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Studies on *Madhuca butyraceae* seed proteins

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Abstract. Defatted *Madhuca butyraceae* seeds contain 24% of crude protein and 10.4% of saponins. The solubility of *Madhuca* seed proteins was determined in water and NaCl as a function of pH and minimum solubility occurred at pH 4.0. The proteins consist of three components with $S_{20,w}$ values of 2.2, 9.8 and 15.4. On gel filtration the proteins gave three peaks and on diethylaminoethyl cellulose chromatography they resolved into two components. The *in vitro* digestibility of *Madhuca* seed protein was found to be 69% when assayed with a pepsin-pancreatin system.

Keywords. *Madhuca* proteins; physicochemical studies; *in vitro* digestibility.

Introduction

Madhuca butyraceae is grown in the Himalayan areas (such as Sikkim and Bhutan) and about 1.2 lakh tonnes of its seeds are estimated to be available annually. The flower of *Madhuca* are used as a source of alcohol The seed contains considerable amount of fat, known as Phulwara butter (Annon, 1952) and is used in the treatment of rheumatism (Kirtikar and Basu, 1935). The defatted meal contains 25% protein (Mitra and Awasthi, 1962) and saponins (10-25%) which are toxic. The present investigation forms a part of an overall study of the chemical characteristics of defatted *Madhuca* seed flour and its possible use as a dietary constituent. The extractability and physico-chemical characteristics of the *Madhuca* seed proteins are presented here.

Materials and methods

The seeds and preparation of meal

Madhuca butyraceae seed kernels were obtained from the National Botanical Research Institute, Lucknow. Lipids were removed by crushing the kernels in a "Hander" crusher. The meal was then ground to 0.2 mm size and extracted at least six times with *n*-hexane to remove the residual fat. The defatted meal was powdered and passed through a 60 mesh sieve before use.

Chemical composition

Moisture, ash, crude fibre, fat and carbohydrates were estimated by standard AOAC methods (1975). Total and non-protein nitrogen were estimated by the Kjeldahl method (Pearson, 1970).

Extractability of proteins

Two g of defatted flour was suspended in 20 ml of the aqueous solvent and the slurry pH was adjusted to the desired value by adding 2 N NaOH or 2N HCl. The suspension was stirred for 1 h at room temperature (~28°C) and centrifuged at 5000 *g* for 20 min. The nitrogen content of the supernatant was estimated by the Kjeidahl method.

Since the extractability of the proteins is low in water and NaCl, a two stage repeated extraction procedure using 1 M NaCl (pH 8) was adopted. The supernatant was first concentrated and dialysed against the buffer solutions for 48 h.

Gel filtration

Sepharose-6B 100 was packed into a 1.5 r 100 cm column. The dialysed sample (3 ml) containing about 50 mg of protein was loaded on the column and the protein eluted with 0.025 M tris-glycine buffer of pH 8.3 containing 1M NaCl. Fractions (3 ml) were collected and the absorbance measured at 280 run.

DEAE-cellulose chromatogrphy

Diethylaminoethyl-(DEAE)-cellulose after regeneration (Peterson, 1970) was packed into a 2×22 cm column under pressure and equilibrated with 0.02 M sodium phosphate buffer of pH 7.6. About 50 mg of the protein was loaded on the column. It was then eluted with a linear gradient of 0.0 — 0.8 M NaCl. Three ml fractions were collected and the absorbance measured at 280 nm. The concentration of NaCl was estimated as described by Rieman *et al* (1951).

Sedimentation velocity experiment

The experiment was performed using 1% protein solution in 0.1 M phosphate buffer of pH 7.8 containing 1 M NaCl at room temperature (~28°C) at 56,100 rpm in a Spinco Model E Analytical Ultracentrifuge equipped with a rotor temperature indicator unit and phase plate schlieren optics. $S_{20,w}$ was calculated by the standard procedure (Schachmann, 1959).

Polyacrylamide gel electrophoresis

Polyacrylamide gels (7.5%) in 0.01 M tris-glycine buffer of pH 8.3 were prepared by the standard procedure. About 100 µg of the protein was loaded on each gel in tubes of 0.5 X 7.5 cm and the electrophoresis was carried out for 1 h at 3 mA/tube. The protein components on the gels were then identified by staining with 0.5% Amido Black for 1 h followed by destaining in 7.5% acetic acid. The gels were then scanned in a Joyce Lobel scanner.

In vitro digestibility

In vitro digestibility was determined by the method of Akeson and Stahmann (1964). The protein was incubated with pepsin for 3 h, followed by pancreatin for a total period of 24 h at 37°C. The reaction was arrested by 10% trichloroacetic acid

and the nitrogen content was determined by the microKjeldahl procedure. The estimations were made at 4 h intervals.

Results and discussion

The chemical composition of the defatted meal is given in table 1. Defatted meal contains 24% protein. A comparable value has been reported by Mitra and Awasthi

Table 1. Chemical composition of defatted *Madhuca* flour (g%).

Moisture	12.0
Total protein (N×6.25)	24.0
Lipids	1.0
Non-protein Nitrogen	0.5
Saponins	10.4
Total carbohydrates	34.0
Ash	6.0
Crude fibre	8.0

(1962). The solubility profile of *Madhuca* seed protein in water shows a "U" shaped pattern (figure 1), indicating only one solubility minimum which is characteristic of most plant proteins (Fontaine *et al.,* 1944; Smith and Circle, 1938).

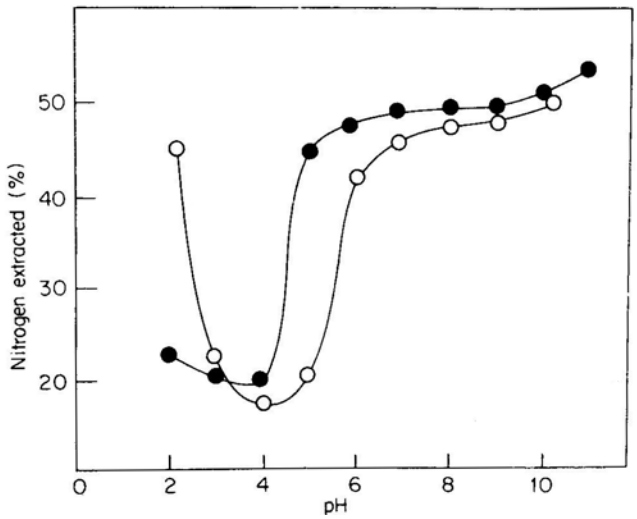

Figure 1. Extractability of *Madhuca* seed as a function of pH. Water O) ; 1 M NaCl (●)

In water, the minimum solubility was found to be at pH 4. At this point, about 16% of the total nitrogen was found to be soluble, whereas at pH 8, about 50% of the nitrogen was solubilized. This appears distinctly different from most plant proteins which show 80-90% solubility at pH 8 (Fontaine *et al.,* 1944, Smith and Circle, 1938). The lower extractibility of *Madhuca* proteins may be due to the presence of saponin-protein complexes. The solubility in 1 M NaCl was slightly higher than in water at pH 8, but the minimum solubility pH was not significantly altered, with only 20% of the nitrogen remaining soluble.

On gel filtration, the proteins were separated into three fractions (figure 2) with V_e/V_o values of 0.97 (I), 2.3 (II) and 3.13 (III) respectively. The first fraction was turbid and eluted near the void volume.

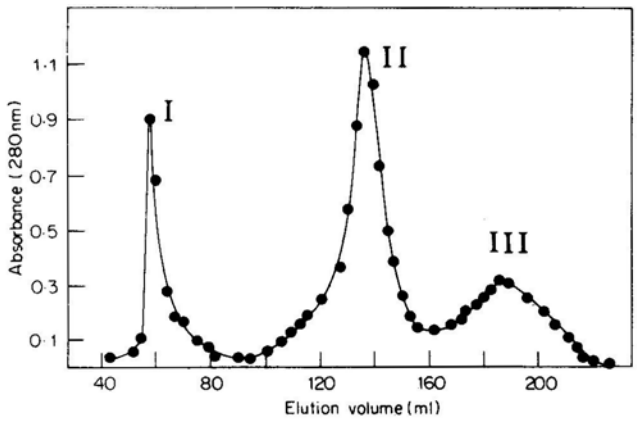

Figure 2. Gel filtration pattern of *Madhuca* seed proteins on Sepharose-6B-l00.

The total proteins were fractionated into two components on DEAE-cellulose chromatography (figure 3) (0.0 — 0.8 M NaCl gradient). One fraction was eluted unadsorbed while the other eluted at 0.29 M NaCl concentration.

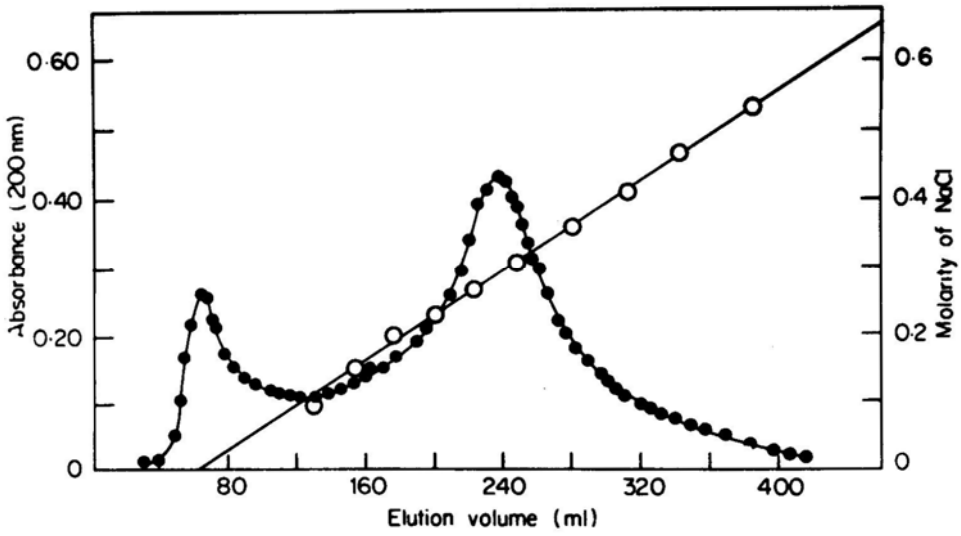

Figure 3. DEAE-cellulose ion-exchange chromatographic pattern of *Madhuca* seed proteins.

The sedimentation velocity pattern of the total proteins showed the presence of three peaks having $S_{20,w}$ values of 2.2, 9.8 and 15.4 (figure 4). The relative proportions of the three fractions were 75.8%, 21.3 % and 2.9% respectively.

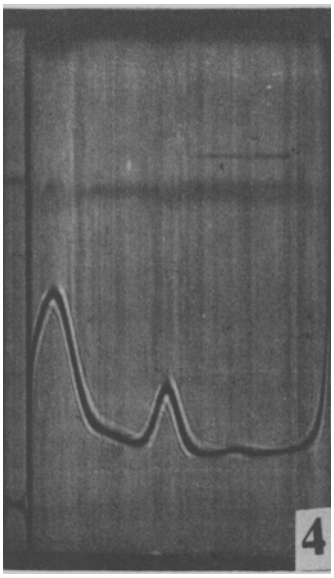

Figure 4. Sedimentation velocity pattern of *Madhuca* seed proteins. Sedimentation proceeds from left to right.

Gel electrophoresis of the total proteins in 0.01 M tris-glycine buffer showed six bands (figure 5) Two of them had higher mobility. The major fraction contributes about 30% of the proteins as read on microdensitometric scanning.

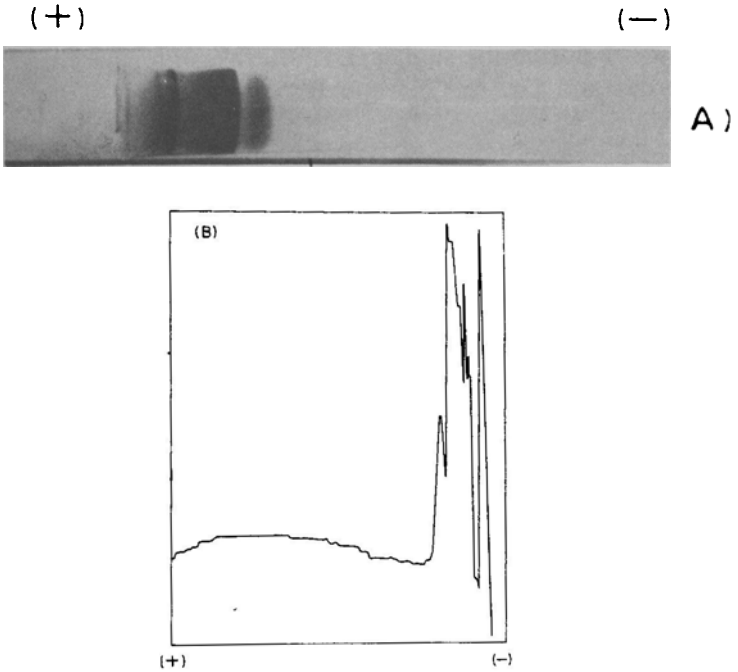

Figure 5. Polyacrylamide gel electrophoretic pattern of *Madhuca* seed proteins A. Gel pattern B. Microdensitometric scanning of the gel.

In the pepsin-pancreatin system, the initial digestibility was ~3 0 % due to non-protein components. The protein hydrolysis reached a maximum level (69%) at 11 h (figure 6). After this, there was no further increase in digestibility.

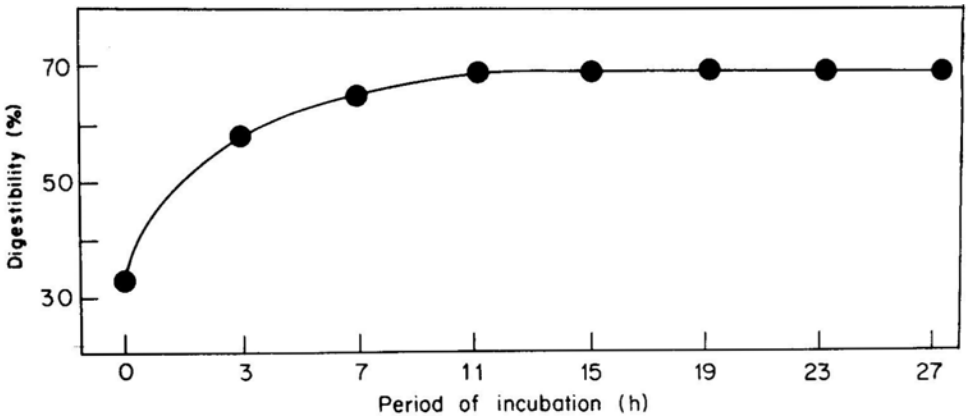

Figure 6. *In vitro* digestibility of *Madhuca* protein.

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References

Akeson, W. R. and Stahmann, M. A. (1964) *J. Nutr.,* **83,** 257.

AOAC Methods of Analysis (1975) *Association of Official Analytical Chemists,* 12th edition, Washington DC 20014.

Anonymous (1952) *The Wealth of India,* Vol. III, Council of Scientific and Industrial Research, New Delhi, India.

Fontaine, T. D., Samuels, C. and Irving, G. W. Jr. (1944) *Ind. Eng, Chem.,* **36,** 625.

Kirtikar, K. R. and Basu, B. D. (1935) *Indian Medicinal Plants,* Second edition (Published by Lalit Mohan Basu, Allahabad, India) Vol. II, p. 1492.

Mitra, C. R. and Awasthi, Y. C. (1962) *J. Sci. Ind. Res.,* **21,** 102.

Pearson, D. (1970) *The Chemical Analysis of Food,* 6th edition, (London: Churchill).

Peterson, E. A. (1970) in *Laboratory Techniques in Biochemistry and Molecular Biology,* eds. T. S. Work and E. Work (Amsterdam: North Holland Publishing Co.) Vol. 2, p. 223.

Rieman, W., Newses, J. D. and Naiman, B. (1951) *Quantitative Analysis* (New York: McGraw-Hill), p. 70.

Schachmann, H. K. (1959) *Ultracentrifugation in Biochemistry,* (New York: Academic Press) p. 92.

Smith, A. K. and Circle S. J. (1938) *Ind. Eng. Chem.,* **30,** 1414.