pH was adjusted by 10% H_3PO_4 containing 5% v/v acetonitrile). The samples were analyzed at 38 °C as per the Pico-Tag manual, using a Pico-Tag C_{18} hydrophobic column (5 μm 3.9 × 150 mm, Waters) and detection at 254 nm. Amino acids present in the unknown samples were determined quantitatively by comparing the peak areas of amino acids present in the "Standard H" (Pierce, Rockford, IL, USA). Tryptophan was not detected. The ratio of essential to total amino acids was reported as E/T (%).

Scanning Electron Microscopy (SEM)

The structural morphologies of defatted seed flour, TPI and the protein fractions were studied using SEM. Dried samples were mounted on circular aluminum stubs with double-sided sticky tape and coated with 20 nm gold using an IB_2 ion coater. The samples were then examined and photographed in a Hitachi S-530 scanning electron microscope (Hitachi Ltd, Tokyo, Japan) at an accelerating potential of 20 kv.

Gel Filtration

The method of Whitaker [20] was used with a slight modification for gel filtration chromatography. The protein extract in 0.1 M NaCl at pH 7.0 was dialyzed against 0.01 M phosphate buffer (pH 7.0) for 48 h at 4 °C. It was then freeze-dried. The proteins thus obtained were again dissolved in 0.01 M phosphate buffer (pH 7.0) containing 0.2 M NaCl, resulting in a protein sample of concentration 4 mg/ml. The actual method was carried out at 20 °C in a 2.5 cm i.d × 42 cm Sephadex G-200 column. The void volume of the column was determined (52 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 (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) [20]. The molecular weights of each of the components (A–G) were also calculated by the following equation, as described by Leach and O'Shea [21]:

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

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

Polyacrylamide Gel Electrophoresis (PAGE)

Total protein isolate, protein fractions that had been separated as described in the above section, and protein extracted with 0.01 M phosphate buffer (pH 7.0) containing 0.2 M NaCl according to the modified method of Marcone [22] were all used for PAGE studies.

SDS–PAGE was performed according to the method of Laemmli [23] on Bio-Rad gels consisting 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.

Nondenaturing polyacrylamide gel electrophoresis (NPAGE) was performed according to the method of Davis [24]. It was performed on 10% (w/v) polyacrylamide gel but without using SDS and reducing agent (2-mercapto ethanol) in each step, as described above for the SDS–PAGE studies. Following electrophoresis, gels were stained with 0.2% (w/v) $AgNO_3$ solution after being treated with fixing solution (methanol–acetic acid–H_2O–*p*-formaldehyde) and sodium thiosulfate solution. They were then treated with developer (Na_2CO_3–sodium thiosulfate–37% *p*-formaldehyde) until the bands came out. The gels were soaked with stop solution and stored in 30% methanol (v/v) at 4 °C.

Results and Discussion

Nitrogen Solubility

The solubility profile (Table 1) showed that the protein was more soluble in alkaline pH. Protein solubilities in the presence of different inorganic salts (0.1–1.0 M) are also shown in Table 1. The nitrogen solubility of the protein was found to be maximum at concentrations of 0.3, 0.1 and 0.2 M of the salts NaCl, Na_2SO_3 and $CaCl_2 \cdot 2H_2O$, respectively, and then decreased with

Table 1 Nitrogen solubility of *Ailanthus excelsa* seed protein in aqueous solutions at various pHs and in solutions with various concentrations of different salts at pH 7.0

pH of solution	Nitrogen solubility (%)	Molar concentration of each salt	Nitrogen solubility (%) in the presence of NaCl	Nitrogen solubility (%) in the presence of Na_2SO_3	Nitrogen solubility (%) in the presence of $CaCl_2{\cdot}2H_2O$	Nitrogen solubility (%) in the presence of $MgCl_2{\cdot}6H_2O$
2	36.23 ± 0.36	0.1	52.83 ± 0.13	14.94 ± 0.04	53.10 ± 0.03	66.72 ± 0.04
3	34.82 ± 0.32	0.2	57.13 ± 0.19	14.82 ± 0.05	56.46 ± 0.04	68.49 ± 0.07
4	33.46 ± 0.11	0.3	59.01 ± 0.04	14.72 ± 0.04	51.1 ± 0.06	67.26 ± 0.04
5	34.78 ± 0.23	0.4	56.70 ± 0.03	14.51 ± 0.03	45.30 ± 0.04	65.73 ± 0.01
6	38.65 ± 0.39	0.5	54.16 ± 0.06	14.49 ± 0.02	34.21 ± 0.04	66.31 ± 0.04
7	44.16 ± 0.05	0.6	53.17 ± 0.07	14.35 ± 0.01	17.65 ± 0.06	69.54 ± 0.06
8	37.34 ± 0.33	0.7	47.89 ± 0.06	14.34 ± 0.02	17.24 ± 0.02	72.41 ± 0.06
9	43.87 ± 0.39	0.8	41.08 ± 0.06	14.34 ± 0.04	17.16 ± 0.04	75.34 ± 0.05
10	55.70 ± 0.11	0.9	43.12 ± 0.06	13.31 ± 0.04	19.21 ± 0.05	75.5 ± 0.04
11	58.20 ± 0.08	1.0	48.65 ± 0.05	12.30 ± 0.03	24.27 ± 0.01	75.74 ± 0.04
12	59.20 ± 0.22	–	–	–	–	–

Values are mean ± SD, $n = 2$

increasing salt concentrations; a similar result was reported by Hazra and Laskar [25]. This may be due to the low ionic strengths of these salts, which allow dissociation and consequent interaction with the proteins, thereby increasing solubility ("salting in effect"). However, at higher concentrations, these salts produce a dehydrating effect on the protein, which then tends to aggregate, resulting in a decrease in solubility ("salting out effect") [26]. The solubility of the seed protein increased with increasing molar concentrations (0.1–1.0 M) of $MgCl_2{\cdot}6H_2O$. Nitrogen solubility in the presence of Na_2SO_3 was lower than it was with the other three inorganic salts. This was probably due to the fact that different salts have different dissociation abilities and undergo different interactions with the protein. It was also observed that the protein solubility was more pronounced for bivalent salts, because bivalent cations have higher tendency than monovalent ones to bind with the –COOH groups in the protein. This reduces the net negative charge and creates the most favorable conditions for protein solubility [27].

Proximate Chemical Analysis

The proximate chemical compositions of de-oiled seed and protein isolate are shown in Table 2. The nitrogen and protein contents of the de-oiled seeds were found to be 2.53 and 15.81% (micro-Kjeldahl N × 6.25), respectively. The protein content of the de-oiled seed was lower than the protein contents of high-protein seeds such as cowpea (23.8%) [28] and moth bean (23.3%) [29]. It is comparable to the 17.1% protein reported for hyacinth bean [30]. The protein content of total protein isolate was also quite high

Table 2 Proximate chemical analysis of the de-oiled seed and the total protein isolate (TPI) of *Ailanthus excelsa* Roxb. seed

Parameter	De-oiled seed[a]	TPI[a]
Moisture (%)	11.43 ± 0.63	7.6 ± 0.20
Ash (%)	1.79 ± 0.05	1.37 ± 0.08
Nitrogen (%)	2.53 ± 0.05	10.54 ± 0.08
Protein (%)	15.81 ± 0.28	65.88 ± 0.53
Extracted protein as TPI (%)	–	67.03 ± 0.39

[a] Values are mean ± SD, $n = 3$

(65.88%), and about 67.03% of the protein can be extracted as TPI.

Protein Fractionation

Osborne solubility-based protein fractionation (Table 3) data indicated that globulins (51.31% of the total soluble protein) were the most dominant, followed by albumins (30.30%) and glutelins (15.17%), with a small quantities of prolamin also observed (3.21%). The dominance of globulins in the total seed protein is similar to that observed for

Table 3 Protein fractionation yields

Osborn protein fraction	Percentage of total soluble protein[a]
Albumin	30.30 ± 1.20
Globulin	51.31 ± 0.39
Prolamin	3.21 ± 0.13
Glutelin	15.17 ± 0.32

[a] Values are mean ± SD, $n = 3$

Table 4 Amino acid composition of *A. excelsa* seed proteins

Name of amino acids	TPI (g/16 g N)	Albumin (g/16 g N)	Globulin (g/16 g N)	Prolamin (g/16 g N)	Glutelin (g/16 g N)	Soybean protein (g/16 g N)[c]
Aspartic acid + asparagine	7.16 ± 0.01	25.43 ± 0.17	15.24 ± 0.23	15.42 ± 0.23	16.88 ± 0.30	11.60
Glutamic acid + glutamine	13.29 ± 0.12	–	14.76 ± 0.18	29.39 ± 0.27	31.22 ± 0.21	19.10
Serine	5.44 ± 0.10	2.44 ± 0.02	2.86 ± 0.03	5.12 ± 0.01	5.13 ± 0.08	5.60
Glycine	5.63 ± 0.08	21.03 ± 0.35	6.67 ± 0.05	5.70 ± 0.09	6.08 ± 0.04	4.20
Histidine[a]	3.31 ± 0.05	–	–	–	2.07 ± 0.02	2.80
Arginine[a]	8.76 ± 0.13	10.02 ± 0.22	14.28 ± 0.25	3.57 ± 0.05	3.58 ± 0.10	7.70
Threonine[a]	2.32 ± 0.24	–	–	4.44 ± 0.02	3.56 ± 0.07	4.10
Alanine	6.58 ± 0.15	15.16 ± 0.18	9.05 ± 0.07	5.15 ± 0.06	5.00 ± 0.10	4.30
Proline	6.09 ± 0.05	2.20 ± 0.03	3.81 ± 0.02	4.77 ± 0.12	4.16 ± 0.05	–
Tyrosine	4.95 ± 0.01	10.51 ± 0.17	3.81 ± 0.02	2.65 ± 0.03	2.52 ± 0.03	3.80
Valine[a]	6.70 ± 0.07	2.69 ± 0.08	6.19 ± 0.05	6.24 ± 0.15	4.86 ± 0.12	5.00
Methionine[a]	2.32 ± 0.14	0.49 ± 0.04	1.90 ± 0.01	0.81 ± 0.03	0.66 ± 0.02	1.40
Cysteine	5.71 ± 0.15	0.73 ± 0.01	0.48 ± 0.01	0.87 ± 0.02	0.69 ± 0.03	1.80
Isoleucine[a]	3.69 ± 0.03	3.18 ± 0.13	2.86 ± 0.13	3.16 ± 0.04	3.04 ± 0.05	4.00
Leucine[a]	6.78 ± 0.16	2.45 ± 0.11	6.19 ± 0.15	6.43 ± 0.11	5.83 ± 0.07	7.80
Phenylalanine[a]	5.60 ± 0.12	1.22 ± 0.02	4.28 ± 0.06	4.17 ± 0.03	3.34 ± 0.06	5.20
Lysine[a]	5.67 ± 0.04	2.45 ± 0.07	7.62 ± 0.17	2.11 ± 0.04	1.38 ± 0.04	6.40
E/T (%)[b]	45.15	23.23	43.32	30.93	28.32	46.83

Values are mean ± SD, $n = 3$

[a] Essential amino acids

[b] Ratio of total essential amino acids to total amino acids

[c] From [32]

moth beans (63.93%) [31] and cowpea seeds (66.6%) [28]. Hence, it can be concluded that globulins are the major seed storage protein of *A. excelsa*.

Amino Acid Composition

The amino acid compositions of TPI and four protein fractions prepared from *A. excelsa* are given in Table 4, along with data for soybean protein given as a reference [32]. The globulin fraction contained the highest amount of sulfur-containing amino acids, followed by prolamin, glutelin, and albumin. The characteristic amino acid profile showed high aspartic acid and asparagine contents in all four fractions, but glutamic acid was only absent in the albumin fraction. Among the four fractions, the essential amino acid histidine was absent in the albumin, globulin and prolamin fractions, but threonine was only absent in the albumin and globulin fractions. The total protein isolate contained a high content (45.15%) of essential amino acids. The amino acid composition of *A. excelsa* showed that globulin was present in the greatest amounts among the essential amino acids. The amino acid composition and E/T (%) of TPI were comparable with those of the reference soybean protein. Based on the E/T (%) ratio, the present study showed that globulin and albumin fractions may be considered the most and least nutritious, respectively.

Scanning Electron Microscopy (SEM)

The SEM structures of flours, total protein isolate and different protein fractions of *A. excelsa* are shown in Fig. 1. The SEM results indicate that albumins of *A. excelsa* exhibit small, flaky particles of a porous nature, while the globulins are irregular in shape with a small, particle-like structure. Prolamins are also flaky particles with low porosity. However, the albumins showed a higher porosity than the prolamins, even at a lower magnification. The glutelin fractions showed a thin, rod-and-plate-like surface topography. Seed flours and total protein isolates, as expected, consisted of these four protein fractions. Therefore, their morphological structures showed that seed flour was small, flaky plates with particle-like surfaces and that TPI were flaky and plate-shaped, with rougher surfaces and high porosity. The different topographical characteristics of the total protein isolate and protein fractions may contribute to the overall physiochemical and functional properties of *A. excelsa* seed proteins [18].

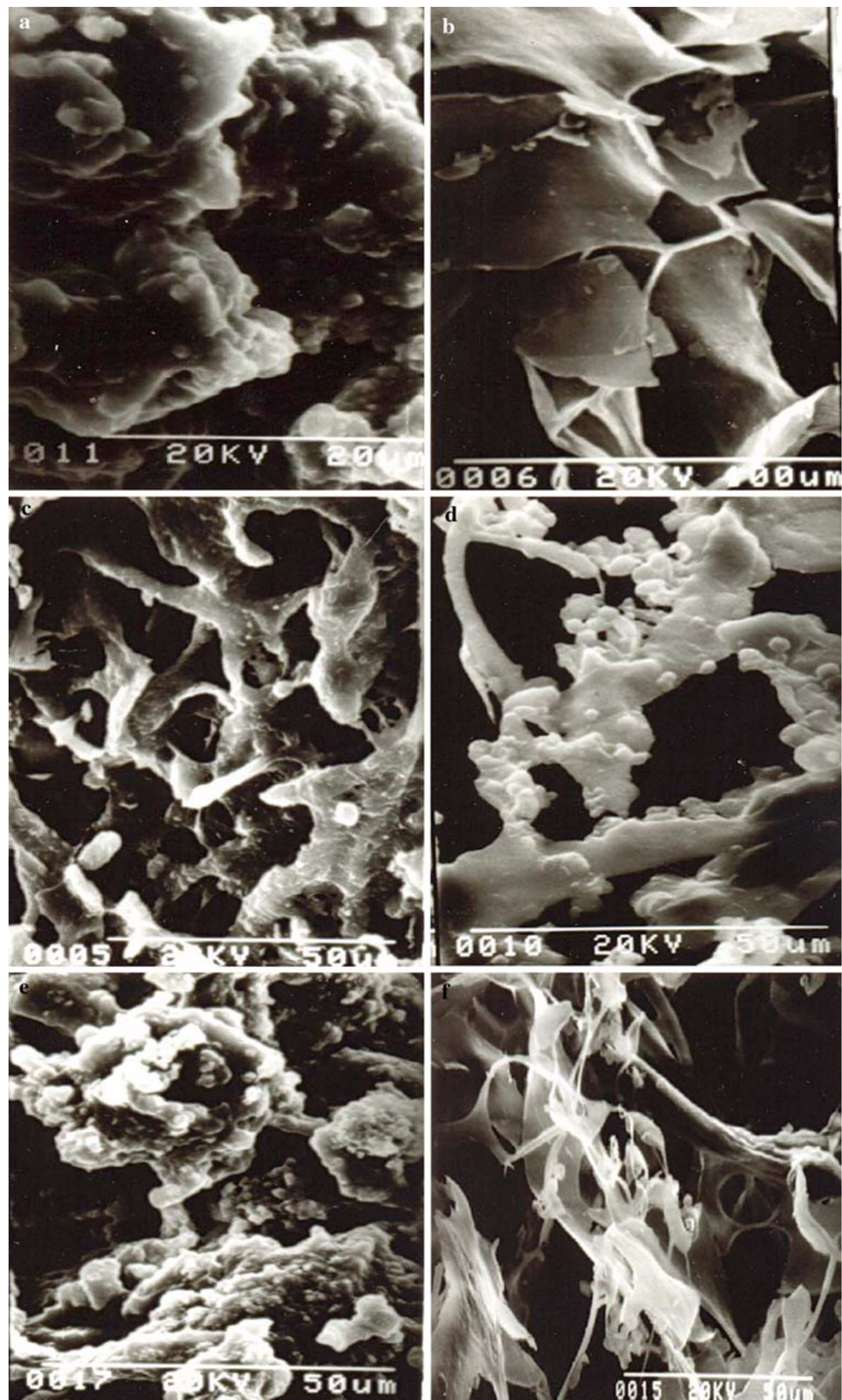

Fig. 1 Scanning electron micrographs of *A. excelsa* (**a**) seed flour, (**b**) total protein isolate (*TPI*) and protein fractions (**c**, albumin; **d**, globulin; **e**, prolamin; **f**, glutelin)

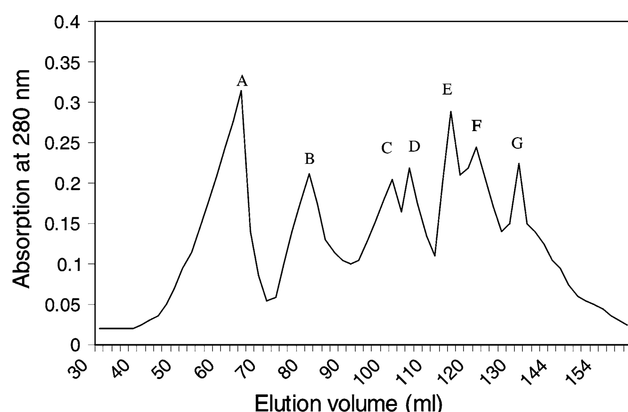

Fig. 2 Gel filtration chromatography of *A. excelsa* seed protein in Sephadex G-200

Gel Filtration

Ailanthus excelsa seed proteins were fractionated into seven components (A–G) by gel filtration chromatography (Fig. 2). The two methods used to determine the molecular weights of each of the seven components were not exactly the same, but were similar to each other (Table 5). Based on the results of the gel filtration of the extractable *A. excelsa* seed protein, it can be assumed that the seed protein is a mixture of at least seven polypeptides.

Polyacrylamide Gel Electrophoresis

NPAGE results for the total protein isolate and the solubility-based protein fractions (Fig. 3a) confirmed the dominance of globulins in the total protein. It is also confirmed that prolamin fractions accounted for only a small percentage of the protein compared to the other three fractions.

SDS–PAGE results for TPI, the four fractions and the total protein extracted by phosphate buffer are shown in Fig. 3b. SDS–PAGE of TPI and phosphate buffer-extracted protein showed very similar electrophoretic subunit compositions and very typical pattern characteristics upon visual examination of the gels. SDS–PAGE of TPI (lane 1) indicated a broad range of polypeptides (28.9–141.2 kDa) with eight major subunits (28.9, 33.9, 36.3, 39.8, 60.3, 67.6, 117.5 and 141.2 kDa). On the other hand, buffer-extracted proteins (lane 6) resolved into ten distinct subunits ranging from 28.8 to 154.9 kDa. Eight prominent polypeptide subunits of molecular mass 28.8, 33.1, 33.9, 39.8, 57.5, 63.1, 66.0, 67.6 kDa and two less prominent subunits of molecular mass 131.8 and 154.9 kDa were identified. The extraction of additional proteins from the seeds of *A. excelsa* by the phosphate buffer solution (pH 7.0) in comparison to that achieved by the total protein isolation process was demonstrated by the appearance of two additional protein bands (Fig. 3b, lane 6). The molecular weight distribution of the total protein isolate in this study was quite similar to that observed in the gel filtration study (Table 5). The major soluble protein fraction, globulin (Fig. 3b, lane 3), was characterized by several polypeptide subunits in the mass range 29.5–63.0 kDa. The three most distinct subunits, those at molecular masses of 39.8, 43.6 and 63.0 kDa, along with one less prominent subunit at 29.5 kDa, were separated by SDS–PAGE. The albumin fractions were found to have three major polypeptide subunits (Fig. 3b, lane 2) of molecular

Table 5 Molecular weights of *A. excelsa* seed proteins, as determined by a gel filtration procedure

Proteins	Elution volume/void volume (V/V_0)	Molecular weight determined from the standard curve (Daltons)[a]	Molecular weight determined by equation (Daltons)	Literature molecular weight (Daltons)[b]
BSA[c]	1.88	–	–	66,000
Ovalbumin	2.07	–	–	45,000
Pepsin	2.20	–	–	34,700
Lysozyme	2.62	–	–	14,300
Ailanthus excelsa seed proteins				
Component A	1.23	2,63,000	3,01,000	–
Component B	1.54	1,38,000	1,52,000	–
Component C	1.92	61,700	65,800	–
Component D	2.00	52,500	55,100	–
Component E	2.19	35,500	36,200	–
Component F	2.31	27,500	27,800	–
Component G	2.50	18,200	18,300	–

[a] From [20]

[b] Molecular weights of standard proteins obtained from Sigma Chemical Co. (St. Louis, MO, USA)

[c] Bovine serum albumin

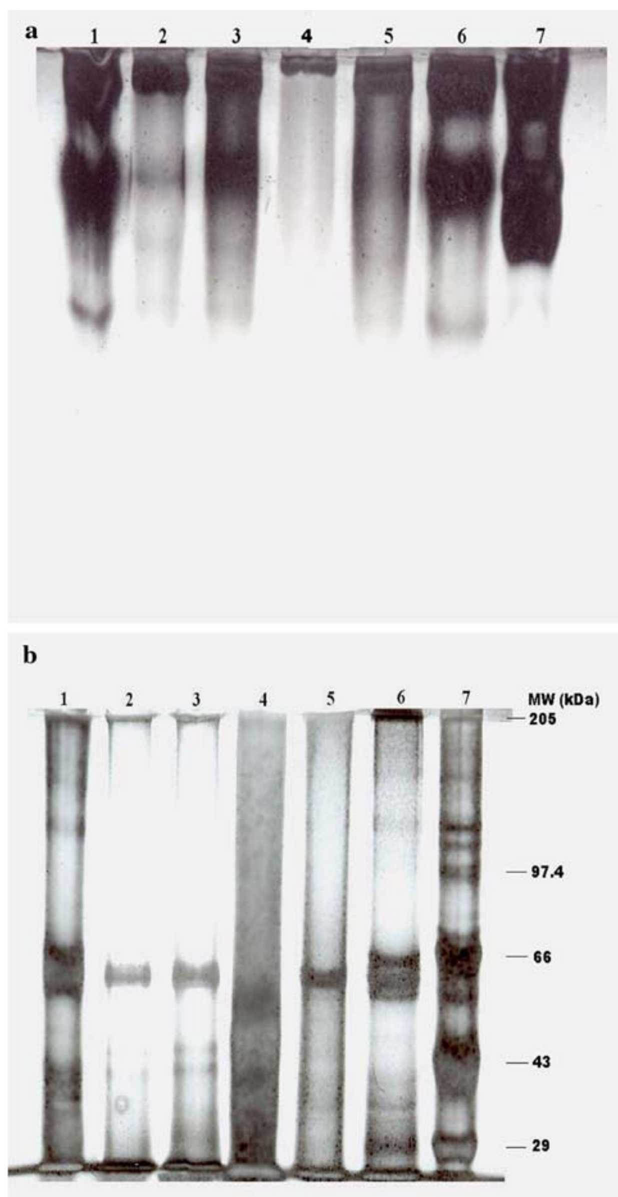

Fig. 3 NPAGE (**a**) and SDS–PAGE (**b**) of *A. excelsa* seed proteins: *1*, total protein isolate; *2*, albumin fraction; *3*, globulin fraction; *4*, prolamin fraction; *5*, glutelin fraction; *6*, buffer-extracted protein isolate; *7*, molecular weight marker; 25 μg of protein was loaded into each *lane*

mass 39.0, 42.65 and 63.0 kDa, which were similar to those for the globulin fraction. The prolamin fraction exhibited a diffuse banding pattern on SDS–PAGE (Fig. 3b, lane 4), and at least two dominant polypeptides of molecular mass 33.1 and 39.8 kDa were found in this study. The alkali-extracted fraction, glutelin, had only one prominent polypeptide of molecular mass of 60.3 kDa. The results show that *A. excelsa* seed proteins are structurally very simple in comparison with other leguminous plant seed proteins.

The present study showed that *A. excelsa* seed protein displays good solubility in different aqueous solution and contains a high percentage of proteins that were extracted by a simple method. Based on these two characteristics of *A. excelsa* seed protein, we concluded that it could be used in the food industry as an unconventional protein source. Furthermore, the abundance of low molecular weight protein and the presence of most of the essential amino acids (except tryptophan, which was not determined) indicates that it is nutritionally significant and could be used for dietary purposes. However, this material can only be considered a feed supplement after a proper toxicological analysis has been carried out on the material.

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