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Chemical Evaluation of Mahua (*Madhuca indica*) Seed

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ABSTRACT

Seeds of mahua fruits contain 16·9 and 51·5% protein and oil, respectively. Fatty acid composition of oil revealed the presence of oleic acid (46·3%) and linoleic acid (17·9%) as the major unsaturated and palmitic acid (17·8%) and stearic acid (14·0%) as major saturated fatty acids. The defatted mahua seed meal contains 29·4 % protein and 9·8% saponins which are toxic at this level. However, the levels of saponins could be reduced by treatment with isopropanol. The defatted flour showed good oil absorption and emulsification properties. The solubility of protein was high at both acidic and alkaline pH with a minimum at 4·0. The in-vitro digestibility of mahua seed flour after treatment with isopropanol was found to be 81%. Polyacrylamide gel electrophoresis showed five bands with different relative mobilities and they contained both high and low molecular weight protein fractions. Detoxified mahua seed flour appears to be a good source of protein for food and feed products.

INTRODUCTION

Mahua (*Madhuca indica*) is a forest tree found in central and northern India and Malaysia. Mahua is valued for its oil-bearing seeds and flowers which are utilized for alcoholic beverage production. Spent flowers (after fermentation) are also used as animal feed. Annual availability of seeds in the country is about 0·12 million tonnes which are being collected in organized sectors and utilized for oil extraction.

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The seed contains a considerable amount of fat (50–60%) which is used in preparation of bakery fat and vanaspati after suitable modifications, and also in the treatment of rheumatism (Kirtikar & Basu, 1935; Anon., 1952). The defatted meal is used for livestock feeding and also as manure due to its nitrogen content. The defatted meal, though it contains a good amount of protein, is not edible due to the presence of saponins which are toxic (Mulky & Gandhi, 1977). The present communication deals with the chemical characterization of mahua seed and its possible use as a dietary constituent.

MATERIALS AND METHODS

Materials

The mahua fruits were obtained from the local market of Faizabad city during the summer season (2nd week of July) of 1988.

Preparation of defatted seed meal and isopropanol treatment

Moisture-free seeds of mahua were crushed (in a crusher) to remove oil. The remaining cake was then extracted with petroleum ether (b.p. 40–60°C) to remove the residual fat. The defatted meal was agitated on a rotary shaker (120 rpm) with the given concentration of isopropanol (10 ml per g meal) for 1 h. The contents were filtered and the residue was extracted similarly for another 1 h. The contents were filtered and the residue was dried at 60°C. The defatted meal was powdered and passed through a 60 mesh sieve.

Analytical methods

Protein, oil, crude fibre, ash and tannin contents were determined by AOAC (1980) methods. Total carbohydrates were calculated by difference. Saponins were estimated gravimetrically by the method of Wall *et al.* (1952).

Physico-chemical properties of oil

The extracted oil was immediately analysed for saponification value, iodine number, refractive index, specific gravity, peroxide value and usaponifiable matter by the conventional methods recommended by AOCS (1973).

Fatty acid analysis

The methyl esters were prepared using methanol–sulphuric acid by the method of Johnson and Stocks (1971) and identified by GLC (Chemito 3800).

Functional properties of defatted mahua seed meal

Water and oil absorption capacities were determined following the methods of Sosulski (1962) and Sosulski *et al.* (1976), respectively. Methods of Huffman *et al.* (1975) were followed for the measurement of emulsifying capacity and foaming properties. Refined corn oil was used for oil absorption and emulsifying capacity studies. The measurements were made at room temperature ($25 \pm 2°C$). Foaming capacity is expressed as increase in the foam volume after 30 s and foam stability is expressed as the foam volume determined from 15–60 min standing at room temperature.

Protein extractability

Protein extractability was determined by suspending 2 g of defatted meal in 20 ml distilled water followed by adjusting the pH (2–10) by 2N HCl or 2N NaOH. The suspension was stirred for 1 h and then centrifuged (5000 rpm) for 20 min at 4°C. The nitrogen content of the supernatant was estimated by the Kjeldahl method (AOAC, 1980).

In-vitro protein digestibility

The in-vitro protein digestibility was determined using a pepsin–pancreatin system following the procedure of Akeson and Stahman (1964). The defatted meal was incubated with pepsin for 4 h followed by pancreatin for 24 h at 37°C. The proteins were precipitated by 10% trichloroacetic acid at different time intervals and nitrogen in the supernatant was determined.

Polyacrylamide gel electrophoresis (PAGE)

PAGE was performed by the method of Davis (1964) on 7% acrylamide gel using Tris–glycine (pH 8·3) as electrophoretic buffer. Protein bands were visualized by Coomassie Brilliant Blue dye.

RESULTS AND DISCUSSION

The proximate chemical composition of whole mahua seed and defatted mahua seed flour is given in Table 1. Oil represents the major component, i.e. thrice the amount of protein. Most stone fruit kernels have been reported to contain oil and protein as major components (Cruess, 1958). Removal of fats from seed resulted in a considerable increase in protein content (29·4%). The increase in other constituents after removal of fats was also expected.

TABLE 1
Proximate Composition (%) of Mahua Seed and Defatted Seed Flour

Constituent	*Whole seed*	*Defatted flour*
Protein (N × 6·25)	16·9	29·4
Oil	51·5	1·1
Fibre	3·2	8·6
Carbohydrates	22·0	42·8
Ash	3·4	6·0
Saponins	2·5	9·8
Tannins	0·5	1·0

Average of three determinations.

Defatting of mahua seed also increased the saponin and tannin contents. Saponins, pentacyclic triterpenoids existing as glycosides, were found at the level of 2·5% in whole seed and 9·8% in defatted seed flour. Many authors have reported the toxic effects of saponins (Birk, 1969; Pederson *et al.*, 1972). Rats fed with *Madhuca latifolia* meal containing 5–6% saponin at the level of 10–12% died in a month (Mulky, 1976). Thus, detoxification of mahua seed flour is necessary before utilization in food or feed products.

TABLE 2
Properties of Mahua Seed Oil

Property	*Value*
Physico-chemical	
Refractive index	1·479 5
Specific gravity	0·915 0
Saponification value	196·0
Iodine value	80·2
Peroxide value (meq/kg)	0·24
Unsaponifiable matter (%)	1·0
Fatty acids composition (%)	
Palmitic	17·8
Stearic	14·0
Oleic	46·3
Linoleic	17·9
Linolenic	1·7
Arachidic	0·9
Total unsaturated	65·9
Total saturated	32·7

Average of three determinations.

Oil characteristics

The fresh mahua seed oil was yellow in colour with an acceptable taste and odour (Table 2). On the basis of iodine value (80·2), mahua seed oil could be classified as a non-drying oil. The peroxide value is a good index for the stability of the oil and its susceptibility to rancidity during storage. The peroxide value (0·24 meq/kg) of mahua seed oil indicates that mahua seed may have low levels of oxidative and lipolytic activities or contains high levels of natural antioxidants.

Fatty acid composition

The fatty acid profile of mahua seed oil is also illustrated in Table 2. Palmitic acid (16:0) was the major saturated fatty acid. The total unsaturated fatty acid content was 65·9% with oleic (18:1) and linoleic as major unsaturated fatty acids. This might focus interest on the use of mahua seed oil, since unsaturated vegetable oils have an ability to reduce serum cholesterol level.

Functional properties

The functional properties of defatted mahua seed flour are presented in Table 3. Oil absorption capacity was found to be about twice water

TABLE 3
Functional Properties of Defatted Mahua Seed Flour

Property	
Water absorption (g/g flour)	2·3
Oil absorption (ml/g flour)	4·5
Emulsification capacity (ml/g)	55·4
Foam capacity (ml)	34·5
Foam stability (ml) at:	
15 min	31·4
30 min	28·6
45 min	27·0
60 min	20·4
Protein extractability (%) at:	
pH 2	45
pH 4	18
pH 6	32
pH 8	59
pH 10	71

Average of three determinations.

TABLE 4
Effect of Isopropanol Treatment on Protein, Saponin and Tannin Contents of Defatted Mahua Seed Flour

Isopropanol (%)	*Protein* (%)	*Saponin* (%)	*Tannin* (%)
Control	29·4	9·8	1·0
20	30·9	6·1	0·7
40	35·4	3·5	0·3
60	36·5	1·0	0·2
80	37·2	1·0	0·1

Average of three determinations.

absorption capacity. This shows the suitability of mahua seed flour in some bakery products which require flour with good absorption capacity. The emulsification capacity of flour was also high, which may be advantageous in products such as sausage and other meat products. The foam capacity of the flour was 34·5 ml. After standing for 1 h at room temperature, foam was decreased about 40%.

The solubility of the proteins at extreme pH values was high (45 and 71% at pH 2·0 and 10·0, respectively). It showed only one solubility minimum (18% at pH 4·0) which is characteristic of most plant proteins. The lower extractability of mahua seed proteins may be due to the presence of a saponin–protein complex.

Table 4 presents the effect of isopropanol treatment on protein, saponin and tannin contents of mahua seed flour. An increase in protein content was observed due to isopropanol treatment which was probably due to the leaching out of soluble carbohydrates and other substances like saponins and tannins. About 90% removal of saponins was achieved by treating the flour with 60–80% isopropanol. Isopropanol-treated mahua flour contains

TABLE 5
In-vitro Digestibility of Mahua Seed Protein

Incubation period (*h*)	*Digestibility* (%)	
	Defatted flour	*Isopropanol-treated flour*
6	46	46
12	64	75
18	72	80
24	72	81

Average of three determinations.

1% saponin which may not be toxic as the LD_{50} of saponin for mice has been reported to be 1 g/kg body weight (Mulky & Gandhi, 1977). However, the gravimetric method was employed for the determination of saponins in the present investigation. Hence, the estimate of the amount of saponins is semiquantitative if non-steroidal materials are present in significant amount (Wall *et al.*, 1952).

The in-vitro digestibility of mahua seed protein using a pepsin–pancreatin system is shown in Table 5. The digestibility increased with the increase in treatment period till 18 h and then remained constant. The observed low digestibility of mahua seed protein was possibly due to the presence of a high level of saponins and tannins. However, the treatment of defatted meal with isopropanol (60%) considerably improved the in-vitro protein digestibility (81%).

The polyacrylamide gel electrophoresis (PAGE) of mahua seed protein in Tris–glycine buffer (pH 8·3) showed five bands wherein three bands were major with higher relative mobilities. The PAGE pattern reveals that mahua seed protein contains both low and high molecular weight proteins. No significant difference was observed between the PAGE pattern of defatted flour and isopropanol-treated flour.

Besides the use of mahua flowers in making alcoholic beverages, its oil is used for edible purposes. Mahua fat is also a rich source of palmitic acid, which is used in cosmetic and pharmaceutical industries. The saponins obtained after extraction may have industrial and commercial applications such as in pharmaceutical and soap industries (Mishra & Nigam, 1979). Based on the foregoing results it appears that mahua seed meal, after detoxification with isopropanol, has a potential for use as a food and feed component.

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