Extraction and Chemical Investigation of Jute (*Corchorus olitorius*, Linn.) Seed Protein

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ABSTRACT

Studies were made on the protein solubility of deoiled jute (*Corchorus olitorius*, Linn.) seed in aqueous solution over various pHs and in different concentrations of NaCl at pH 8.0. Chemical analysis of the seed protein showed 16 amino acids, of which 9 were essential. Gel filtration on Sephadex G-200 revealed the presence of four components, and their molecular weights were determined by two standard methods. Extractable jute seed proteins in salt solution were separated into six fractions electrophoretically (SDS-PAGE). The molecular weights of the six fractions were found to be 118,000; 103,000; 96,000; 67,500; 48,500; and 15,000.

Index Entries: *Corchorus olitorius*; seed proteins; amino acids; gel filtration; SDS-PAG electrophoresis.

INTRODUCTION

Jute (*Corchorus olitorius* Linn.; family, Tiliaceae), an indigenous plant to India (1), is well reputed for its cambium fiber and for its application as a folk medicine (2). Though its oil and protein content is very high (1), no report on its use has been published, except the fact that its protein is well tolerated by albino rats (3,4). Very scanty reports (5,6) have been published on its chemical analysis, which is of immense importance for further investigation.

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The present communication is mainly concerned with the chemical analysis of the seed protein, with special reference to the extraction of protein from the deoiled seed (jute seed), amino acid composition of isolated protein, and determination of its molecular weight by Gel-filtration and SDS-PAGE.

EXPERIMENTAL PROCEDURES

Finely ground jute seeds were extracted with petroleum ether $(40-60^{\circ}\text{C})$ in a Soxhlet for 48 h. The seeds were then washed well with acetone and air dried. Nitrogen content (3.54%) of the deoiled seed was determined by the micro-Kjeldahl method (7), and the protein content (20.18%) was also determined (8). Extraction of proteins (9,10) from the deoiled seeds was carried out using 20 vol (w/v) of extractant for 30 min either at various pHs (2-12) or by using gradient NaCl solution (0.05-1.00M) at pH 8.0. The micro-Kjeldahl method (7) (Table 1) revealed that the seed protein was substantially insoluble at low pH and very soluble at high pH and low concentrations of NaCl (up to 0.1M) at pH 8.0. High solubility characteristics of the protein in alkaline NaCl solution could be used to prepare protein isolate, followed by precipitation at pH 5.0-6.0

Amino acid composition of the seed protein was determined according to the method recommended by Matsubara and Sasaki (11), using a Beckman Multichrome 4255 amino acid analyzer. Sixteen amino acids were found to be present, of which nine were essential (Table 2): Glutamic acid was present in greater amounts (18.20 g/16 g N) in comparison to other amino acids.

TABLE 1
Solubility of Jute Seed Protein in Aqueous Solution at Various pHs and in Different Concentrations of Aqueous NaCl Solutions at pH 8.0

| pH of solution | Nitrogen solubility, % | Different concentration, M of NaCl at pH 8.0 | Nitrogen solubility, % |
|----------------|------------------------|--|---------------------------|
| 2 | 15.75 | 0.05 | 58.97 |
| 3 | 20.26 | 0.10 | 60.80 |
| 4 | 22.74 | 0.20 | 53.62 |
| 5 | 23.19 | 0.30 | 52.92 |
| 6 | 23.64 | 0.40 | 51.78 |
| 7 | 27.95 | 0.50 | 50.69 |
| 8 | 60.59 | 0.60 | 50.24 |
| 9 | 76.56 | 0.70 | 48.41 |
| 10 | 82.85 | 0.80 | 47.32 |
| 11 | 84.24 | 0.90 | 43.26 |
| 12 | 84.24 | 1.00 | 27.94 |

TABLE 2 Amino Acid Composition of Isolated Jute Seed Protein

| Amino acids ^a | g/16 g N |
|----------------------------|----------|
| Aspartic acid | 9.52 |
| Threonine ^b | 3.70 |
| Serine | 5.82 |
| Glutamic acid | 18.25 |
| Proline | 7.14 |
| Glycine | 7.94 |
| Alanine | 7.14 |
| Valine ^b | 6.35 |
| Methionine ⁶ | 1.59 |
| Isoleucine ^b | 4.23 |
| Leucine ⁶ | 6.88 |
| Tyrosine | 1.85 |
| Phenylalanine ^b | 6.35 |
| Histidine ^b | 2.12 |
| Lysine ^b | 4.76 |
| Arginine ^b | 6.35 |

[&]quot;Amide content not determined.

Seed protein extract in 0.1M NaCl at pH 8.0 was dialyzed against 0.01M phosphate buffer (pH 7.0) for 48 h at 4° C. Freeze-dried protein was then dissolved in 0.01M phosphate buffer (pH 7.0) containing 0.2M NaCl to obtain a protein concentration of 4 mg/mL. Gel filtration (12) on Sephadex G-200 was carried out on a 2.5×40 cm column and operated at 25° C. The protein mixture was resolved into only five peaks, as shown in Fig. 1. Peak A emerged at the void volume and was not taken into consideration (13). The elution volumes of peaks B–E corresponded to mol wt 95,500 (101,300), 64,500 (69,000), 46,800 (47,300), and 15,100 (15,100). The values within the bracket are the mol wt of the components and were obtained using the equation of Leach and O'Shea (14). The two methods (12,14) used for the determination of the mol wt of each of the four components were in fair agreement with each other.

Extractable jute seed protein in salt solution (0.1*M* NaCl; pH 8.0) was dialyzed against PBS buffer (pH 7.0), and the sample was freeze dried. It was then subjected to SDS-PAGE, recommended by Weber and Osborn (15) for further evaluation of mol wt. The gel system was calibrated using standard proteins (BSA, ovalbumin, pepsin, and lysozyme). The mol wt of the seed protein bands (six in number), determined on the basis of their mobilities, were in the following order: 118,000; 103,000; 96,000, 67,500; 48,500; and 15,000.

^bEssential amino acid.

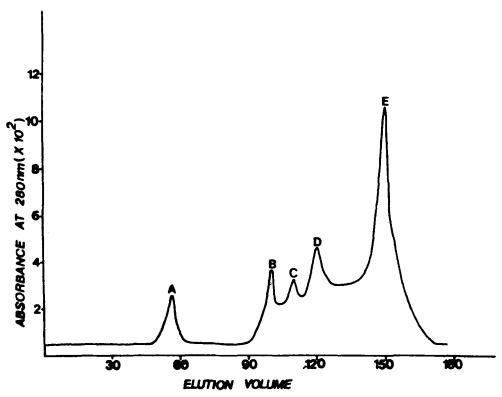


Fig. 1. Gel filtration of jute seed proteins on a Sephadex G-200 column (40 cm long, with 2.5 cm id).

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