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Extraction and Chemical Investigation of Kulthi (*Macrotylona uniflorus*, Lam.) Seed Protein

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ABSTRACT

Studies have been carried out on the protein solubility profile of Kulthi (*Macrotylona uniflorus*, Lam.) seed in aqueous solution over various pHs and at different concentrations of NaCl, Na_2SO_3, $CaCl_2$, and $MgCl_2$ at pH 8.0. Amino acid analysis of isolated protein identified 17 amino acids, 9 of which are essential. Gel-permeation chromatography on Sephadex G-200 revealed the presence of seven components in the protein fraction. Their molecular weights were determined by two comparable standard methods. Extractable Kulthi seed proteins in salt solutions were separated electrophoretically into eight fractions whose molecular weights were found to be 186,200, 131,800, 108,400, 91,200, 53,700, 44,700, 38,000, and 27,500.

Index Entries: *Macrotylona uniflorus* (*Dolichos biflorus*); seed proteins; amino acids; gel filtrations; SDS-PAGE.

INTRODUCTION

Horse gram or Kulthi (*Macrotylona uniflorus*, Lam., formerly known as *Dolichos biflorus*, Fam: Leguminosae) is widely cultivated in two drought-prone districts (Bankura and Purulia) of West Bengal, India, and also in Midnapore (West Bengal, India) as a companion crop.

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The plant is well known for its medicinal properties (*1*). It is also rich in carbohydrate and protein (*2*). In addition, the seed has a remarkable agglutination property that is specific for A_1 cells (*3*). Analysis of this property reveals the presence of two glycoproteins. The nature of these glycoproteins and their molecular weights, carbohydrate content, and N-terminal residues were reported earlier (*4–8*). Although the seed contains a high precentage of protein, few reports (*9,10*) have been published on its chemical analysis. The present article deals with the chemical analysis of the isolated seed protein with special reference to solubility studies, amino acid composition, fractionation, and molecular-weight determination by gel filtration and electrophoresis.

EXPERIMENTAL PROCEDURES

Finely powdered Kulthi seeds were defatted with petroleum ether (40–60°C) by Soxhleting for 48 h. They were then washed well with acetone and air-dried. Nitrogen content of the defatted seeds was estimated by the usual micro-Kjeldahl method (*11*). Protein solubility of defatted seeds was determined at several pH values ranging between 2 and 12. The solubility at each pH value was determined by stirring defatted seeds with water (1:20, w/v) at room temperature for 30 min, after which the pH of solution was adjusted by adding 0.5*M* HCl or 0.5*M* NaOH. The adjusted pH was maintained throughout the experiment (*12–14*).

A solubility profile of Kulthi seed proteins was also determined by a similar process in the presence of NaCl, Na_2SO_3, $CaCl_2$, and $MgCl_2$ at different molar concentrations (0.1–1.0*M*) at a fixed pH of 8.0 (*12,14*). In all the cases, nitrogen solubility were monitored by the micro-Kjeldahl method (*11*).

Amino acid analysis of the seed protein was performed in a Pharmacia LKB Alpha Plus amino acid analyzer (UK) taking precautions for cystine, methionine, and tyrosine by using proper protecting reagents. (Analysis was done at the Centre for Cellular and Molecular Biology, Hyderabad, India.)

Preparation of Protein Sample for Gelfiltration

The protein extract at pH 8.0, in presence of 0.1*M* NaCl, was dialyzed against 0.01*M* phosphate buffer (pH 7.0) for 48 h at 4°C. It was then freeze-dried. The proteins so obtained were again dissolved in 0.01*M* phosphate buffer (pH 7.0) containing 0.2*M* NaCl at a protein concentration 4 mg/mL.

Gel filtration (*15*) was then carried out with this protein solution (1 mL) on a Sephadex G-200 column (2.5 cm id × 40 cm) at 25°C using 0.01*M* phosphate buffer (pH 7.0) containing 0.2*M* NaCl as eluting solvent. Two-milliliter fractions were collected at the rate of 0.4 mL/min.

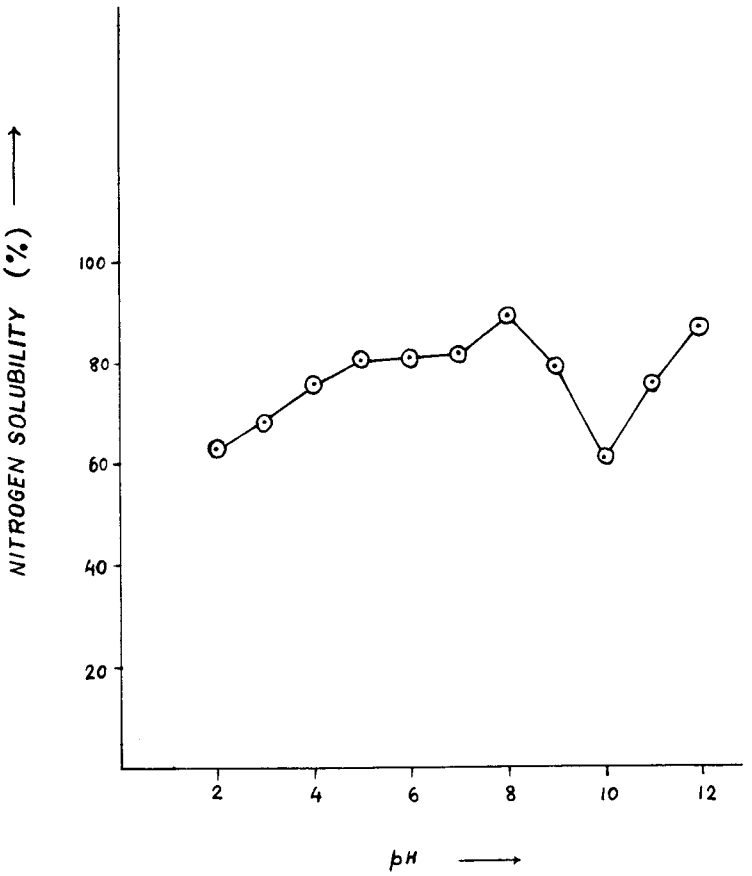

Fig. 1. Solubility of Kulthi (*M. uniflorus*) seed protein at different pHs.

Preparation of Protein Sample for Gel (SDS-PAGE)

Protein extracts in various salt solutions (NaCl, Na_2SO_3, $CaCl_2$, and $MgCl_2$) of 0.1*M* concentration and at pH 8.0 were dialyzed against 0.01*M* phosphate buffer (pH 7.0). A protein sample containing 5 mg/mL protein was subjected directly to SDS-PAGE according to the method of Weber and Osborn (*16*).

RESULTS AND DISCUSSION

From the value of nitrogen content (5.095%), the protein content (29.04%) of the defatted Kulthi seed was also determined (*17*). It was also observed that nitrogen solubility increased with increasing pH and reached a maxima at pH 8.0 (Fig. 1). However, the solubility decreases with increase in concentrations (Fig. 2) of inorganic salts, except $CaCl_2$ (Table 1).

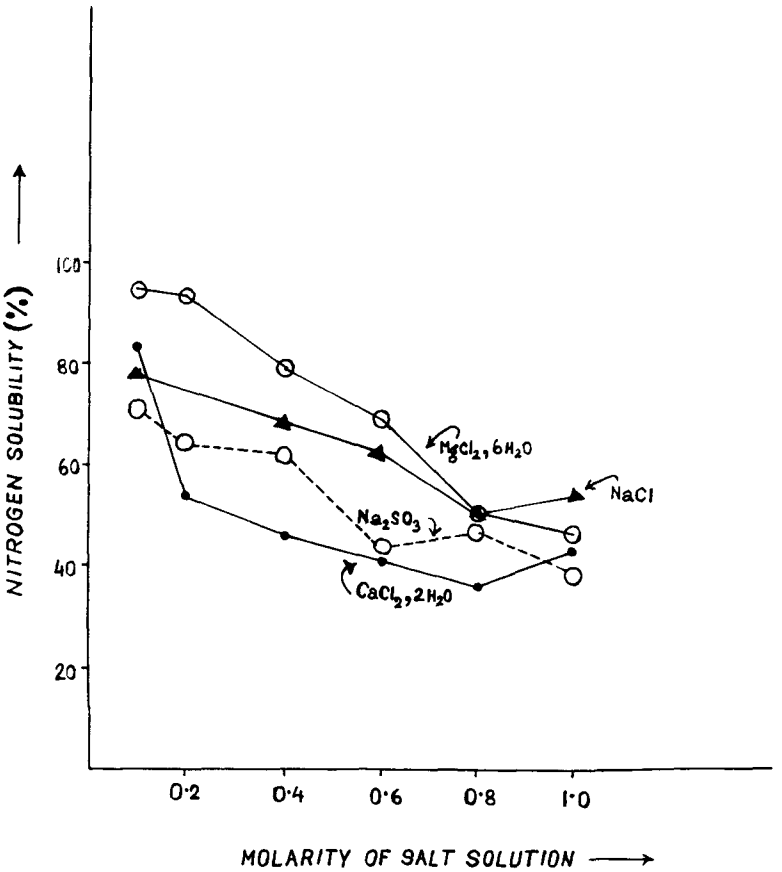

Fig. 2. Solubility study of Kulthi (*M. uniflorus*) seed protein in different salt solutions of various concentrations at pH 8.0.

Amino acid analysis revealed that this seed protein contains 17 amino acids of which 9 were essential. The amount of glutamic acid was the highest (26.62 g/16 g N), whereas that of methionine (0.15 g/16 g N) was at the lowest concentration (Table 2).

Seven components were observed (components A-G) by gel-filtration procedure (Fig. 3), indicating that the isolated protein was a mixture of at least seven components. The molecular weights of the components corresponding to the seven peaks (Fig. 3) were determined (*15*) from a linear curve (Fig. 4), which is 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 (*18*).

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

Table 1
Solubility of Kulthi (*M. uniflorus*) Seed Protein in Aqueous Solution at Various pHs and in Varied Concentrations of Different Salt Solutions at pH 8.0

pH of solution	Nitrogen solubility, %	Molar concn. of NaCl, pH 8	Nitrogen solubility, %	Molar concn. of Na_2SO_3, pH 8	Nitrogen solubility, %	Molar concn. of $MgCl_2$, pH 8	Nitrogen solubility, %	Molar concn. of $CaCl_2$, pH 8	Nitrogen solubility %
2	63.66	0.1	77.64	0.1	70.67	0.1	94.82	0.1	82.57
3	68.72	0.2	74.95	0.2	64.26	0.2	93.44	0.2	53.71
4	76.32	0.3	72.00	0.3	62.90	0.3	86.50	0.3	50.00
5	80.91	0.4	68.22	0.4	62.63	0.4	79.30	0.4	45.76
6	81.50	0.5	65.20	0.5	52.50	0.5	73.90	0.5	43.50
7	81.91	0.6	62.81	0.6	44.13	0.6	69.10	0.6	41.21
8	89.73	0.7	55.25	0.7	45.10	0.7	59.90	0.7	38.50
9	79.99	0.8	60.80	0.8	47.30	0.8	50.47	0.8	35.96
10	61.34	0.9	52.00	0.9	42.65	0.9	48.50	0.9	39.00
11	76.48	1.0	64.16	1.0	38.13	1.0	46.55	1.0	42.71
12	87.69	—	—	—	—	—	—	—	—

Table 2
Amino Acid Composition
of Isolated *M. uniflorus* Seed Protein

Amino acids	g/16 g N
Aspartic acid	14.54
Threonine*	2.83
Serine	6.23
Glutamic acid	26.62
Proline	4.11
Glycine	3.26
Alanine	3.45
Cystine	0.27
Valine*	3.83
Methionine*	0.15
Isoleucine*	3.73
Leucine*	8.31
Tyrosine	3.38
Phenylalanine*	5.83
Histidine*	2.43
Lysine*	6.05
Arginine*	4.98

* Essential amino acids.

The two methods used for the molecular-weight determination of each of the seven components are similar to each other (Table 3).

Further evaluation of molecular weights of extractable Kulthi seed proteins (0.1*M* NaCl, 0.1*M* Na_2SO_3, O.1*M* $MgCl_2$, and 0.1*M* $CaCl_2$) was made by SDS-PAGE. Each of the protein isolates consisted of eight fractions or components (Fig. 5). Actually there were no differences in the number of protein fractions of various salt solutions. Relative mobilities of the fractions were calculated, and the molecular weights of the components were determined from standard curve (Fig. 5) using standard proteins (BSA, ovalbumin, pepsin, and lysozyme) purchased from Sigma Chemical Co., St. Louis, MO. The molecular weights of the fractions (components) as determined were 186,200, 131,800, 108,400, 91,200, 53,700, 44,700, 38,000, and 27,500 (Table 4). From the above observations, a conclusion may be drawn in such a manner that Kulthi (*M. uniflorus*) seed protein contains several fractions or components, either seven or eight in number.

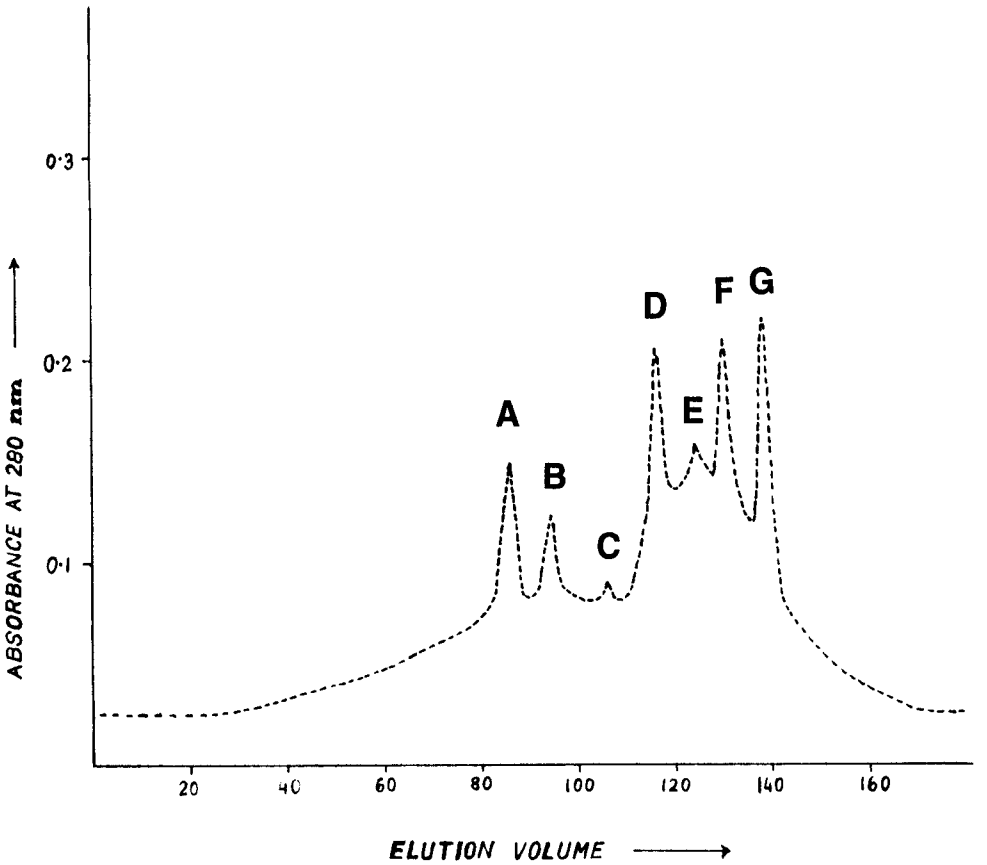

Fig. 3. Gel filtration of Kulthi (*M. uniflorus*) seed proteins in Sephadex G-200 column (2.5 × 40 cm).

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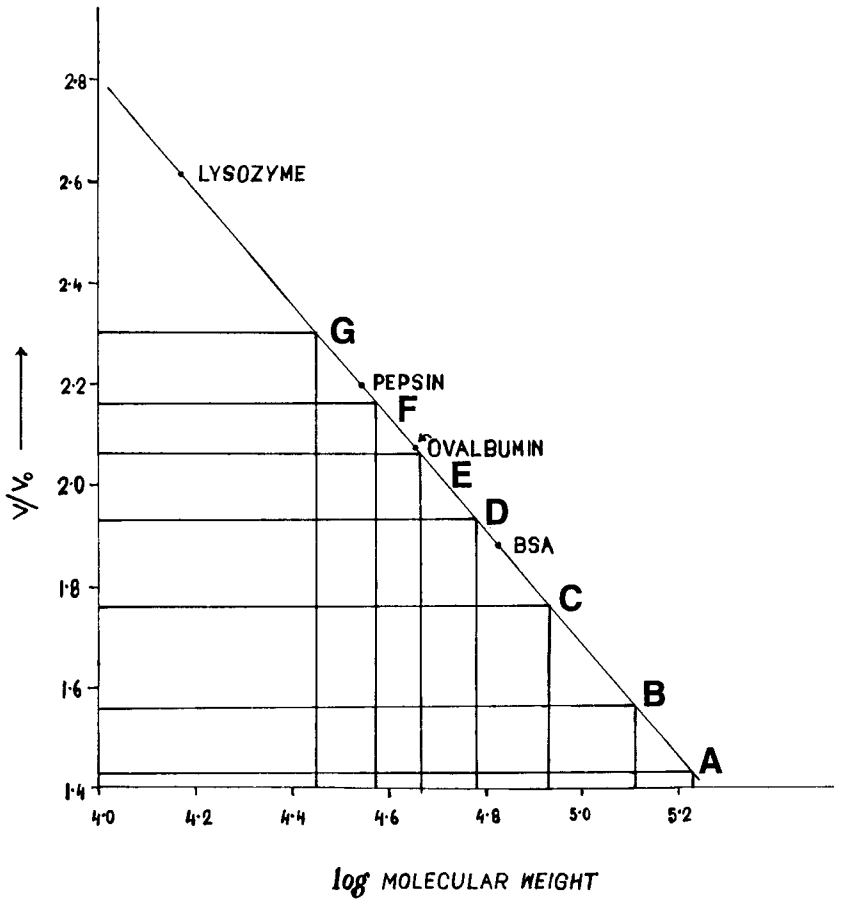

Fig. 4. Determination of molecular weights of Kulthi (*M. uniflorus*) seed proteins by gel filtration.

Table 3
Determination of Molecular Weights of Kulthi (*M. uniflorus*) Seed Proteins by Gel-Filtration Procedure

Proteins	Elution vol/ void vol V/V_0	Molecular weight determination from the curve, daltons	Molecular weight determination by equation, daltons	Literature[a] molecular weight daltons
BSA[b]	1.880	—	—	66,000
Ovalbumin	2.071	—	—	45,000
Pepsin	2.195	—	—	34,700
Lysozyme	2.618	—	—	14,300
M. uniflorus seed proteins				
Component A	1.433	1,69,800	1,90,500	—
Component B	1.567	1,28,800	1,43,200	—
Component C	1.767	85,100	91,200	—
Component D	1.933	60,300	63,100	—
Component E	2.067	45,700	47,500	—
Component F	2.167	37,200	38.000	—
Component G	2.300	28,200	28,100	—

[a]Literature of molecular weight of standard proteins from Sigma Chemical Co., St. Louis, MO.
[b]Bovine serum albumin.

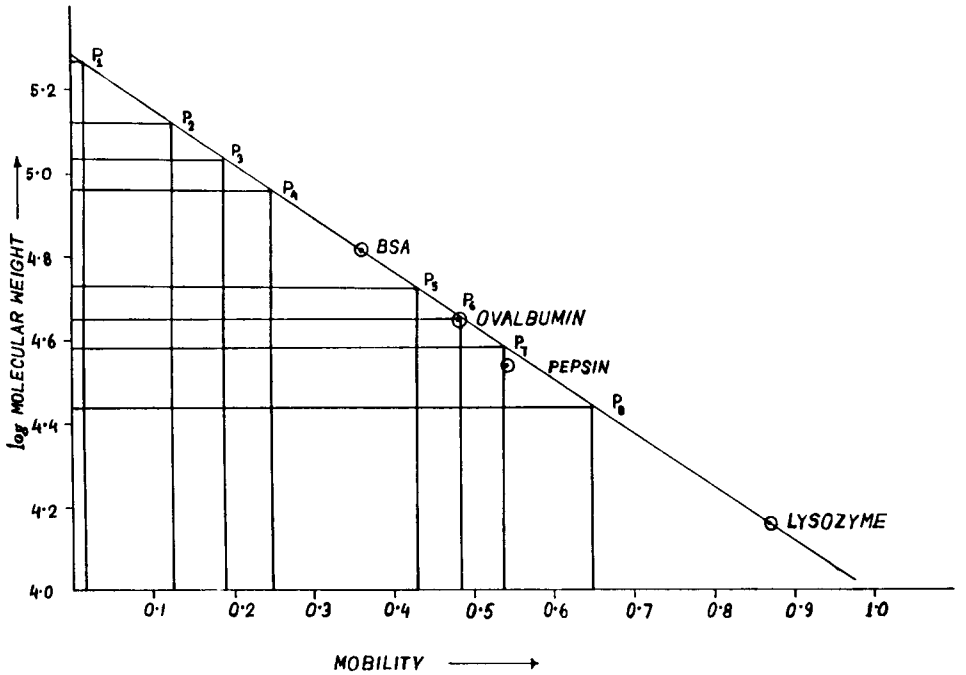

Fig. 5. Determination of molecular weights of Kulthi (*M. uniflorus*) seed proteins by SDS-PAGE using some standard proteins.

Table 4
Molecular Weight Determination
of Kulthi (*M. uniflorus*) Seed Proteins by SDS-PAGE

Proteins	Mobility	Molecular weight from the literature[a], daltons	Molecular weight from fig., daltons
BSA[b]	0.36	66,000	—
Ovalbumin	0.48	45,000	—
Pepsin	0.54	34,700	—
Lysozyme	0.87	14,300	—
Kulthi (*M. uniflorus*) seed proteins			
(P_1)	0.01	—	1,86,200
(P_2)	0.12	—	1,31,800
(P_3)	0.19	—	1,08,400
(P_4)	0.25	—	91,200
(P_5)	0.43	—	53,700
(P_6)	0.48	—	44,700
(P_7)	0.54	—	38,000
(P_8)	0.65	—	27,500

[a]Literature of molecular weight of standard proteins from Sigma Chemical Co., St. Louis, MO.
[b]Bovine serum albumin.

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