



Functional and nutritional properties of tamarind (*Tamarindus indica*) kernel protein

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Tamarind (*Tamarindus indica*) is one of the important tree legumes in tropical and sub-tropical countries. The functional as well as the nutritional properties of the meal and concentrate from tamarind kernels (raw and roasted) were determined. The concentrates were obtained by using the micellization process of protein isolation. The functional properties determined were the nitrogen-solubility index, water-absorption capacity, emulsifying capacity, foaming capacity, and foam stability. The nutritional properties estimated included *in-vitro* protein digestibility and amino-acid composition. The proteins were also fractionated according to their solubility in water, salt solution, ethanol, and sodium hydroxide solution. The *in-vitro* digestibility was 71.3; the kernel protein was rich in lysine, glutamic acid, aspartic acid, glycine, and leucine but deficient in sulphur-containing amino acids.

INTRODUCTION

Tree legumes grow extensively in tropical and sub-tropical regions of the world because of their ability to grow in poor soils and because they can withstand long spans of droughts (Felker & Bandurski, 1977). The high content of protein and of certain minerals and vitamins (Fordham *et al.*, 1975) makes them a good source of nutrients. A sizeable amount of research has been carried out on tropical grain legumes (peas, beans, lentils) (Manan *et al.*, 1987), but little work has been done on the seeds of tree legumes.

Tamarind (*Tamarindus indica*) is one of the most important and common trees in tropical countries. In addition to being a forest tree, it is grown throughout India, Bangladesh, Sri Lanka and Burma, and in many other parts of the world (CSIR, 1976). It is grown mainly for its sour fruits. The fruit consists of the seed

(33.9%), pulp (55.0%), and shell and fibre (11.1%) (Rao & Srivastava, 1974). The seed, a by-product of the tamarind pulp industry, is a typical under-utilized, or waste, material. For edible uses, it is essential to remove the testa completely (usually by roasting followed by decortication) from the edible kernel, in order to avoid undesirable effects, such as depression, constipation, and diarrhoea (Rao & Srivastava, 1974).

Tamarind seed has many uses. Its major industrial use, however, is as tamarind-kernel powder (TKP) as a sizing material in the textile and jute industries (Lewis & Neelakantan, 1964). The powder is also recommended as a valuable remedy in diarrhoea and dysentery (Rao & Srivastava, 1974), as feed for pigs (Reddy *et al.*, 1986), as a base in the cosmetics and pharmaceutical industries, and as a curative against rheumatism (Forest Research Institute, 1955).

Despite its many uses, there has been little investigation on tamarind kernel powder (TKP) to date. Only recently, Rao & Subramanian (1984) and Marangoni *et al.* (1988) have attempted the production of protein concentrates or meals and studied some functional properties of kernel protein. However, data from scientific research are still lacking, and there is a need for better utilization than as a mere by-product of little value, or as a waste material.

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The chemical composition, as well as the functional and nutritional properties, of tamarind kernel indicates that it could be useful as a food or food ingredient. The proteins in roasted TKP can be used to prepare fortified bread and fortified biscuit (Bhattacharya, 1990). Other possible uses of the kernel protein may include development of bread spread, beverage powder, and protein gel. In a previous publication (Bhattacharya *et al.*, 1991), the present authors have described the rheological behaviour of TKP suspensions. The present paper deals with the isolation and characterization of protein concentrate from the kernel and determination of the functional and nutritional properties of tamarind kernel protein.

MATERIALS AND METHODS

Materials

Tamarind seeds (about 100 kg) were procured in bulk from the local markets of villages in the neighbourhood of Kharagpur, West Bengal (India). Raw seeds were washed with water to remove dust and adhering pulp and separated from infested seeds that float during washing with water. The cleaned seeds were dried (12–14% moisture, dry basis) in the shade for about a week, during which period they were turned and stirred to prevent mould infestation. The seeds were then stored for one month, in an airtight metal container, at room temperature.

Proportion of seed coat (testa) and kernel

Raw seeds (about 20 g) were carefully broken into between six and ten pieces by use of a hammer. The brown testa was removed from the white kernel manually by means of a small knife. The weight of the kernels was then noted. The difference in weight of the raw whole seed and kernels gave the weight of testa. The reported values are means of five replications.

Proximate composition

The protein, fat, ash, crude fibre, and moisture contents of seed and kernel were estimated according to the method described by AOAC (1984) and were reported as the arithmetic mean \pm the standard deviation of five observations.

Roasting of seeds

The details of the roasting and dehusking of seeds and grinding of kernels have been described in a previous publication (Bhattacharya *et al.*, 1991).

Preparation of meal and protein concentrate from TKP

The raw or roasted tamarind kernel powder (80–100 mesh) was defatted by solvent extraction by using *n*-

hexane to obtain tamarind kernel meal. The extracted meal was desolventized by using a vacuum drier (70-cm vacuum) at room temperature ($29 \pm 2^\circ\text{C}$) and ground to 80–100 mesh size in a laboratory grinder. These defatted samples were used for the extraction and preparation of protein concentrates. Generally, the isolation or extraction of protein is effected by the precipitation of protein at the isoelectric pH. In the present study, however, this method failed to give satisfactory results, and yields were appreciably low. The formation of a gel at low pH (4–5) hindered the process of filtration. The proteins were therefore isolated by the process of micellization in sodium chloride solution, a technique developed by Lopez & Falomir (1986). This process was found to be effective. Details of the process as standardized are shown in Fig. 1. Defatted TKP (100 g) was mixed well with 1 M NaCl solution (800 ml) and the pH of the suspension was adjusted to about 10. After stirring for 30 min, the mixture was centrifuged at 6000 r/min for 15 min in a laboratory centrifuge; it was then extracted twice with 1 M NaCl solution (200 ml). The supernatant was brought to a pH of 4–6 by adding 1 N HCl solution, and solid ammonium sulphate (salting out) was added to saturation, at which point proteins of TKP were precipitated. The suspension was centrifuged at 8000 r/min for 20 min to obtain the precipitated protein. The process of salting out was repeated once more. The precipitated protein was purified by dialysis against distilled water at 2–4°C for 48 h, a cellophane bag being used as the dialysing membrane. A volume of 9 litre of distilled water was used for dialysis, and the water was changed every 8 h. The contents of the cellophane bag were then freeze-dried to obtain the protein concentrate. It was stored at 4–6°C in a refrigerator until used. The protein-recovery values reported are the means of three replications.

Characterization of tamarind kernel protein/meal

Functional properties, such as the nitrogen-solubility index (NSI), water-absorption capacity (WAC), emulsifying capacity (EC), foam capacity (FC), and foam stability (FS) were measured for tamarind kernel protein meal and concentrate, obtained from both raw and roasted seed and from raw and roasted meal. The nitrogen-solubility index was determined by the procedure suggested by Warriar & Ninjoor (1981). The water-absorption capacity was determined according to the method of Sosulski (1962). The method suggested by Beuchat *et al.* (1975) was used to determine the emulsifying capacity. The method of Hoffman *et al.* (1975) was used for the determination of foam capacity and foam stability. The values reported here are the means of three replications.

Fractionation of proteins

Cereal proteins are usually divided into four groups according to the solubility-fractionation scheme of

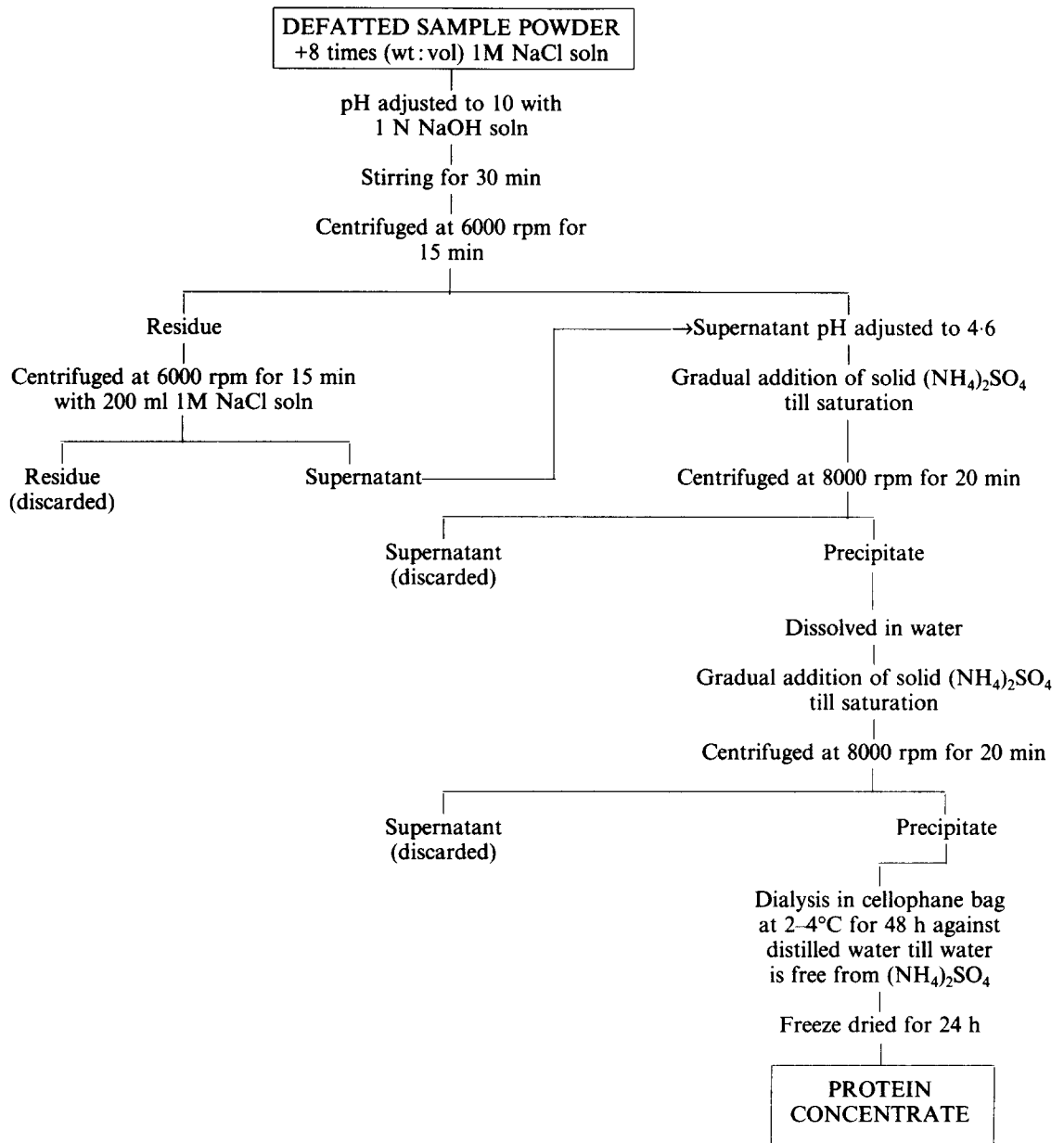


Fig. 1. Flow chart for the isolation of protein from tamarind kernel powder.

Osborne (Ewart, 1968). The proteins were fractionated into albumin, globulin, prolamine, and glutelins according to their solubility in distilled water (pH 7.0), 5% NaCl solution (pH 7.0), 70% ethanol, and 0.25% NaOH (pH 10.0) solution, respectively, by using the method suggested by Marfo *et al.* (1986).

Protein-digestibility index (PDI)

The method followed for the estimation of *in-vitro* protein digestibility was the same as that described by Hsu *et al.* (1977). The digestibility values reported here are the means of three replications.

Amino-acid analysis

A 10-mg protein sample (freeze-dried concentrate, obtained from raw kernel) was hydrolysed by 6 N HCl (5 ml) in an evacuated sealed glass tube, which was

kept in an air oven maintained at 110°C for 24 h. The sealed tube was broken, and the acid removed completely by repeated flash evaporation after the addition of deionized water. Dilution was effected by means of a citrate buffer, pH 2.2, to such an extent that the solution contained 0.5 mg protein/ml. The solution was passed through a millipore filter. A 20 ml sample was applied on the column of the amino-acid analyser (LKB Alpha Plus Analyser, Produkter AB, Stockholm, Sweden), equipped with autoloader, programmer, and integrator. The sample was autoloader on the analytical column containing a cation-exchange resin and eluted with a buffer sequence of pH 3.0, 4.24, and 6.25 and then with 0.4 N NaOH. The amino acids were detected spectrophotometrically after complexing with ninhydrin reagent. An LKB standard amino-acid kit was used as an internal standard for calibration of the integrator. The amino-acid composition of the sample was directly computed by the integrator by comparison

with the standard; each amino acid is expressed as g/16 g of nitrogen. The amino-acid scores were calculated by dividing the content of each amino acid in the FAO/WHO (1973) reference amino-acid pattern to determine the amino-acid score of the protein.

RESULTS AND DISCUSSION

Composition of seed and kernel

The proximate composition of the tamarind seed and kernel is shown in Table 1. The weight of the whole seed was 3.5 or 1.4 times the weight of the hull or the kernel, respectively, and the hull and kernel weights were in the ratio of 1:2.5 (g/g wet basis) in the whole seed. The protein content of the kernel ($18.1 \pm 1.3\%$) was quite high. The hull contained a considerable amount of crude fibre (21.6%) and ash (7.4%).

Isolation of protein from tamarind kernel

Raw and roasted, defatted, kernel powders were used for the isolation of protein. The yield, as measured in terms of protein recovery (ratio of protein present in isolate and defatted input samples), was 38.3, and 33.3% for raw and roasted defatted kernel, respectively. The protein and moisture contents of the isolated materials were 40.5 and 6.1%, and 38.3 and 5.9%, respectively. It may be mentioned here that the yield, in the case of sunflower-protein isolate under similar conditions, was reported to be 44.2% (Lopez & Falomir, 1986). Arntfield *et al.* (1985) have also reported similar results for the faba bean. The yield was found to be lower for roasted samples, than for raw ones, perhaps owing to the formation of a complex between the protein and carbohydrate or protein and lipid during the roasting process. Kashani & Guy Valadon (1984) have observed a decrease in the contents of total available carbohydrate, total starches and dextrans, and total free sugars during roasting of Iranian pistachio kernels. Furthermore, Salem (1975) has also reported a reduction in total carbohydrates, sugars, and starches after the cooking of broad beans.

Foam capacity (FC) and foam stability (FS)

Foam capacity, calculated as the percentage volume increase of 5% suspensions of defatted raw and roasted TKP and of protein concentrates from raw and roasted TKP is shown in Table 2 along with the protein contents of the samples. The protein concentrates possessed higher foam-capacity values than the defatted samples. Roasting markedly decreased the foam capacity of the samples. It may be mentioned here that tamarind kernel proteins have a lower foam capacity than several other proteins, including concentrates from rice bran (about 33%: Bera &

Table 1. Composition of tamarind seed (wet basis)

	Whole seed (%)	Hull (%)	Kernel (%)
Moisture	11.3 ± 1.4	11.0 ± 0.9	11.4 ± 0.9
Protein (N*6.25)	13.3 ± 1.2	—	18.1 ± 1.3
Fat	5.4 ± 0.9	—	7.2 ± 0.6
Ash	4.1 ± 1.0	7.4 ± 0.7	2.6 ± 0.5
Crude fibre	8.8 ± 0.9	21.6 ± 1.9	2.5 ± 0.5
Carbohydrate (by difference)	57.1	—	58.5

—Not determined.

Hull = $28.6 \pm 2.3\%$ of whole seed.

Kernel = $71.4 \pm 3.4\%$ of whole seed.

Mukherjee, 1989), soybean meal (about 56%: Marfo *et al.* 1986), sunflower-isoelectric-protein isolate (about 43%: Lopez & Falomir, 1986) and defatted groundnut flour and protein concentrate (75% and 80%, respectively: Ihekoronye, 1986) but has greater foaming capacity than papaya-seed meal and protein concentrate (about 5 and 20%, respectively: Marfo *et al.* 1986).

The stability of foam was measured by allowing the whipped 5% suspension to stand for 0.5, 5, 10, 15, 20, 30, 60, 90, and 120 min and is illustrated in Table 3. It was observed that the foams of both tamarind-kernel-protein concentrate and defatted meal were stable, and the foam volume decreased very slowly. The foam volume remained fairly constant in the case of raw and roasted samples of concentrates and of raw-meal samples (the extent of change being 13–17% after 120 min), but the roasted meal showed a marked decrease (about 40%) in foam volume after 120 min. Protein concentrates from groundnut (Ihekoronye, 1986) and papaya-seed meal and concentrate (Mayo *et al.* 1986) produced a foam whose volume decreased rapidly.

Emulsifying capacity (EC)

The emulsifying capacity (EC) was determined in raw and roasted kernel meal and freeze-dried raw and roasted kernel concentrates and is shown in Table 4. It may be observed from the table that roasting reduced the EC values, i.e. a smaller amount of fat could be emulsified by the kernel protein. Denaturation of protein owing to heating (roasting) may be responsible for the decrease in the EC values. It is reported that, for defatted meals of peanut, field-pea, and pecan flours, the EC values are low at a pH close to 7 (McWatters & Cherry, 1977). Table 4 also shows that

Table 2. Foam capacity (FC) of tamarind kernel protein

	Raw meal	Roasted meal	Raw protein concentrate	Roasted protein concentrate
Foam capacity (% volume increase)	21.9 ± 1.1	10.8 ± 0.4	28.3 ± 0.6	14.9 ± 0.5
Protein content(%)	20.2 ± 0.6	20.1 ± 0.5	40.5 ± 0.8	38.3 ± 0.6

Table 3. Foam stability (FS) of tamarind kernel meal and concentrate for raw and roasted powder suspensions at different times of standing

Time (min)	Raw meal	Roasted meal	Raw protein concentrate	Roasted protein concentrate
0.5	21.8 ± 1.0	10.7 ± 0.5	27.1 ± 0.9	14.0 ± 0.6
5	21.8 ± 0.8	10.7 ± 0.5	27.1 ± 1.0	14.0 ± 0.6
10	21.8 ± 1.0	10.7 ± 0.4	27.1 ± 0.8	13.9 ± 0.6
15	21.6 ± 0.7	9.5 ± 0.6	27.1 ± 0.9	13.9 ± 0.5
20	20.0 ± 0.6	9.0 ± 0.3	27.1 ± 0.5	13.7 ± 0.4
30	20.0 ± 0.7	9.0 ± 0.4	25.9 ± 0.4	13.4 ± 0.6
60	18.5 ± 0.8	7.8 ± 0.4	25.9 ± 0.6	12.9 ± 0.5
90	18.5 ± 0.4	7.6 ± 0.3	24.1 ± 0.8	12.6 ± 0.4
120	18.4 ± 0.5	7.6 ± 0.3	24.1 ± 0.4	12.5 ± 0.3

the defatted (meal) samples exhibited higher EC values than the concentrates. Similar results were reported for great-northern-bean proteins by Sathe & Salunkhe (1981), who observed that protein concentrates (protein content 85.4%, db) exhibit higher EC values than the isolates (protein content 92.4%, db). These researchers indicated that this is due to a difference in the method of preparation of the protein fractions.

Water-absorption capacity (WAC)

The WAC values for raw and roasted tamarind kernel meal and concentrate, as well as their protein contents, are shown in Table 4. Roasting of the seeds decreased the WAC values. Rao & Subramanian (1984) also observed a decrease in WAC values from 246 to 185 g/100 g of defatted meal owing to roasting. The concentrates had higher WAC values than their corresponding meals. Similar results were reported for sesame, soybean, and great-northern-bean flour by Dench *et al.* (1981), Marfo *et al.* (1986), and Sathe

Table 4. Emulsifying capacity (EC) and water-absorption (WAC) of tamarind kernel meal and concentrate for raw and roasted powders

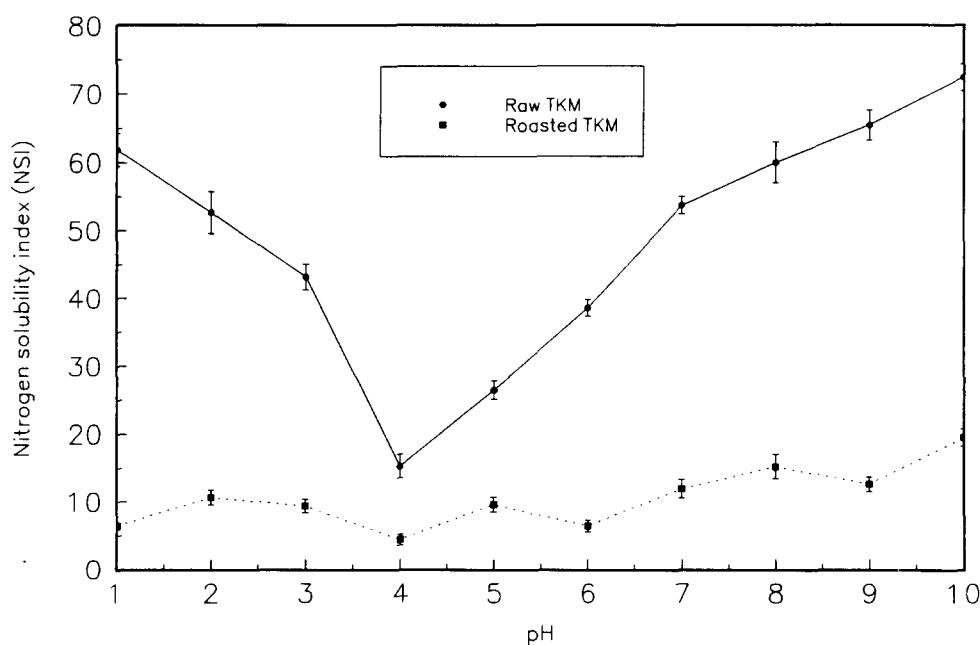
	Raw meal	Roasted meal	Raw protein concentrate	Roasted protein concentrate
Emulsifying capacity (ml oil/100 g powder)	122.0 ± 3.1	107.8 ± 2.2	68.7 ± 2.4	51.7 ± 1.6
Water-absorption capacity (g/100 g powder)	245.7 ± 6.2	196.6 ± 5.0	336.0 ± 8.1	324.1 ± 8.3

& Salunkhe (1981), respectively. A suggestion that arises from such results is that protein concentrates, being rich in protein content, have more hydrophilic groups exposed than the meals. It may be mentioned that Bull & Bresse (1968) had observed a linear relationship between the content of hydrophilic groups of a protein and WAC values.

Nitrogen-solubility index (NSI)

Nitrogen-solubility index (NSI), an important functional property, was measured for the raw (protein content 20.2%) and roasted (protein content 20.1%) kernel meal with water and with 1 M NaCl solution. NSI was also measured for the protein concentrates of the raw (protein content 40.5%) and roasted (protein content 38.3%) kernel powders.

Figure 2 represents the NSI of raw and roasted kernel meals in water. The raw meal exhibited a V-shaped curve with a minimum NSI value of about 15% around pH 4 (isoelectric pH). The NSI values were observed to be high at highly acidic and alkaline pH

**Fig. 2. Nitrogen-solubility index (NSI) of raw and roasted TKM in water.**

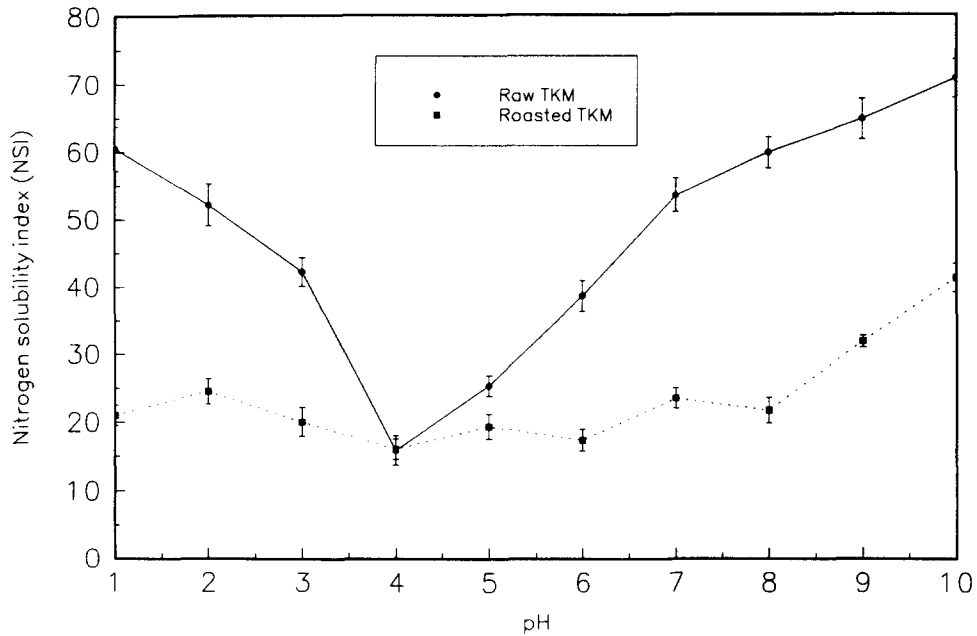


Fig. 3. Nitrogen-solubility index (NSI) of raw and roasted TKM in 1 M NaCl solution.

values; the maximum solubility of about 74% was observed at pH 10. The regression equation between the NSI and pH can be expressed as

$$\text{NSI} = 15.04 - 3.48 (\text{pH}) + 0.36 (\text{pH})^2 \quad (1)$$

Equation (1) is able to predict 75.2% of the total variation in NSI ($r = 0.867$, $p \leq 0.01$). The regression equation for roasted tamarind-kernel meal (TKM) is shown by eqn (2) ($r = 0.79$, $p \leq 0.01$):

$$\text{NSI} = 9.82 - 1.49 (\text{pH}) + 0.23 (\text{pH})^2 \quad (2)$$

The isoelectric pH obtained in the present study was found to compare well with the observations of Rao &

Subramanian (1984), who found the minimum NSI value (about 13%) near pH 4. Bera & Mukherjee (1989) reported the minimum solubility at pH 4.5 for defatted and full-fat, rice-bran protein. Dev and Quensel (1986) reported isoelectric-pH values in the region of 4.0–4.5 for linseed flour and isolate, and for soybean flour and isolate. Oat proteins and their succinylated and acetylated forms have an isoelectric pH between 4 and 5 (Ma, 1984).

Figure 2 also shows the NSI at different pH values for roasted TKM. The NSI values were much smaller than those in raw meals. The NSI values were nearly the same in the range of pH 1–6, with the minimum

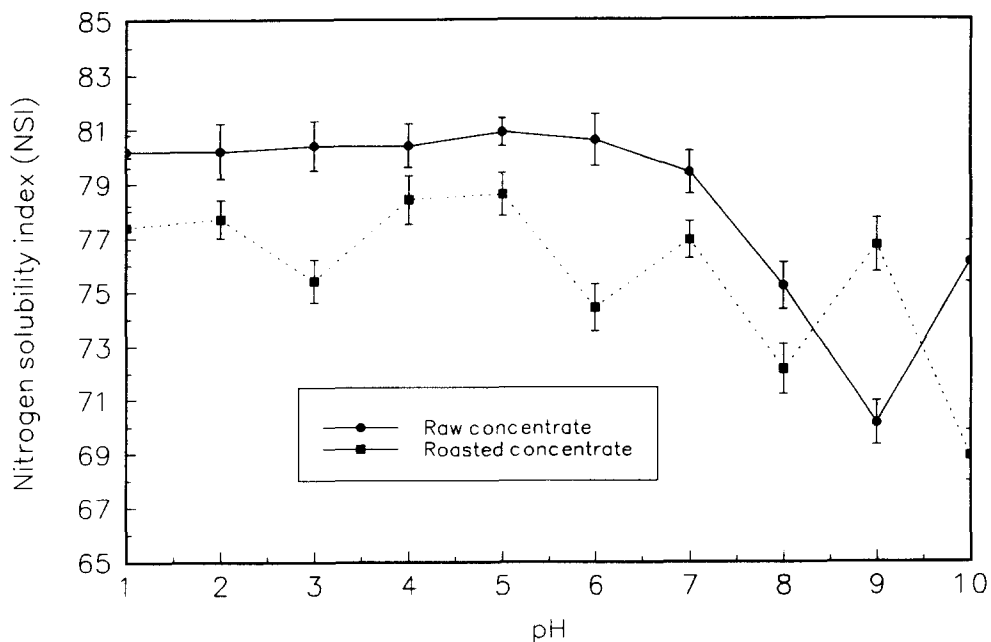


Fig. 4. Nitrogen-solubility index (NSI) of raw and roasted tamarind protein concentrate in water.

Table 5. Classical fractionation of protein from defatted TKP meal

Extraction solvent	Protein type	% Extraction of sample protein
Distilled water	Albumin	19.6
5% NaCl solution	Globulin	21.7
70% Ethanol	Prolamine	3.9
0.25% NaOH solution	Glutelins	16.1

at pH 4, but showed higher values between pH 6 and pH 10.

Figure 3 shows the NSI of tamarind-seed proteins (raw and roasted TKM) in 1 M NaCl solution. Similar curves to those in Fig. 2 were observed. The V shaped curve for raw TKM showed a maximum solubility of about 70% at pH 10. The minimum solubility was observed at pH 4 for both raw and roasted TKM. The maximum solubility of roasted TKM at pH 10, in 1 M NaCl, was about twice that in water (Figs 2 and 3).

The regression equation (eqn (3)) between NSI and pH for raw TKM was found to be:

$$\text{NSI} = 13.69 - 2.38 (\text{pH}) + 0.23 (\text{pH})^2 \quad (3)$$

Equation (3) accounts for 64% of the total variation in NSI ($r = 0.80$, $p \leq 0.01$). The regression equation for roasted TKM in 1 M NaCl is shown by eqn (4) ($r = 0.92$, $p \leq 0.01$):

$$\text{NSI} = 29.81 - 6.16 (\text{pH}) + 0.71 (\text{pH})^2 \quad (4)$$

The NSI at different pH values for raw and roasted protein concentrates in water is shown in Fig. 4. The pattern of the curves is different from the previous NSI curves (Figs 2 and 3). The NSI values are higher than those for meals (raw and roasted). For raw concentrate, the NSI values remained constant at about 81% in the pH range of 1–6 but decreased gradually to reach the minimum value of about 70% at pH 9. The solubility increased again with an increase in pH. For roasted concentrates, a zigzag curve was obtained with the lowest value of about 72% at pH 9.

Fractionation of proteins

Distilled water (pH 7.0), 5% NaCl solution (pH 7.0), 70% ethanol, and 0.25% NaOH solution were used in sequence for the extraction of protein from TKP. Table 5 shows the protein types of defatted TKP meal together with the values for protein extraction in these four solvents. A total of 61.3% of protein can be extracted by these solvents, so that the sum of non-protein nitrogen (NPNM) and insoluble proteins accounted for the remaining 38.7%. The amounts of different proteins in decreasing order of solubility are: salt-soluble protein 21.7%, > water-soluble protein 19.6% alkali-soluble protein 16.1% > alcohol-soluble protein 3.9%. The present findings tally with those obtained for rice bran by Betschart *et al.* (1977), who

Table 6. Amino-acid composition of the tamarind-seed protein

Amino acid	mg/16 g N
Lysine	5.96
Histidine	2.01
Arginine	4.20
Aspartic acid	11.59
Threonine	3.75
Serine	7.71
Glutamic acid	18.53
Proline	6.19
Glycine	9.12
Alanine	6.96
Cysteine	0.30
Valine	4.60
Methionine	0.33
Isoleucine	4.12
Leucine	8.21
Tyrosine	1.99
Phenylalanine	4.33

reported a total nitrogen extraction of 65.9%, with prolamine accounting for the smallest quantity. Papaya seeds showed a higher extraction of protein (73.4%) with a very high content of globulins (53.9%) and the smallest quantity of prolamine (3.0%) (Marfo *et al.* 1986).

Nutritional status of kernel protein

In-vitro protein-digestibility tests and amino-acid analysis were performed to determine the nutritional status of the tamarind kernel protein. The *in-vitro* protein-digestibility index was found to be 71.3. Marangoni *et al.* (1988) have reported an *in-vitro* digestibility value of 69.1 for tamarind seed protein (of West Indian origin).

Amino-acid analysis

The amino-acid composition of the tamarind seed protein is given in Table 6. Tamarind seed protein is rich in glutamic acid (18.5%), aspartic acid (11.6%), glycine (9.1%) and leucine (8.2%) but deficient in sulphur-containing amino acids (methionine and cysteine together amount to only 0.63%). The proportions of hydrophobic amino acids (alanine, valine, leucine, isoleucine, and phenylalanine) and hydrophilic amino acids (lysine, histidine, aspartic acid, glutamic acid, and arginine) are 28.2% and 42.3%, respectively.

Table 7 shows the profile of essential amino acids in tamarind seed protein along with the FAO/WHO provisional amino-acid scoring pattern and the amino-acid score. The proportion of total essential amino acids in tamarind kernel protein is 33.6%. It is also observed from this table that lysine, isoleucine, and leucine are present in excess amounts in tamarind kernel protein, whereas the amounts of threonine, valine, and sulphur-containing essential amino acids are lower than that of the FAO/WHO (1973) reference pattern. The sulphur-containing amino acids are the limiting amino acids and are present in very low quantity (the amino-acid

Table 7. Essential amino-acid profile of the tamarind seed protein and FAO/WHO reference protein, and amino-acid score

Amino acid	Amino-acid content (g amino acid/ g protein)		Amino-acid score* (%)
	Tamarind kernel protein	FAO/WHO reference protein	
Lysine	59.6	55	108.4
Threonine	37.5	40	93.8
Valine	46.0	50	92.0
Methionine + cysteine	6.3	35	18.0†
Isoleucine	41.2	40	103.0
Leucine	82.1	70	117.3
Tyrosine + phenylalanine	63.3	60	105.3

$$* \text{ Amino-acid score} = \frac{\text{Essential amino-acid content in tamarind kernel protein}}{\text{Corresponding essential amino acid in FAO/WHO reference protein}} \times 100$$

† Chemical score.

score is only 18%), resulting in a very low protein or chemical score (18%).

It can be concluded from the preliminary nutritional studies that tamarind kernel protein is a low-quality protein. The low content of sulphur-containing amino acids and high level of lysine make it suitable for use as a food ingredient but only when supplemented with cereal flours, which are usually rich in sulphur-containing amino acids but deficient in lysine.

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