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Chemical composition and functional properties of raw, heat-treated and partially proteolysed wild mango (*Irvingia gabonensis*) seed flour

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The chemical composition and functional properties of raw, and heat-processed wild mango (*Irvingia gabonensis*) seed flour were studied. The effect of partial proteolysis on some functional properties of the raw flour was also determined. There was not much variation between the values obtained for the proximate chemical composition and mineral contents of the raw and heat-treated samples, although significant decreases in ascorbic acid, carotenoids and polyphenols were noted. Nitrogen solubility was pH-dependent with a minimum value of 14% at pH 4·0 and a maximum of 66% at pH 10·0 for the raw flour. Heat-treatment diminished nitrogen solubility, bulk density, emulsion and foam capacities but increased the water and fat absorption capacities and gelation properties of the flour. Partial proteolysis increased nitrogen solubility and bulk density but reduced water and fat absorption capacities of the flour. The foam of the raw flour was more stable than those of the heat-treated or enzyme-hydrolysed samples.

Calculated on crude protein basis, raw wild mango flour showed comparatively better water and fat absorption properties than raw soya flour. Hence it may prove to have useful applications in fabricated foods such as bakery products and ground meat formulations.

INTRODUCTION

Wild mango (*Irvingia gabonensis* Aubry Lecomte ex O'Rork-Baill) belongs to the family Simarubaceae and is botanically unrelated to cultivated mango (*Mangiffera indica* L.) a member of the Anacardiaceae (Okafor, 1981). Wild mango grows naturally in the forest habitat of parts of Africa, extending from Senegal to the Sudan and South to Angola (Keay *et al.*, 1964). The seed has about 10·6% protein and 54–67% fat and hence it ranks as an oilseed (Oke and Umoh, 1978). Oilseed proteins are gaining increased acceptance as food ingredients world-wide. Soybean has been a primary source of plant protein for use as a functional ingredient in food systems. Studies on the functional potentials of oilseeds such as cottonseed, sunflower, groundnut and fluted pumpkin flours have been made (Lawhon and Cater, 1971; Lin *et al.*, 1974; McWatters and Cherry, 1977; Giami and Bekebain, 1992).

Wild mango seed is used widely in Nigeria as a flavouring ingredient in soups and to impart desired consistency, because of its viscous properties. Although there are limited reports on the chemical composition and nutritive value of wild mango seed (Abaelu and Akinrimisi, 1980; Eka, 1980), information on its functional properties is lacking. Essential in determining potential uses for wild mango seed flour is the identification and improvement of its functional properties. Partial proteolysis of proteins by enzymes and heat-treatment have been shown to alter certain functional behaviour of oilseed and legume flours. Proteolysis has been reported to increase the nitrogen solubility and foaming capacity of peanut flour (Beuchat *et al.*, 1975), cottonseed flour (Arzu *et al.*, 1972; Rahma and Narasinga Rao, 1983) and winged bean flour (Narayana and Narasinga Rao, 1984). Improvement in water absorption capacity of oilseed and legume flours as a result of heat treatments has also been reported (Lin *et al.*, 1974; Narayana and Narasinga Rao, 1982; Giami, 1993). This study was carried out to examine the chemical composition and functional properties of raw and heat-processed wild mango seed flour. The effect of partial proteolysis on some functional properties of the raw flour was also investigated. The potential food uses for the flour were evaluated by laboratory tests for protein solubility, water and fat absorption, bulk density, emulsion and foaming properties, and the results obtained compared with those of raw soyflour.

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MATERIALS AND METHODS

Materials

Wild mango (*Irvingia gabonensis*) seeds were bought from a local market in Port Harcourt, Nigeria, and stored at 5°C throughout the experimental period.

Heat-treatment

The samples to be heat-treated were first ground in the raw form, then autoclaved (121°C, 1·05 kg cm^{-2}, 15 min). The autoclaved samples were dried in a vacuum oven (60°C, 24 h) and then milled to pass through a 0·5 mm sieve.

Raw flours

Raw wild mango flour was made from ground seeds which were dried in a vacuum oven (60°C, 24 h) and then milled to pass through a 0·5 mm sieve. Raw and heat-treated samples to be used for studies on functional properties were defatted by solvent extraction using *n*-hexane. Soybean (*Glycine max* L.) obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria was processed in a similar manner to obtain defatted raw flour. All samples were stored in capped bottles at 4°C until required for use.

Proteolysis of flour

Proteolysis was carried out with pepsin powder supplied by Riedel-De Haen AG Seelze—Hannover (activity of 10 000 units g^{-1} enzyme; activity is defined as the quantity of enzyme required to produce amino acid equivalent to 100 g tyrosine in 1 ml filtrate when 1 ml 1·5% milk casein solution is incubated with 1 ml enzyme solution for 60 min at 37°C).

The pH of a 25% aqueous dispersion of raw defatted wild mango flour was adjusted to pH 7·0 with 2 M NaOH. Enzyme, supplied as a powder, was added at different levels ranging from 5 to 25% (w/w, based on flour protein) and the mixture incubated at 45°C for 3 h, with occasional shaking. At the end of 3 h the mixture was heated to 75°C and held at that temperature for 10 min to inactivate the enzyme. Then it was dried in a vacuum oven (60°C, 24 h) and milled to pass through a 0·5 mm sieve. The protein dispersion, which was kept at 45°C for 3 h without added enzyme, and subsequently dried in a vacuum oven (60°C, 24 h), served as the control.

The degree of hydrolysis was determined by incubating 5 ml aliquots of enzyme-treated flour with an equal volume of 10% trichloroacetic acid (TCA) for 15 min at room temperature (28°C). The suspension was centrifuged for 20 min at 5000 *g* and nitrogen in the supernatant was estimated by the Kjeldahl method.

The flour, suspension to which 10% TCA solution was added, followed by the enzyme served, as control. Hydrolysis was expressed as the percentage of the flour nitrogen rendered TCA-soluble by the enzyme.

Chemical analysis

Proximate chemical composition and ascorbic acid by the colorimetric method were determined using AOAC (1984) methods, except that 1% oxalic acid was substituted for 3% metaphosphoric acid in the ascorbic acid determination. The calorific value was obtained by standard calculation (Osborne and Voogt, 1978). Total polyphenols were determined using the vanillin–H_2SO_4 assay as described by Wilson and Blunden (1983). The results were expressed as g phloroglucinol equivalents 100 g^{-1} dry weight of flour. Total carotenoids were determined by the procedure outlined by Thomas and Janave (1975) with reference to a standard graph based on beta-carotene. All reagents used were of analytical grade.

Functional properties

Nitrogen solubility

Nitrogen solubility index was determined in the pH range 2–12 for the raw and heat-treated samples and at pH 7·0 for the partially proteolysed samples at room temperature (28°C) using the method described by Narayana and Narasinga Rao (1982). The flour sample (1 g) was dispersed in 60 ml distilled water and the dispersion was shaken for 2 h at room temperature. It was then centrifuged at 5000 *g* for 20 min and nitrogen in the supernatant was estimated at the Kjeldahl method. The nitrogen extracted was expressed as a percentage of the flour nitrogen.

Water and fat absorption

These were determined as described by Beuchat (1977) with slight modifications. The flour sample (1 g) was mixed with 10 ml distilled water or refined groundnut oil (density = 0·86 g ml^{-1}) in a weighed 20-ml centrifuge tube. The slurry was agitated on a vortex mixer for 2 min, allowed to stand at 28°C for 30 min and then centrifuged at 5000 g for 20 min.

The clear supernatant was decanted and discarded. The adhering drops of water or oil were removed and the tube was weighed. The weight of water or oil absorbed by 1 g of flour or protein was calculated and expressed as water or fat absorption capacity.

Bulk density

Bulk density was determined according to the method described by Narayana and Narasinga Rao (1984). A calibrated centrifuge tube was weighed and samples were filled to 5 ml by constant tapping until there was no further change in volume. The contents were weighed and from the difference in weight the bulk density of the sample was calculated.

Foam capacity and stability

This was determined as described by Coffman and Garcia (1977) with minor modifications. A 2-g sample was whipped with 100 ml distilled water for 5 min in a Kenwood blender (Model A 907 D) at speed of 5000 rev min^{-1} poured into a 250 ml graduated

cylinder. The volume of foam at 30 s after whipping was expressed as the foam capacity and the volume of the foam over 10–120 min as the foam stability for the respective time periods. The volume increase (%) was calculated according to the following equation:

$$\text{Volume increase} = \frac{\text{volume after whipping (ml)} - \text{volume before whipping (ml)}}{\text{volume before whipping (ml)}} \times 100$$

The effect of concentration on foaming was evaluated by whipping 2–10% (w/v) slurries as described above. Foam capacity measurements were also done on 2% (w/v) slurries using NaCl concentrations of 0·2–1·2% (w/v). The effect of pH on foaming properties was also studied using 2% (w/v) slurries. The pH was adjusted to a desired volume with either N HCl or N NaOH prior to whipping. All experiments were conducted at room temperature (28°C).

Emulsion capacity and stability

Emulsions were prepared according to the method of Beuchat (1977) with small variations. The sample (2 g) was blended in a Kenwood blender with 100 ml of distilled water for 30 s at a speed of 5000 rev min^{-1}. After complete dispersion, vegetable oil was added continuously in 5 ml portions from a burette. Blending continued until the emulsion breakpoint, separation into two layers was reached. The amount of oil added up to this point was interpreted as the emulsion capacity of the sample. The emulsion capacity was determined at 28°C and the values expressed in grams of oil emulsified by 1 g flour. The stability of the emulsion was evaluated for up to 20 h at 28°C by noting the separation of water in graduated cylinders.

Gelation

The method of Coffman and Garcia (1977) was employed with small modifications. Sample suspensions of 2–20% (w/w) were prepared in 5 ml distilled water. The test tubes containing these suspensions were then heated for 1 h in a boiling water bath followed by rapid cooling under cold running tap water. The test tubes were then further cooled for 2 h at 4°C. The least gelation concentration (LGC) was determined as that concentration when the sample from the inverted test tube did not fall or slip.

Table 1. Chemical composition of raw and heat-treated wild mango flour[a]

Components	Sample	
	Raw	Heat-treated
Moisture (%)	4·1 ± 0·2	5·2 ± 0·3
Crude protein (%), N × 6·25	10·9 ± 0·1	8·3 ± 0·4
Ether extract (%)	64·2 ± 0·6	63·1 ± 0·5
Total ash (%)	2·2 ± 0·3	2·7 ± 0·2
Crude fibre (%)	3·4 ± 0·1	3·6 ± 0·3
Carbohydrate (%), by difference	15·2 ± 0·6	17·1 ± 0·4
Calorific value (kcal per 100 g)	682	670
Total carotenoids (mg per 100 g)	3·6 ± 0·2	2·4 ± 0·1
Total polyphenols (mg per 100 g)	2·6 ± 0·5	0·2 ± 0·1
Ascorbic acid (mg per 100 g)	6·2 ± 0·4	2·2 ± 0·3
Total phosphorus (mg per 100 g)	195 ± 2·1	185 ± 1·6
Iron (mg per 100 g)	3·8 ± 0·3	3·5 ± 0·2
Calcium (mg per 100 g)	127 ± 1·0	120 ± 0·9

[a] Mean ± standard deviation of the mean for three determinations; values except moisture expressed on dry weight basis.

Statistical analysis

All the experiments were conducted in triplicate and the means ± standard deviation of three values are reported. Data were subjected to analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960) to determine the significance of differences between the means.

RESULTS AND DISCUSSION

Chemical composition

The results of the chemical composition of raw and heat-treated wild mango flour are presented in Table 1. The fat content of the raw flour (64·2%) falls within the range of values (54–67%) earlier reported by Oke and Umoh (1978), but lower than the value (70·8%) reported by Eka (1980). The protein content of the raw flour (10·9%) is low compared to the values reported for other Nigerian oilseeds (Achinewhu, 1982; Giami and Bekebain, 1992). This suggests that wild mango seed may not be very useful as a protein supplement in diets. There was not much variation between the values obtained for the proximate chemical composition and mineral contents of the raw and heat-processed flours.

Table 2. Water and fat absorption of raw and heat-treated wild mango seed flour[a]

Sample	Crude Protein[b] (%)	Water absorption capacity (g g^{-1})		Fat absorption capacity (g g^{-1})	
		Flour	Protein[c]	Flour	Protein[c]
Raw wild mango flour	12·6 ± 0·3	3·6 ± 2[b]	28·6 ± 0·8[b]	1·5 ± 0·1[b]	11·9 ± 0·6[b]
Heat-treated wild mango flour	12·0 ± 0·2	4·2 ± 0·1[a]	35·0 ± 0·7[a]	2·4 ± 0·2[a]	20·0 ± 0·9[a]
Raw soya flour	45·4 ± 0·5	3·0 ± 0·3[a]	6·6 ± 0·4[c]	1·2 ± 0·2[b]	2·7 ± 0·1[c]

[a] Mean ± standard deviation of the mean for 3 determinations.
[b] Expressed on dry weight basis, defatted flour.
[c] Expressed on crude protein basis.
a,b,c: Means with the same roman superscript within the same column do not differ ($p < 0·05$).

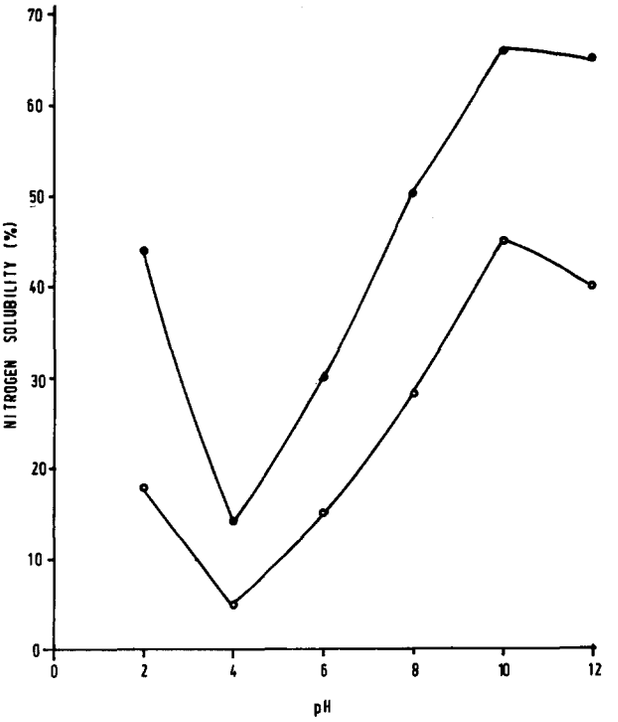

Fig. 1. Effect of pH on nitrogen solubility of raw and heat-treated wild mango seed flour (●, raw flour; ○, heat-treated flour).

However, on a dry weight basis, heat-processing resulted in a decrease in ascorbic acid (64·5%), carotenoids (33·3%) and polyphenols (92·3%). Losses in polyphenols varying between 78·7 and 95·8% as a result of heat-treatment have been reported for Pinto and Kidney beans (Iyer *et al.*, 1980).

Nitrogen solubility

The nitrogen solubility profiles of the raw and heat-treated flours are shown in Fig. 1. Nitrogen solubility was observed to be pH-dependent. Compared with soya flour with a minimum nitrogen solubility of 10% at pH 4·5 (Smith & Circle, 1972), wild mango seed flour had a minimum nitrogen solubility of 14% at pH 4·0. Maximum nitrogen solubilities of 66·0% and 45·0% were recorded for raw and heat-treated flour samples, respectively. Heat-treatment resulted in a 31·8% reduction in nitrogen solubility at pH 10·0, compared to the raw flour. A reduction in nitrogen solubility due to heat-treatment has been reported for winged bean, peanut and cowpea flours (Narayana and Narasinga Rao, 1982; Singh and Singh, 1991; Giami, 1993).

Water and fat absorption

The heat-treated flour was significantly higher in water absorption capacity ($p < 0.05$) with differences between samples (Table 2). The water absorption capacity of raw wild mango flour was 3·6 g^{-1} flour. Since wild mango and soya flour had different protein contents, water and fat absorption capacities were also calculated on a crude protein basis. The values obtained showed that raw wild mango flour had a higher water absorption capacity (28·6 g g^{-1} protein) than raw soya flour (6·6 g g^{-1} protein). Heat-treatment improved the water absorption capacity of wild mango flour. Similar effects of heat-treatment on absorption of oilseed flours such as sunflower and peanut flour have been reported (Lin *et al.*, 1974; Singh and Singh, 1991).

The fat absorption capacity of 1·5 g g^{-1} flour obtained for the raw wild mango flour was similar to the value of 1·2 g g^{-1} flour obtained for raw soya flour and both values were significantly lower ($p < 0.05$) than the value of 2·4 g g^{-1} flour obtained for heat-treated wild mango flour (see Table 2).

Bulk density, emulsion, gelation and foaming properties

The emulsion capacity and stability, bulk density, least gelation concentration, foam capacity and stability of raw and heat-treated wild mango flour are presented in Table 3. Heat-treatment reduced the emulsion capacity of the flour by 16·7%. Singh and Singh (1991) reported that the emulsion capacity of peanut flour was reduced by 26·5% as a result of boiling. An emulsion prepared from raw wild mango flour was more stable than that prepared from heat-treated flour. The bulk density was reduced by heat-treatment from 0·08 to 0·05 g ml^{-1}. The least gelation concentrations for the raw and heat-treated flour samples were found to be 6·0% and 8·0% (w/v) respectively (see Table 3). The smallest gelation concentrations reported for both mung bean

Table 3. Emulsion properties, gelation, bulk density, foam capacity and stability of raw and heat-treated wild mango seed flour[a]

Sample	Emulsion capacity (g g^{-1})	Emulsion				Least gelation concentration (% w/v)	Bulk density (g ml^{-1})	Foam capacity[c] (ml)	Foam volume (ml) after		
		0 h	1 h	3 h	20 h				30 min	60 min	120 min
Raw flour	25·8 ± 1·5	0·0	45·0 ± 0·5	55·5 ± 0·8	62·0 ± 1·0	6·0	0·08 ± 0·02	120 ± 1·0	119 ± 1·3	116 ± 1·0	109 ± 1·5
Heat-treated flour	21·5 ± 12·0	0·0	52·0 ± 1·2	68·5 ± 1·3	70·5 ± 0·8	8·0	0·05 ± 0·03	110 ± 0·5	108·5 ± 1·3	105 ± 1·0	103 ± 1·0
LSD[d] (p = 0·05)	3·8	0·0	6·5	10·5	7·5	2·2	0·02	9·5	6·0	10·0	4·2

[a] Mean ± standard deviation of the mean for three determinations.
[b] Volume (ml) of water separated at 28°C.
[c] Determined at pH = 7·0.
[d] LSD: differences of two means between samples exceeding this value are significant.

Table 4. Effect of concentration on foaming capacity of wild mango seed flour[a]

Concentration % (w/v)	Raw flour		Heat-treated flour	
	Final foam volume (ml)	Volume increase (%)	Final foam volume (ml)	Volume increase (%)
2	120 ± 0·5	20[c]	110 ± 0·5	10[c]
4	126 ± 1·0	26[b]	113 ± 0·5	13[c]
6	126 ± 1·0	28[b]	118 ± 1·5	18[b]
8	134 ± 1·5	34[a]	120 ± 1·0	20[b]
10	138 ± 1·0	36[a]	128 ± 1·5	28[a]

[a] Mean ± standard deviation of the mean for three determinations.
a,b,c,: Means with the same superscripts within the same column do not differ ($p < 0·05$).

and peanut flours were 10·0% (del Rosario and Flores, 1981; Singh and Singh, 1991) and for cowpea flour was 16·0% (Abbey and Ibeh, 1983). This study showed that wild mango flour required a lower concentration for gel formation than most oilseed and legume flours and may find useful applications in food systems such as sausage emulsions, custard type puddings and sauces, which require thickening and gelling. Protein concentration, especially globulin fraction, and interactions between proteins, carbohydrates and lipids have been reported to be responsible for the gelation capacity of legume and oilseed protein (Fleming *et al.*, 1975).

The foam of the raw flour was more stable than that of the heat-treated sample. It has been suggested that foam stability is related to the amount of native protein (Lin *et al.*, 1974). Native protein has been shown to give higher foam stability than denaturated protein (Yatsumatsu *et al.*, 1972). The foamability of flour was observed to be concentration-dependent (Table 4). Both the raw and heat-treated samples showed increasing foamability with increase in concentration of the flour. Addition of salt (NaCl) up to 0·8% (w/v) for the raw flour and up to 0·6% (w/v) for the heat-treated flour increased the foam capacity; greater concentrations of NaCl reduced it considerably (Fig. 2(a)). For both raw and heat-processed flour, the foam capacity at 1·2% (w/v) NaCl were higher than in water. The beneficial effect of low concentrations of NaCl on the foam capacity of raw winged bean flour and soya flour has been reported (Narayana and Narasinga Rao, 1982). The foam capacity against pH profile of raw wild mango seed flour closely resembled in shape its nitrogen solubility against pH profile (Fig. 2(b)), suggesting that the foaming property was also dependent on the solubilised protein. Compared to heat-treated flour, raw flour had a higher foam capacity at all pHs studied.

Effect of proteolysis

The effects of partial proteolysis on the nitrogen solubility, bulk density, foam capacity, water and oil absorption capacities of raw wild mango flour are presented in Table 5. The nitrogen solubility of the enzyme-treated flour increased with the degree of hydrolysis. A similar effect of partial proteolysis on nitrogen solubility has been reported for peanut flour (Beuchat *et al.*, 1975) and cottonseed flour (Rahma and Narasinga Rao, 1983).

For beverage applications where high protein solubility is desired, partially proteolysed wild mango seed proteins seem to have an advantage over unproteolysed proteins. The bulk density of wild mango flour increased with the extent of hydrolysis. The unproteolysed flour had a value of 0·08 g ml^{-1} and 48% hydrolysed flour of 0·31 g ml^{-1}, showing an increase of 288%. The foam capacity of the flour increased with the extent of hydrolysis up to 36% and then decreased. However, the foam stability (foam volume at 120 min)

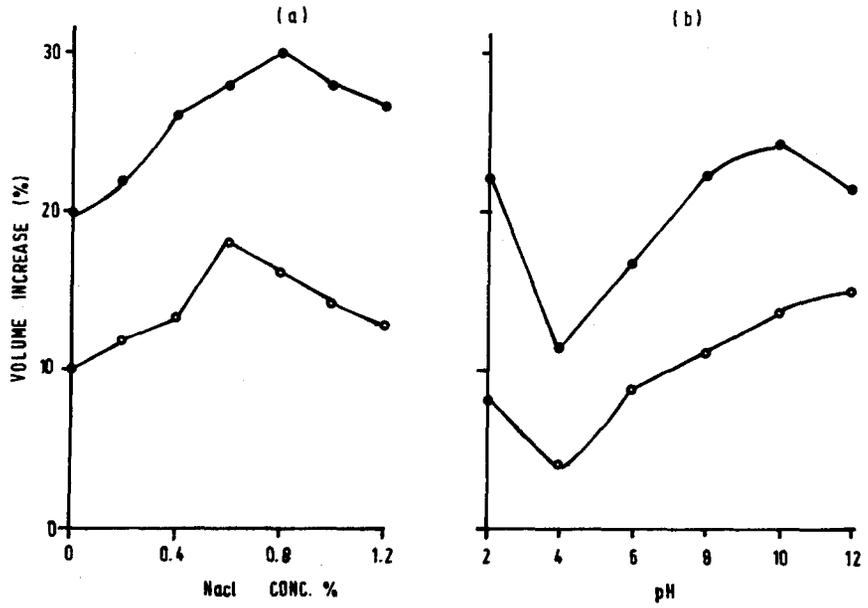

Fig. 2. Foam volume increase of raw and heat-treated wild mango seed flour as influenced by (a) NaCl concentration; (b) pH. (●, raw flour; ○, heat-treated flour).

Table 5. Effect of partial proteolysis on some functional properties of wild mango seed flour[a]

Enzyme concentration[b] (%)	Degree of hydrolysis (%)	Nitrogen solubility[c] (%)	Bulk density (g ml^{-1})	Water absorption capacity (g g^{-1})	Fat absorption capacity (g g^{-1})	Foam volume[c] (ml)	
						30 s	120 min
0 (unproteolyzed)	0	38·2 ± 0·4	0·08 ± 0·02	3·6 ± 0·2	1·5 ± 0·4	120 ± 1·0	109 ± 1·5
5	13	42·4 ± 0·8	0·14 ± 0·04	3·2 ± 0·2	1·3 ± 0·2	128 ± 1·5	105 ± 1·0
10	24	48·6 ± 1·2	0·20 ± 0·03	2·8 ± 0·1	1·1 ± 0·1	135 ± 2·0	102 ± 0·5
15	36	54·7 ± 1·0	0·24 ± 0·02	2·1 ± 0·2	0·9 ± 0·1	160 ± 2·0	100 ± 0·5
20	42	61·6 ± 1·3	0·27 ± 0·04	1·7 ± 0·3	0·7 ± 0·2	125 ± 1·5	100 ± 0·5
25	48	67·5 ± 1·0	0·31 ± 0·03	31·2 ± 0·1	0·5 ± 0·1	110 ± 1·0	100 ± 0·5

[a] Mean ± standard deviation of the mean for three determinations.
[b] Percentages indicate amounts of enzyme (w/w, based on flour protein).
[c] Determined at pH = 7·0.

decreased continuously with the extent of hydrolysis. The water absorption capacity of partially proteolysed wild mango flour decreased with an increase in the extent of hydrolysis (see Table 5). A similar effect in fat absorption capacity was observed. Beuchat *et al.* (1975) have reported that water and oil absorption capacities of partially proteolysed peanut flour decreased. Though hydrolysis may be expected to increase the number of hydrophilic groups on the protein molecule, conformational changes that may occur due to proteolysis may reduce the water absorption capacity of the enzyme-treated flours.

This study has shown that wild mango seed flour has great potential for incorporation into human food products as a functional agent in a variety of formulated foods. Further studies are needed to evaluate the functionality of the flour in real food systems.

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