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Isolation and Chemical Investigation of Teak (*Tectona grandis* Linn) Seed Proteins

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ABSTRACT

Proteins were extracted from the deoiled seeds of *Tectona grandis* Linn., Fam. Verbenaceae, a quality lumber source, in aqueous solutions of various pH's or by different concentrations of NaCl at pH 8.0. Chemical analysis of isolated protein identified 15 amino acids, of which eight were essential. Gel filtration on Sephadex G-200 revealed the presence of six components, whose molecular weights were determined by two comparable standard methods. Seven components were resolved electrophoretically (SDS-PAG electrophoresis) and their molecular weights were found to be 118,900, 92,300, 72,400, 62,400, 43,600, 39,800 and 32,400.

INTRODUCTION

Teak (*Tectona grandis* L.) is a species of a small genus *Tectona* belonging to the family Verbenaceae. It enjoys a worldwide reputation as a quality lumber resistant to attack by termites (1). It is generally used in aesthetic crafts and is widely cultivated in tropical countries. This plant is indigenous to India, Burma and the western part of Thailand (2). Handling of its wood causes contact dermatitis (3), and different parts of the plant are used as medicines.

The seed kernel contains 40% oil (4), and the total seed only 7.3%. Seed oil may be used for edible purposes (5) and contains no unusual fatty acids. In addition to oil, the seed kernel contains a high concentration of protein (4). The seed protein and oil have not been used extensively until now, although a small amount of oil has been used in remote villages of India to prevent falling of hair (2). Recently, it has been observed that the seed protein is well tolerated by albino rats (6).

This report summarizes the extraction (7) and isolation of protein from deoiled Teak seed, the amino acid composition of isolated protein and determination of molecular weights of its different fractions by gel filtration and by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis.

EXPERIMENTAL PROCEDURE

Materials

All reagents used in this investigation were of analytical grade. Reagents for SDS-Polyacrylamide gel electrophoresis, Sephadex G-200 (for gel filtration) and proteins used for standard calibration (BSA, Ovalbumin, Pepsin and Lysozyme) were purchased from Sigma Chemical Co., St. Louis, Missouri.

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Extraction of Proteins from Teak Seed

Finely powdered teak seeds were extracted with petroleum ether (40-60°) in a Soxhlet for 48 hrs. Then the seeds were washed well with acetone and air dried. The nitrogen content of the seed was estimated by the micro-Kjeldahl method (8) and the protein content was determined (9).

Extraction of proteins (7) from deoiled seeds was carried out using 20 volumes (w/v) of extractant at room temperature for 30 min, either at various pH's (2-12) or by using gradient NaCl solution (2-10%) at pH 8.0. The nitrogen content of each extract was monitored by the micro-Kjeldahl method (9).

Determination of Amino Acid Composition of Teak Seed Protein

The deoiled teak seed was stirred with 20 volumes (w/v) cold aqueous 4% sodium chloride solution at pH 10-11 (adjusted by adding 0.5M sodium hydroxide solution) for one hour. It was then centrifuged at 5000 RPM for 10 min, and the suspension was filtered through Whatman No. 1 filter paper. The protein solution was dialyzed against distilled water and freeze dried. The resulting protein product was deep brown in color.

Acid hydrolysis of the seed protein was conducted by hydrolyzing freeze dried sample with 3 ml 6 (N) HCl, containing 5% thioglycolic acid (10), in a sealed evacuated tube at 110 C for 24 hr. Amino acid composition of the hydrolysate was determined using a Beckman Multichrome 4255 amino acid analyzer.

Preparation of Protein Sample and Gel Filtration on Sephadex G-200

For gel filtration, the protein was extracted with 4% NaCl at pH 8.0 and was dialyzed against 0.01M phosphate buffer (pH 7.2) for 48 hr at 4 C. The protein was obtained by freeze drying the dialyzed solution. Then it was dissolved in 0.01M phosphate buffer (pH 7.0) containing 0.2M NaCl to obtain a protein concentration of 4 mg/ml.

Gel filtration (11) of teak seed proteins on Sephadex G-200 was conducted on a 2.5 x 40 cm column at 25 C. 1.5 ml of the protein sample was applied to the top of the gel bed. The eluting buffer was 0.01M sodium phosphate (pH 7.0) containing 0.2M NaCl. Fractions of 2 ml were collected at the rate of 0.4 ml/min and monitored at 280 nm. The molecular weights of the components corresponding to the peaks A, B, C, D, E and F (Fig. 1) were determined from a linear plot (V/V_0 against log mol wt, Fig. 2) and calibrated with reference protein standards

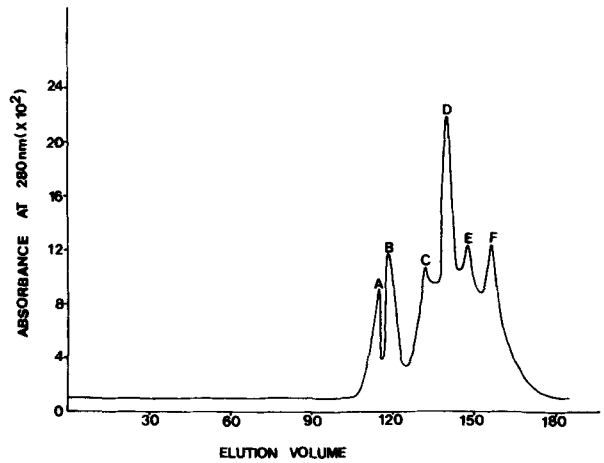

FIG. 1. Gel filtration of teak seed proteins on a Sephadex G-200 column (40 cm X 2.5 cm) marking the fractions by letter A-F.

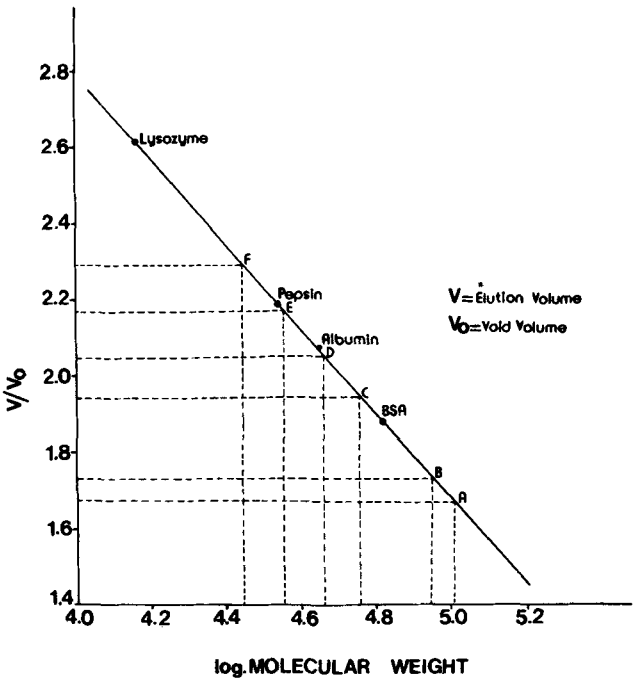

FIG. 2. Determination of molecular weights of six fractions (A-F) of teak seed proteins by gel filtration procedure using some standard proteins.

TABLE I

Protein Solubility of Teak Seed Meal in Aqueous Solution at Various pH and in Different Concentrations of Aqueous NaCl Solution at pH 8.0

pH of solution	Nitrogen solubility (in %)	Concentration (%) of NaCl at pH 8.0	Nitrogen solubility (in %)
2	43.77	2	72.39
3	50.38	3	77.45
4	57.24	4	82.49
5	65.60	5	76.50
6	74.07	6	71.38
7	76.60	7	66.25
8	79.13	8	61.27
9	85.50	9	61.00
10	92.93	10	60.59
11	93.00	–	–
12	93.00	–	–

(BSA, Ovalbumin, Pepsin and Lysozyme). The molecular weight of each of the components (A-F) also was calculated by the following equation (12):

$$\log \text{mol wt} = -0.959(V/V_O - 1) + 5.7 \quad [1]$$

where V = elution volume of the component, V_O = void volume of the column.

SDS-Polyacrylamide Gel Electrophoresis

The protein extract in salt (4% NaCl) solution at alkaline pH (8.0) was dialyzed against phosphate buffer (pH 7.0) containing 0.9% NaCl solution, and the sample was freeze dried. It was then incubated at 37 C overnight in 0.01M phosphate buffer (pH 7.0) containing 1% SDS and 1% β-mercaptoethanol. The protein concentration was 1 mg/ml. After incubation, 0.1 ml sample was mixed with 10 μl 0.05% bromophenol blue solution and 0.1 ml 40% sucrose solution, then subjected to SDS-PAG gel electrophoresis (13). The gel system was calibrated using standard proteins (BSA, Ovalbumin, Pepsin and Lysozyme). 50 μl of the protein sample was applied on each of the gels.

After electrophoresis, gels were removed from the tubes and placed in a staining solution of 1.25 g Coomasie Brilliant Blue in 454 ml 50% methanol and 46 ml glacial acetic acid for 2 hr at room temperature. The gels then were destained electrophoretically using a destaining solution (75 ml CH_3COOH, 50 ml CH_3OH and 875 ml water). Mobility of the protein fractions was calculated as:

$$\text{Mobility} = \frac{\text{Distance of protein migration}}{\text{Length after destaining}} \times \frac{\text{Length before staining}}{\text{Distance of dye migration}}$$

Molecular weights of the protein fractions (bands) were determined from a linear curve obtained by plotting log mol wts against mobilities of standard proteins.

RESULTS AND DISCUSSION

It was found that nitrogen and protein contents in deoiled seeds were 2.13% and 12.14%, respectively. Table I shows the nitrogen solubility profile of deoiled teak seed in aqueous solution at various pH's and in different salt (NaCl) concentrations at pH 8.0. It can be seen that the seed protein's solubility increases with an increase in pH of the extractant.

This method gave about 95% recovery (11.57 g/100 g of deoiled seed) of the protein content. Amino acid composition of the isolated protein hydrolysate showed that 15 amino acids, of which eight were essential (Table II), were present. Methionine and tryptophan were not found, but

TABLE II

Amino Acid Composition of Teak *(Tectona grandis)* Seed Protein

Amino acids	g/16 g Nitrogen
Aspartic acid	8.00
Threonine[a]	2.91
Serine	3.63
Glutamic acid	25.09
Proline	8.73
Glycine	10.18
Alanine	6.54
Valine[a]	6.18
Isoleucine[a]	4.73
Leucine[a]	8.00
Tyrosine	0.73
Phenylalanine[a]	4.00
Histidine[a]	1.82
Lysine[a]	3.64
Arginine[a]	5.82

[a]Essential amino acids.

other essential amino acids were present in considerable amounts. The isolated protein is rich in glutamic acid (25.09 g/16 g N).

Six peaks (Components A-F) were observed by gel filtration (Fig. 1), indicating that the protein was a mixture of at least six components. It is evident from Table III that the two methods used for determination of the molecular weight of each of the six components are in fair agreement with each other.

Further evaluation of molecular weights of extractable teak seed proteins in alkaline (pH 8.0) salt solution (4% aqueous NaCl solution) by SDS-PAG electrophoresis revealed seven protein bands. Relative mobilities for these fractions (components) were calculated and compared with the standard curve (Fig. 3). Mol wts as determined on the basis of their mobilities (Table IV) were 118,900 (T_1), 92,300 (T_2), 72,400 (T_3), 62,400 (T_4), 43,600 (T_5), 39,800 (T_6) and 32,400 (T_7).

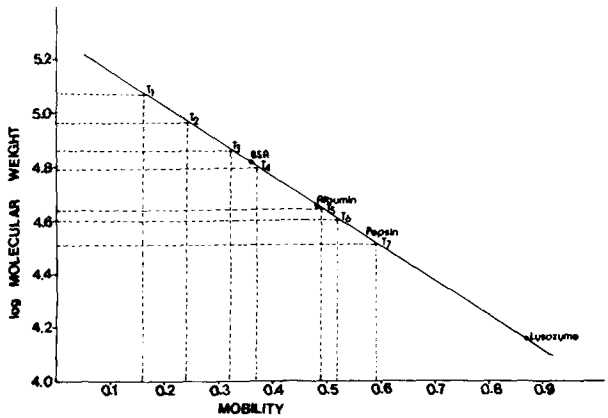

FIG. 3. Comparison of molecular weights of standard proteins and seven different fractions (T_1-T_7) of teak seed proteins by SDS-PAG gel electrophoresis.

TABLE III

Determination of Molecular Weights of Teak Seed Proteins by Gel Filtration Procedure

Proteins	Elution vol. / Void vol. (V/V_O)	Molecular wt determined from the curve (Fig. 2)	Molecular wt calculated from the equation [1]	Literature mol wt[a]
BSA	1.880	–	–	66,000
Ovalbumin	2.081	–	–	45,000
Pepsin	2.198	–	–	34,700
Lysozyme	2.616	–	–	14,300
Teak seed proteins:				
Component A	1.676	102,300	112,500	–
Component B	1.735	89,100	96,900	–
Component C	1.941	57,500	62,700	–
Component D	2.059	46,700	48,400	–
Component E	2.176	36,300	37,300	–
Component F	2.294	31,600	28,300	–

[a]Literature of mol wts of standard proteins, from Sigma Chemical Co., St. Louis, Missouri.

TABLE IV

Molecular Weight Determinations of Teak Seed Proteins by SDS-PAG Electrophoresis

Proteins	Mobility	Molecular weight from the literature[a]	Molecular weight from Figure 3
Bovine Serum Albumin (BSA)	0.36	66,000	–
Ovalbumin	0.48	45,000	–
Pepsin	0.54	34,700	–
Lysozyme	0.87	14,300	–
Teak seed proteins:			
Protein (T_1)	0.16	–	118,900
Protein (T_2)	0.24	–	92,300
Protein (T_3)	0.32	–	72,400
Protein (T_4)	0.37	–	62,400
Protein (T_5)	0.49	–	43,600
Protein (T_6)	0.52	–	39,800
Protein (T_7)	0.59	–	32,400

[a]Literature of mol wts of standard proteins, from Sigma Chemical Co., St. Louis, Missouri.

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