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Effect of detoxification on the functional and nutritional quality of proteins of karanja seed meal

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Abstract

Meal from karanja, an unconventional oilseed, is a good source of proteins (33.2%). The presence of anti-nutritional constituents, such as phytates, tannins and protease inhibitors, glabrin and karanjin (a furano-flavonoid) is a formidable constraint. The effects of various treatments on the "functional and nutritional" quality of the proteins were evaluated. Treatments, such as water leaching, mild acid and mild alkali were found to bring down the levels of anti-nutrient components, while 2% hydrochloric acid improved the nutritional value by reducing the content of phytate (81%), tannin (69%) and trypsin inhibitor activity (84%). Effective removal of the residual oil from the meal ensured complete reduction of karanjin, a fat-soluble constituent. The functional characteristics of proteins are affected with respect to solubility at various pH values from 2 to 11. There is a decrease in water and fat absorption capacities (50% and 35%). Emulsification capacity is found to reduced by 50% while the foam capacity decreases drastically (>60%). The available lysine content (3.46%) in acid-treated meal was comparable with the control (3.6%). The amino acid composition of the meal was not affected by the treatments. The protein digestibility corrected amino acid score (PDCAAS) of the meal was 0.6. Detoxification methods may potentially lead to effective value addition to these agro-resources.

Keywords: *Pongamia*; Karanja; Detoxification; Anti-nutrients; Functional–nutritional; Characteristics; Proteins quality

1. Introduction

Karanja (*Pongamia pinnata*) is a forest tree belonging to the family *Leguminosae*, grown in all parts of India, particularly in Tamil Nadu, Andhra Pradesh and Karnataka, for its ecological advantages. The seed contains 27–39% oil, 20–30% protein and a group of furano-flavonoids that constitutes 5–6% by weight of the oil (Bringi, 1987). The estimated production of seed is nearly 0.11 million tons per annum in India (National Oilseed and Vegetable Oils Development Board, 1995). The projected production of oil is nearly 30,000 tons per annum, at present (De, Rakshit, Sen, & Bhattacharyya, 1998). The oil is known for its curative effect on skin problems, such as, leucoderma, psoriasis, scabies and skin itches (Bringi, 1987). The oil is extracted by conventional ghani or expeller pressing, leaving 15–20% of residual oil in the cake. The oil is dark in colour, with an unpleasant odour. Technologies are developed to upgrade oil quality for soap manufacture and other industrial purposes. Presently, the use of karanja oil for the production of biodiesel is being explored (De & Bhattacharyya, 1999; Meher, Vidya, Dharmagadda, & Naik, 2006; Srivastava & Prasad, 2000; Vivek & Gupta, 2004). This may enhance the availability of karanja cake.

The cake, which is bitter and pungent, is used as manure, fungicide and insecticide. Although the cake is rich in proteins, it is unpalatable and toxic due to the presence of karanjin, pongamol and an unusual amino acid, glabrin. However, raw cake is not normally used as feed for poultry and livestock since the presence of its toxic factors resulted in poor intake (Anon, 1984; Parmar, Sahrawat, & Mukherjee, 1976). Gupta, Srivastava, Tripathy, Verma, and Thakur (1981) have reported that complete de-oiling of the cake by

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solvent extraction removes bitter taste and pungent odour. Such a cake has been fed to cattle calf up to 160 g/kg level in the diet without any adverse effect. However, significant reduction in the feed intake, growth, and nutrient balance is found in the animals (Mandal & Banerjee, 1974; Natanam, Kedirvel, & Balagopal, 1988; Natanam, Kedirvel, & Ravi, 1989) and reduced protein digestibility in growing goat kids, fed at higher levels of solvent-extracted cake, is observed (Srivastava, Gupta, Thakur, & Verma, 1990). Ravi, Singh, Garg, and Agrawal (2000) have observed significant difference in the digestibility of dry and organic matter in animals fed with expeller pressed cake, revealing that oil-soluble toxic compounds are responsible for the adverse effects in the animals. Various methods, namely, solvent extraction, water leaching, autoclaving, acid and alkali treatment, were adopted to detoxify karanja cake (Prabu, Devakumar, Sastry, & Agrawal, 2002). It is found that solvent extraction removed karanjin efficiently. Besides these, tannins, phytates and protease inhibitors, as meal constituents, are the non-nutrient components that hinder the usage of cake. Attempts have also been made to detoxify karanja seed meal by refluxing with 2% HCl (Mandal, 1985). Rats, fed on the resultant meal, at 30% level, did not suffer any toxic effects. The studies were conducted for assessment of protease inhibitors and digestibility of proteins due to detoxification by refluxing with 2% HCl.

Attempts were made by others to detoxify the meal from oil-soluble materials. But enough attention has not been paid to anti-nutrients, such as phytates, tannins, trypsin inhibitors, karanjin and glabrin, present in meal. Reports on the effect of detoxification on (functional and nutritional) quality of protein are lacking. The present study addresses detoxification of meal for removal of these anti-nutritional components, employing various methods and their effects on (functional and nutritional) quality of proteins.

2. Materials and methods

2.1. Materials

Karanja seeds were procured from M/s. Suresh Forestry Network, Chickballapur, Karnataka. All the solvents used were of HPLC grade or analytical grade (E. Merck). Fine chemicals used were obtained from Sigma Co., USA.

2.2. Preparation of detoxified meal

Karanja seed meal was prepared by de-oiling 15 kg of seeds by pressing, using motor driven ghani, when around 80% oil was removed; the residual oil was removed by hexane extraction through repeated percolation until the meal left had less than 0.5% oil. Karanja seed meal (200 g) was soaked (1:2 w/v) in water or 0.1% and 0.5% $Ca(OH)_2$ for 6–8 h, boiled for 15–20 min, drained, washed with water and dried in an oven at 60 °C for 6–8 h. The dried seeds were powdered in a hand mill and passed through a 60 mesh sieve. Similarly, seed meal (200 g) was soaked (1:2 w/v) in dilute acid (2% HCl) for 1, 4 and 8 h at room temperature (27 °C); then dilute alkali was added to bring it up to isoelectric pH (pH 4.5) and the supernatant was decanted and the residue washed with water. The detoxified meal was dried at 60 °C for 6–8 h in an oven and powdered in a hand mill and passed through a 60 mesh sieve.

2.3. Chemical composition of the meal

Proximate analysis was carried out by the standard methods (Association of Official Analytical Chemists (AOAC), 2000).

2.4. Estimation of anti-nutrients

2.4.1. Estimation of karanjin

The oil (100 ml) was subjected to liquid–liquid (1:2 v/v) extraction with methanol for 36 h. The methanolic extract was separated and concentrated 2.5-fold and made up to 100 ml with methanol. A known volume (20 ml) of the methanolic extract was subjected to column chromatography on silica gel (120–200 mesh) for purification. The column-purified ether fraction was quantitatively analyzed for karanjin content, spectrophotometrically at 259 nm, using the molar extinction coefficient ($\log \varepsilon$ 4.58) (Lakshmi, Srimannarayana, & Subba Rao, 1975).

2.4.2. Estimation of tannins

One gramme of seed meal was boiled with 80 ml water for 30 min, then filtered and made up to 100 ml. Aliquots of sample (10–50 μg) were taken and 0.5 ml of 50% Folin–Ciocalteau reagent and 1 ml of saturated sodium carbonate were added. The reactants were mixed well and incubated for 30 min at room temperature. The colour developed was measured at 760 nm against a reagent blank. The concentration was determined against a standard calibration curve for tannic acid (Ranganna, 1995).

2.4.3. Estimation of phytates

Two grammes of seed meal, along with 100 ml (1.2% hydrochloric acid and 10% Na_2SO_4 solution) were mechanically shaken for 2 h. On filtration, the filtrate (10 ml) along with 12 ml of ferric chloride (0.2%), was kept in a boiling water bath for 75 min. After cooling, the contents were centrifuged at 1000*g* for 15 min. The supernatant was discarded and the residue was washed thrice with 0.6% HCl and 2.5% sodium sulphate; after each wash, the contents were centrifuged at 1000*g* for 10 min and the supernatant was discarded. 10 ml of concentrated HNO_3 were added to the resulting pellet and the contents transferred quantitatively to a 100 ml beaker, four drops of concentrated H_2SO_4 were added, and the whole heated for 30 min until only sulphuric acid remained. 4.5 ml of 30% hydrogen peroxide were added and the mixture boiled at a low heat. The residue was treated with 15 ml of 3 N HCl and kept in boiling water bath for 15 min. The resulting solution was made up to 100 ml and the absorbance was measured at 660 nm.

A standard curve was prepared with a known concentration (10–50 mg) of KH_2PO_4 solution reacted with ferrous sulphate-ammonium molybdate reagent and concentration was determined against a calibration curve (Thompson & Erdman, 1982).

2.4.4. Determination of trypsin inhibitor activity

Trypsin inhibitor was extracted by shaking 1 g of seed meal with 50 ml of 0.1 N sodium hydroxide at room temperature for 1 h and centrifuging at 6000 rpm. The supernatant was collected and diluted 50 times with 0.1 N sodium hydroxide. The aliquots (0.5–2.5 ml) were assayed for TIA according to Kakade, Simon, and Liener (1969).

2.5. Functional characteristics

2.5.1. Nitrogen solubility index (NSI)

One gramme of sample was suspended in 20 ml of water; pH of the suspension was adjusted from 2 to 11 using 1 N hydrochloric acid or 1 N sodium hydroxide. The suspension was then shaken for 1 h in a rotary shaker at room temperature and centrifuged at 5000 rpm for 20 min. The clear supernatant was used to estimate soluble nitrogen by the Kjeldahl method and values were expressed as percent of the total nitrogen in the material.

2.5.2. Water absorption capacity (WAC)

WAC, expressed as the amount of water absorbed by 100 g of sample on a moisture-free basis was determined by the method of Sosulki (1962).

2.5.3. Fat absorption capacity (FAC)

FAC was determined according to the method of Sosulki, Humbert, Bui, and Jones (1976), using 1 g of sample and refined groundnut oil. The values were expressed as the amount of oil (millilitres) absorbed by 100 g of sample.

2.5.4. Emulsification capacity (EC)

Emulsification capacity was determined at pH 7 by the method of Beuchat, Cherry, and Quinn (1975). To the meal, equivalent to 1 g protein, 25 ml of distilled water were added and the whole mixed in a blender at low speed. After complete dispersion, refined groundnut oil was added from a burette at a rate of 0.4 ml/s; blending was continued until phase separation was seen. EC was expressed as millilitres of oil emulsified per gramme of protein.

2.5.5. Foam capacity (FC) and foam stability (FS)

FC and FS were determined according to the method of Lawhon, Cater, and Mattil (1972). Flour (3 g) was dispersed in 100 ml of distilled water, adjusted to pH 7.0, and the contents transferred to a mixer blender and whipped for 5 min at 10,000 rpm. The contents, along with the foam, were poured into a 250 ml measuring cylinder; the foam volume was recorded after 30 s. FC was expressed as percentage increase in volume. After 30 min, the volume of foam was measured and expressed as FS.

2.6. Nutritional quality evaluation

2.6.1. In vitro digestibility

In vitro digestibility was determined, according to the method of Akeson and Stahman (1964), using pepsin and pancreatin.

2.6.2. Amino acid analysis

Samples containing 5 mg of protein, were hydrolyzed with 5.8 M HCl for 24 h under vacuum at 110 °C. Amino acid analysis was performed by precolumn derivatization, using phenylisothiocyanate. Phenyl thiocarbamyl amino acids were used in the analysis system (Bidlingmeyer, Cohen, & Tarvin, 1984).

2.6.3. Chemical score

Chemical score was calculated using the following formula (Food & Agriculture Organization (FAO), 1968):

$$\frac{\text{g essential amino acid in test protein}}{\text{g total essential amino acid in test protein}} \times \frac{\text{g total essential amino acid in egg protein}}{\text{g essential amino acid in egg protein}} \times 100$$

2.6.4. Essential amino acid index (EAAI) and biological value (BV)

EAAI was calculated according to the method of Oser (1951) and BV was calculated by using the formula of Oser (1959).

$$\text{BV} = 1.09\ (\text{EAA Index}) - 11.7$$

2.6.5. Nutritional index (NI)

NI was calculated using formula of Crisan and Sands (1978).

$$\text{NI} = \frac{\text{EAA Index} \times \%\ \text{protein}}{100}$$

2.6.6. Computed protein efficiency ratio (C-PER)

This was calculated according to the method of Satterlee, Marshall, and Tennyson (1979) and the C-PER computed using the formula

$$\text{C-PER} = -2.1074 + 7.1312\ (\text{SPC}) - 2.5188\ (\text{SPC})^2$$

where SPC is the essential amino acid score ratio of sample and casein.

2.6.7. PDCAAS

Protein digestibility corrected amino acid score was calculated using the following formula (Report of FAO/WHO, 1989).

$$\text{PDCAAS} = \frac{\text{Amount of amino acid in test protein}}{\text{Amount of amino acid in reference protein}} \times \text{Digestibility of test protein}$$

2.6.8. Estimation of available lysine

Available lysine was estimated using the modified method of Carpenter and Booth (1980).

3. Results and discussion

3.1. Chemical composition of the meal

Table 1 shows the distribution of chemical constituents in various parts of karanja seed. The seed contained 22% proteins, 33.4% fat and 26.1% (by difference) carbohydrates. The seed was comprised of an outer hull portion, constituting 5.7%, and an inner kernel portion of 94.3%. Though the hull fraction contained 9% (g/100 g) protein, its input to the whole seed was minimal since the amount of hull fraction was less than 6%. Hence, elimination of hulls may contribute little to the chemical composition (Vidya, Vinay, & Sindhu Kanya, 2005).

3.2. Estimation of anti-nutrients

The meal, after entire removal of oil by expeller/ghani pressing, followed by solvent extraction, contained non-nutrients, such as phytates, tannins and protease inhibitors (Table 2). Different methods of detoxification, such as water leaching, mild alkali [0.1% and 0.5% $Ca(OH)_2$] and mild acid [2% HCl (1–8 h)] were employed to remove these non-nutrients. Tannin (0.94%) and phytate (0.65%) contents and trypsin inhibitor activity (31 units/mg sample) were found in the seed meal before the detoxification treatments applied. Detoxification treatments brought down the tannin contents to 0.28–0.52% in the meals. Acid treatment for 8 h reduced tannins by 70%, whereas content of phytate in the detoxified meals was 0.1–0.42%, it being reduced by 85% with 0.5% alkali treatment. However, around 84% decrease of trypsin inhibitor activity was found in 0.5% $Ca(OH)_2$ treated meal (5–8 units/mg). With respect to phytates, either calcium hydroxide or hydrochloric acid treatments were suitable; however, tannins were more efficiently released by the treatment with acid than by alkali treatment. In order to diminish the impact of the treatments on the quality of the proteins, detoxification of the meal by acid-washing was attempted under milder conditions by reducing the contact time to 1 h. Use of 2% HCl treatment for 1–8 h, at room temperature, resulted in the removal of anti-nutritional components (54–70% of tannins, 72–83% of phytates and 74–81% of protease inhibitors). The results indicated that the treatment could reduce anti-nutrients and trypsin inhibitor without subjecting the meal to higher temperature for inactivation. The present approach differs from the reported method (Mandal, 1985) wherein karanja seed meal was detoxified by refluxing with 2% HCl; trypsin inhibitors were reduced by around 50% and there was no evaluation of tannins and phytates. The detoxified seed meal contained 0.03% of karanjin compared to 1.95% in the seed (Vinay et al., 2006).

Table 1
Proximate composition of Karanja (*Pongamia Glabra*) seed

Constituents	Whole seed (%)	Kernel (%)	Hull (%)
Moisture	6.1 ± 0.20	9.4 ± 0.25	6.0 ± 0.02
Protein	22.0 ± 0.55	21.8 ± 0.50	9.5 ± 0.25
Fat	33.4 ± 0.85	39.2 ± 0.85	4.4 ± 0.25
Crude fibre	6.8 ± 0.25	4.2 ± 0.3	5.6 ± 0.25
Total ash	3.3 ± 0.25	3.2 ± 0.25	5.0 ± 0.25
Carbohydrate	26.1 ± 0.60	22.2 ± 0.50	69.5 ± 1.5

Mean ± SD of triplicates.

3.3. Functional characteristics

The functional characteristics of proteins were studied in order to find an application as a new protein meal. The effect of treatments was studied for functional characteristics of proteins. Protein solubility is the most important functional property because it influences other functional properties. The karanja seed proteins were found to vary in solubility (36–90%) with pH change (2–11). The maximum solubility was achieved at pH 10.0, whereas isoelectric precipitation was found at pH 4.0. The detoxification treatments had an effect on the protein solubility in the pH range 2–11 (Fig. 1). Detoxification treatments reduced the solubilities of proteins at various pH values. This decrease in solubility could probably be explained by the increased surface hydrophobicity of protein, due to unfolding of molecules which could have been attributable the effect of detoxification treatments. Jianmei Yu and Ipek (2007) have explained that roasting of peanuts significantly decreases protein solubility in peanut flour in the pH range 3.5–10.0 compared to that in raw peanut flour. This decrease was attributed to the effect of heating which increased the surface hydrophobicity of protein due to unfolding of molecules and molecular size effects through hydrophobic interactions and disulfide formation.

Interactions of water and oil with proteins are very important in food systems because of their influence on the flavour and texture of foods. Intrinsic factors affecting water-binding capacity of food proteins include amino acid composition, protein conformation and surface polarity/hydrophobicity (Barbut, 1999). However, food processing methods have important impacts on the protein conformation and hydrophobicity. With respect to water-holding capacity, the denatured proteins bind more water through exposure of hydrophilic groups (Kinsella, 1982). In contrast there was a decrease in water absorption capacity by around 50%, which might be explained by more hydrophobic groups being exposed during the treatments. Similarly, 35% reduction in fat absorption capacity was observed (Table 3). These results may be supported by a previous report (Jianmei Yu & Ipek, 2007), that, during roasting, peanut proteins were denatured by high temperature, exposing more hydrophobic sites, which explained the reduced water retention of peanut protein.

Table 2
Detoxification of karanja seed meal for removal of anti-nutrients

Treatment	Tannins (%)	Reduction (%)	Phytates (%)	Reduction (%)	TIU/mg sample	Reduction (%)
Untreated meal (control)	0.94	–	0.65	–	31 units	–
Water leaching	0.52	44.7	0.42	35.4	5	83.9
0.1% $Ca(OH)_2$	0.41	56.3	0.20	69.2	6	80.6
0.5% $Ca(OH)_2$	0.38	59.6	0.10	84.6	5	83.9
2% HCl (8 h)	0.28	70.2	0.11	83.1	6	80.6
2% HCl (4 h)	0.45	52.1	0.13	80.0	7	77.4
2% HCl (1 h)	0.43	54.3	0.18	72.3	8	74.2

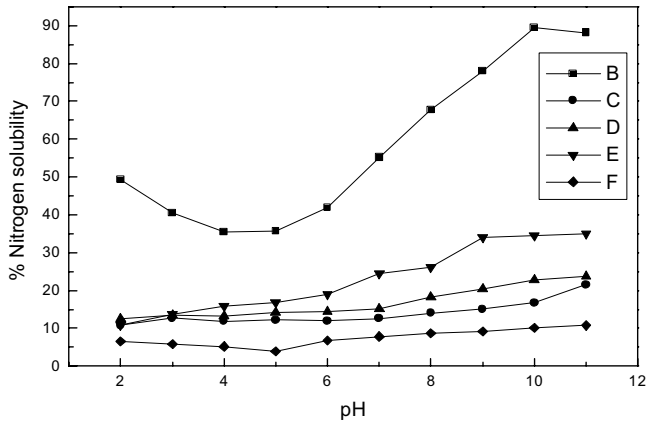

Fig. 1. Effect of detoxification on nitrogen solubility profile. B: control; C: water leaching; D: 0.1% $Ca(OH)_2$ wash; E: 0.5% $Ca(OH)_2$ wash; F: acid wash.

Emulsification of proteins is closely related to the conformation of proteins and interaction of adsorbed molecules at the oil/water interface. The capacity of proteins to unfold at an interface depends on the conformational stability of their tertiary structure. Emulsification capacity of karanja seed proteins was found to decrease by 50% due to the impact of detoxification treatments (Table 3). This indicated that the extent of denaturation of proteins was considerable and they did not retain any sizable tertiary structure during treatments. The type of protein and method of preparation affect the formation and stability of emulsion. Soy protein isolate and soy protein concentrate vary in their emulsification capacities (Hutton & Campbell, 1977a, 1977b) according to their composition and the stress to which they have been subjected.

The formation of foam is analogous to the formation of emulsion. In the case of foam, water molecules surround air droplets, and air is the non-polar phase. The amphipathic character of proteins makes them good foaming agents that work at air–water interfaces to prevent bubble coalescence. Foaming properties of oilseed proteins are described elsewhere (Kinsella, 1979). Soybean proteins are extensively used in the preparation of aerating or whipping agents; however, many such preparations that show excellent foam expansion are of little practical value because of instability. Thermal instability of many oilseed proteins limit their applications. Table 3 shows the foam capacity and stability of karanja seed proteins in both treated and untreated meals. Solubility of proteins affects foaming ability, as reflected in the study; reduced foam capacity of proteins in treated meal parallelled decreased solubility. The foam volume and stability of the protein were affected and were reduced by more than 60%.

3.4. Nutritional quality evaluation

Table 4 shows the amount of protein loss during the anti-nutrient removal process. The protein content was reduced from 33% to 22.1–23.9% in meals obtained from various treatments. The loss of proteins was found to be

Table 3
Functional characteristics of the detoxified karanja seed meal

Treatments	Solubility of proteins at neutral pH (%)	Water absorption capacity (ml/100 g)	Fat absorption capacity (ml/100 g)	Emulsification capacity oil (ml)/ g protein	Foam capacity (ml/g of protein)	Foam stability (ml/g of protein)
Untreated meal (control)	72.0	273	150	45	17.0	16.6
Water leaching	25.0	159	120	26.4	5.6	3.6
0.1% $Ca(OH)_2$	14.0	175	90.0	29.3	9.3	9.6
0.5% $Ca(OH)_2$	15.0	144	110	28.5	8.6	8.3
2% HCl (8 h)	10.0	166	90.0	25.0	6.0	5.0
2% HCl (4 h)	10.0	133	100	24.5	6.6	6.0
2% HCl (1 h)	14.0	127	110	24.8	6.6	6.0

36% during 0.5% $Ca(OH)_2$ treatment and up to 25% protein loss was observed in various other treatments, probably attributable to the solubility of proteins in different pH media (acidic and basic). Due to loss of proteins (up to 36%) and inefficiency in reducing tannins beyond 60% by 0.1% and 0.5% $Ca(OH)_2$ or beyond 35% by water leaching, the approach was restricted to 2% HCl treatment.

The effect of detoxification on nutritional quality of proteins was studied. The nutritional quality of protein was evaluated by assessment of lysine availability, amino acid profile, *in vitro* digestibility of proteins, and computed nutritional indices. The *in vitro* digestibility varied from 82–91% compared to 92% in the control and showed marginal differences in various treated meals (Table 4). The results showed that the karanja seed proteins might not be either complex or bound to any minor constituents through glycosidic or ester linkages, and hence were easily digestible. The good digestibility may even be possibly due to the lower levels of tannins which inhibit the trypsin digestion. In many legume seeds, tannins are reported to inhibit the digestive enzymes.

Table 4 also depicts the results for available lysine content. The available lysine contents of the meals treated with 2% HCl for 1 h and the water leaching treatment showed values similar to those obtained for the control. The available lysine content (3.49%) in acid-treated meal (1 h) was comparable to the control (3.6%). Probably, the ∈-amino acid (lysine) available for reaction might have been destroyed during all the detoxification methods except the mild acid treatment for the short period of 1 h. The amino acid analysis indicated that the karanja seed protein was a rich source of lysine, leucine, tyrosine and phenylalanine. However, with respect to sulphur-containing amino acids, acid hydrolysis had destroyed sulphur amino acids. The amino acid composition shows that karanja protein is rich in sulphur amino acids (Bringi, 1987). The lysine content includes non-bioavailable and bio-available lysine. Acid and alkali treatments with higher strength would lead to decrease in nutritive value of proteins, due to recemization, resulting in the formation of DL-amino acids (partially or completely) and destruction of some of the essential amino acids, with the production of toxic constituents, such as lysino-alanine. However, the treatment using 2% HCl for up to 4 h had no effect on the amino acid composition (Table 5). PDCAAS value of detoxified meal was 0.6, which was calculated from the amino acid composition analysed (except, the sulphur-containing amino acids) and the reported value of (Bringi, 1987). The seed protein was rich in glutamic and aspartic acids and similar to conventional oilseed proteins. Nutritional indices are shown in Table 6, and revealed that detoxification, by using 2% HCl for 1 h at room temperature, did not affect the nutritional quality of proteins.

Table 4
Effect of detoxification on *in vitro* protein digestibility and available lysine

Treated meal	Protein (%)	*In vitro* digestibility (%)	Available lysine (%)
Untreated meal (control)	33.0	92.0	3.60
Water leaching	22.1	89.6	3.50
0.1% $Ca(OH)_2$	23.1	90.5	2.85
0.5% $Ca(OH)_2$	22.4	82.1	2.65
2.0% HCl (8 h)	23.7	87.9	2.40
2.0% HCl (4 h)	23.9	88.8	2.90
2.0% HCl (1 h)	23.2	87.4	3.49

Table 5
Effect of detoxification on amino acid composition

Amino acid (%)	Control	2% HCl (1 h)	2% HCl (4 h)	Reported[a]	FAO/WHO
Essential amino acids					
Lysine	8.42	8.55	8.27	9.3	5.5
Leucine	10.1	10.1	10.2	9.4	7.0
Isoleucine	4.25	4.05	4.23	5.0	4.8
Methionine & Cystine	0.38	0.4	0.48	6.8	3.5
	0.12	0.16	0.12		
Threonine	2.71	2.79	2.9	3.3	4.0
Tyrosine Phenylalanine	2.0	2.29	2.41	8.5	6.0
	6.61	6.72	6.88		
Valine	5.45	5.23	5.29	3.7	5.0
Tryptophan	–	–	–	5.5	–
Non-essential amino acids					
Alanine	4.13	3.84	3.77	4.0	–
Glycine	4.71	4.83	5.03	5.5	–
Aspartic acid	13.3	13.4	13.2	10.5	–
Glutamic acid	19.1	18.6	18.2	15.3	–
Serine	4.47	4.57	4.77	6.8	–
Histidine	2.81	2.84	2.8	5.7	–
Arginine	6.18	6.46	6.44	6.2	–
Proline	5.1	5.18	4.99	–	–

[a] Bringi (1987).

Table 6
Effect of detoxification of karanja seed meal on nutritional indices

Nutritional parameter	Defatted karanja meal	2% HCl treated karanjameal
In vitro digestibility (%)	92.0	89.4
Essential amino acid index	93.5	93.9
Biological value	90.3	90.6
Nutritional index	30.9	21.8
C-PER	2.29	2.19
Available lysine (%)	3.6	3.45

4. Conclusion

Karanja seed, was comprised of an inner kernel portion, constituting 94.3%, and outer hull portion of 5.7% unlike most conventional oilseeds (comprising 15–25% of hulls). The seed was similar to conventional oilseeds in possessing 22.0% of proteins and 33.4% of fat. Tannins (0.94%), phytates (0.65%) and protease inhibitor (31 units/mg sample) were found in the seed meal. HCl (2%) treatment for 1 h at room temperature resulted in removal of anti-nutritional

components, such as tannins up to 54%, phytates up to 72.5% and trypsin inhibitor activity up to 74%. Efficient removal of oil resulted in a karanjin content 0.03% suggesting this as the best detoxification approach. Decreased water absorption capacity, by around 50%, was explained by more exposed hydrophobic groups or hydrophilic embedded groups due to the treatments. Emulsification capacity of the proteins studied indicated that the extent of denaturation of proteins was considerable and proteins did not retain any sizable tertiary structure during treatments. The amino acid analysis disclosed that the karanja seed protein was a rich source of lysine, leucine, tyrosine and phenylanine. Detoxification of karanja seed meal for food/feed would potentially lead to effective value addition to karanja seed.

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