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# Some Studies on the Proteins of *Carica papaya* Seeds

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*ABSTRACT*

*Protein isolates and seed meals made from* Carica papaya *seeds were studied with respect to their composition and functional properties. Studies showed that the seed proteins are most soluble in 5% NaCl (23·77 ± 0·15%). In all concentrations of NaCl tested, the protein has a solubility peak at pH 8·0. Classification of the protein showed that globulins constitute the bulk of the protein (53·9 ± 0·89%). The amino acid pattern of the samples studied is not too different from other plant protein sources. However, the seed appeared deficient in many amino acids.*

*Electrophoretic studies showed that the water extract had only one band with a molecular mass of about 70·7 × $10^3$ daltons. The 5% NaCl extract gave five bands, with molecular mass ranging from 37·6 to 105·9 × $10^3$ daltons while the NaOH-soluble fraction gave six bands with a range of 18·2 to 104·0 × $10^3$ daltons.*

*Compared to soya bean meal and protein concentrate, the papaya products were inferior in terms of functional properties.*

## INTRODUCTION

The importance of plant seeds as food for human and animal nutrition has stimulated a lot of research into their utilization (Del Valle *et al.*, 1983; Latha & Prakash, 1984). However, attention has been focused on edible seeds such as peanuts, beans, soya beans, etc. (Oyenuga, 1968; Poulter,

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1981). In contrast, many potentially utilizable plant seeds have been neglected. One such seed is that of *Carica papaya*. The nutritional evaluation of the seeds was studied in previous work (E. K. Marfo and O. H. Oke, pers. comm.). In the present paper, the protein isolates of the papaya seeds and the seed meal are studied with respect to their composition and functional properties.

## MATERIALS AND METHODS

### Preparation of seed meal

Mature fruits of papaya (*Carica papaya*) were collected from Oyo and Ondo states of Nigeria. Seeds were removed from the fruits, dried and ground into fine meal after removing the testa. The meal was sieved by a 40 mesh screen. The residue was ground and reground until all passed through the 40 mesh screen. Samples of the meal were defatted by continuous Soxhlet extraction with petroleum ether (40–60°C boiling point) for 12 h (AOAC, 1970). Residual solvent was removed by drying the defatted meal in an oven at 60°C for 24 h.

### Protein classification of the defatted seed meal

An 'Osborne classification' of the proteins was done as described by Lund & Sandstrom (1943). Distilled water (pH 7·0), 5% NaCl (pH 7·0), 70% ethanol and 0·25% NaOH (pH 10·0), were used in sequence for extraction, at a solid to solvent ratio of 1:20. Defatted seed meal samples were put in centrifuge tubes and shaken vigorously for 45 min. They were then centrifuged at 3500 rpm for 10 min. The supernatant was filtered using Whatman No. 1 filter paper. The nitrogen contents of the filtrates were determined by the semi-micro Kjeldahl method and the protein value was obtained by multiplying the nitrogen content by a factor of 6·25 (AOAC, 1970). In order to characterise the protein in the supernatants by gel electrophoresis, the supernatants were dialysed against distilled water at 4°C for 48 h, freeze-dried and stored at −10°C until required for analysis.

### Preparation of protein concentrate

The method was essentially as described by Latha & Prakash (1984). Defatted seed meal (1500 g) was suspended in 5% NaCl (3 litres) at pH 7·0 and was shaken vigorously for 45 min. The suspension was centrifuged at 3500 rpm for 10 min. The supernatant was centrifuged through cotton wool. The filtrate was acidified (pH 3·5) using 1M HCl. The solution was stored at 4°C overnight and then centrifuged at 4°C at 3500 rpm. Protein

pellets obtained as the residue were washed (2 ×) with distilled water (pH 7·0) and then lyophilized. The freeze-dried sample was stored at −10°C until required.

**Effect of pH on protein solubility in NaCl solution**

The method used was as described by Rivas *et al.* (1981). Two grams of fatted seed meal were suspended in 40 ml of 5% NaCl in a centrifuge tube. The pH of extraction was adjusted to between 1 and 11 using either 0·5M HCl or 0·5M NaOH. Extraction was done by shaking the suspension for 45 min, after which it was centrifuged for 15 min at 3500 rpm.

During extraction the pH was checked at 15-min intervals and re-adjusted as appropriate. The supernatant was filtered using cotton wool. A 5 ml aliquot of each extract was used for nitrogen determination as described above.

**Amino acid analysis**

Amino acid analysis was done as described by Moore & Stein (1963). Lyophilized protein samples (20 mg) were hydrolyzed under nitrogen with 10 ml 6M HCl at 110°C for 24 h. The amino acids were quantified using a Technicon amino acid analyzer.

**Gel electrophoresis**

Electrophoretic characterization of the proteins was done as described by Weber & Osborne (1969). Protein samples (2 mg/ml SDS buffer, pH 6·8) were dissolved in sodium dodecyl sulphate (SDS). A total of 0·05 ml protein solution was applied per gel. Electrophoresis was run for 12 h at 4 mA per gel. Gels were stained with kenacid blue solution (0·02% w/v) and destained using a destaining solution (75 ml acetic acid + 50 ml methanol + 87·7 ml $H_2O$). Apparent molecular weights of separated sub-units were determined by comparing with molecular weight markers (bovine serum albumin, ovalbumin, cytochrome C and $\gamma$-globulins).

**Bulk density determination**

The apparent bulk densities of seed meals and protein concentrates were determined by the method of Wang & Kinsella (1976).

**Water absorption capacity**

Water absorption capacity was determined as described by Sosulski (1962).

### Fat absorption capacity

Fat absorption capacity was determined as described by Dench *et al.* (1981).

### Emulsification property

Emulsification property was determined using the method of Beuchart (1977). Emulsions containing 2·5 g samples were prepared. Samples suspended in distilled water (50 ml) were rapidly blended for 10 min at pH 7·0, 50 ml soy oil was added gradually and blended rapidly for another 10 min. The emulsions were centrifuged at 3500 rpm for 15 min. The ratio of the height of the emulsified layer to the total height of the fluid was calculated and emulsification ability expressed as a percentage. Emulsification stability was determined by the same method except that the emulsions were heated at 70°C for 30 min in a water bath and then cooled under running tap water for 20 min before centrifugation.

### Whipping and foaming properties

Whipping properties were determined according to the Lawhon *et al.* (1972) procedure; 2·5 g of sample was suspended in distilled water and the pH adjusted to 7·0. The suspension was whipped in a Kenwood Chef food mixer for 10 min. The suspension was immediately poured into a 100 ml measuring cylinder and the foam height and volume of liquid collected at the bottom of the cylinder were measured at intervals.

The percentage foam volume was then calculated as described by Lawhon *et al.* (1972).

### Statistical analysis

Data were subjected to statistical analysis. Analysis of variance and regression analysis were conducted using the MATH/STAT PAC for Hewlett Packard 41CV.

## RESULTS AND DISCUSSION

### Solubility profiles

In order to study the protein solubility profile in NaCl and NaOH, various concentrations of the two solvents were used. Table 1 shows the extent of

**TABLE 1**
Protein Solubility Profile of Papaya Seed Meal in NaOH and NaCl. (Values are means of three determinations ± standard deviation of the means)[a]

| *NaCl concentration (%)* | *Protein extracted (% dry weight)* | *NaOH concentration (%)* | *Protein extracted (% dry weight)* |
|---|---|---|---|
| 1 | 5·33 ± 0·25 | 0·01 | 6·77 ± 0·38 |
| 3 | 12·63 ± 0·21 | 0·05 | 8·33 ± 0·12 |
| 4 | 21·70 ± 0·10 | 0·10 | 11·27 ± 0·15 |
| 5 | 23·77 ± 0·15 | 0·15 | 16·87 ± 0·38 |
| 6 | 22·67 ± 0·15 | 0·17 | 19·53 ± 0·32 |
| 8 | 21·20 ± 0·20 | 0·20 | 21·10 ± 0·17 |
| 9 | 19·17 ± 0·15 | 0·25 | 21·47 ± 0·06 |

[a] Values are significant at $P < 0·001$.

solubility of the papaya seed meal in NaCl and NaOH. The soluble protein concentration in NaCl increased with increasing NaCl concentration until it peaked at 5% NaCl (23·8 ± 0·15) ($P < 0·001$). For the NaOH concentrations examined, the soluble protein concentration increased with increasing NaOH concentration ($r = 0·97$).

Table 2 shows the protein types (Lund & Sandstrom, 1943) of papaya seed meal. The higher solubility of the seed meal proteins in NaCl indicates that the globulins make up the bulk of the proteins present in the seed (53·9 ± 0·89% of total protein).

## pH effect on the NaCl solubility

Since NaCl had a profound solubility effect on papaya seeds (Table 2), it was of interest to further investigate the pH effect in the various concentrations of NaCl on protein solubility. Figure 1 shows the effect of pH on

**TABLE 2**
Classification of Protein Types of Papaya Seed. (Values[a] are means of three determinations ± standard deviation of means)

| *Extraction solvent* | *Protein type* | *Per cent of total protein* |
|---|---|---|
| Water (pH 7·0) | Albumin | 10·97 ± 0·96 |
| 5% NaCl | Globulins | 53·90 ± 0·89 |
| 70% Ethanol | Prolamins | 3·00 ± 0·82 |
| 0·25% NaOH | Glutelins | 5·57 ± 0·65 |
| 10% TCA | Non-protein nitrogen | 8·43 ± 0·59 |
| Residue | Insoluble protein | 18·30 ± 0·70 |

[a] Values are significant at $P < 0·001$.

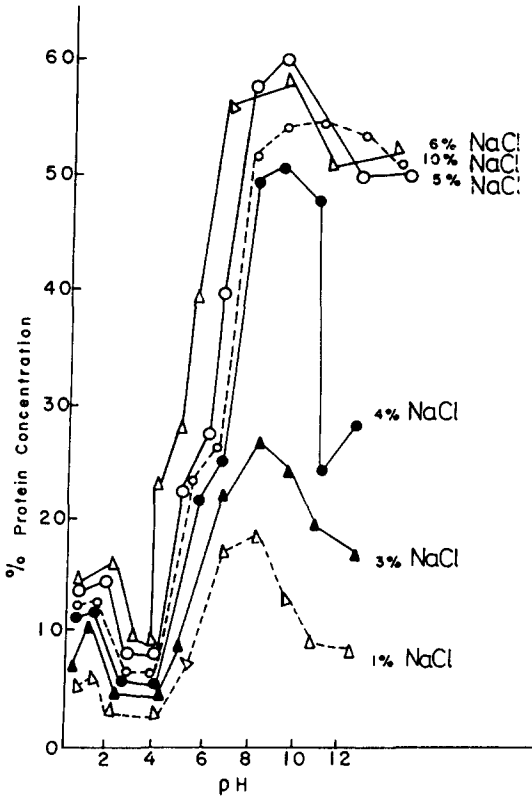


**Fig. 1.** Solubility patterns of papaya seed protein in NaCl solution at various concentrations and pH.

the solubility pattern of the seed soluble proteins. It appears that increase in NaCl concentration enhanced protein solubility, the effect being more pronounced between pH 4·0 and 10·0. The solubility of the protein peaked at pH 8·0 in all NaCl concentrations studied. Guerra & Park (1975) and Rivas *et al.* (1981) made similar observations.

*Amino acid composition*

The amino acid pattern of the extractable proteins is presented in Table 3. Since it was NaCl and water which extracted protein the most from seed meal (Table 2), these two extracts were the only ones considered for amino acid analysis. In general, the amino acid pattern is not too different from other plant protein sources (Oyenuga, 1968; Afolabi *et al.*, 1985). The amino acid pattern of the proteins was compared with FAO reference protein (FAO, 1965) and whole hen's egg. The sulphur and the aromatic amino acid contents appear to be sufficient. However, the seed extracts

**TABLE 3**
Amino Acid Profile of Papaya Seed Extracts. (Values[a] are means of two determinations ± standard deviation of means)

| *Amino acids* | *Extracts* | | | | | |
|---|---|---|---|---|---|---|
| | *Defatted papaya seed meal* | *Protein extracts from 5% NaCl (pH 8·0)* | *Protein extracts from water (pH 7·0)* | *Protein extracts from 0·25% NaOH (pH 10·0)* | *Egg*[b] | *FAO*[c] *protein reference* |
| Lys. | 1·93 ± 0·03 | 2·91 ± 0·02 | 0·43 ± 0·03 | 1·50 ± 0·11 | 7·0 | 4·2 |
| His. | 1·49 ± 0·10 | 2·16 ± 0·06 | 1·26 ± 0·01 | 1·26 ± 0·01 | 2·4 | |
| Arg. | 4·49 ± 0·11 | 6·63 ± 0·12 | 5·27 ± 0·02 | 5·27 ± 0·02 | 6·1 | 2·4 |
| Asp. | 0·54 ± 0·03 | 0·86 ± 0·12 | 0·80 ± 0·01 | 0·80 ± 0·01 | 9·0 | |
| Thr. | 7·82 ± 0·74 | 7·7 ± 0·20 | 2·24 ± 0·02 | 6·05 ± 2·89 | 5·1 | 2·6 |
| Ser. | 2·11 ± 0·81 | 3·09 ± 0·20 | 2·97 ± 0·01 | 1·36 ± 0·01 | 7·7 | |
| Glu. | 6·57 ± 0·31 | 9·45 ± 0·07 | 6·45 ± 0·02 | 2·34 ± 0·03 | 12·7 | |
| Pro. | 7·62 ± 0·36 | 11·16 ± 0·03 | 7·03 ± 0·02 | 10·26 ± 0·01 | 4·2 | |
| Gly. | 5·03 ± 0·01 | 7·48 ± 0·01 | 0·08 ± 0·01 | 7·49 ± 0·03 | 3·3 | |
| Cys. | 3·24 ± 0·05 | 3·24 ± 0·01 | — | 2·35 ± 0·05 | 2·4 | |
| Met. | 2·82 ± 0·01 | 2·76 ± 0·01 | 0·24 ± 0·04 | 1·19 ± 0·02 | 3·4 | 2·2 |
| Leu. | 7·93 ± 0·01 | 11·81 ± 0·01 | 5·19 ± 0·00 | 5·19 ± 0·02 | 8·8 | 4·8 |
| Tyr. | 1·20 ± 0·03 | 1·78 ± 0·01 | 0·83 ± 0·83 | 1·20 ± 0·02 | 4·2 | |
| Phe. | 1·97 ± 0·02 | 2·94 ± 0·03 | 2·12 ± 0·02 | 2·78 ± 0·02 | 5·6 | 2·8 |
| Ala. | 5·09 ± 0·01 | 7·51 ± 0·01 | 2·46 ± 0·01 | 4·26 ± 0·02 | 5·9 | |
| Val. | 3·12 ± 0·02 | 4·65 ± 0·01 | 4·47 ± 0·01 | 1·81 ± 0·01 | 6·9 | 4·2 |
| Ile. | 1·34 ± 0·02 | 1·71 ± 0·43 | 0·96 ± 0·05 | 1·22 ± 0·01 | 6·3 | 4·2 |

[a] Values are significant at $P < 0·001$ except Ile. which is significant at $P < 0·01$.
[b] Afolabi & Oke (1981).
[c] FAO (1965).

appeared deficient in many amino acids. Of particular interest was the aspartic acid (Asp.) content which was less than 1 g/100 g protein.

Compared to the defatted seed meal, there appears to be a general enrichment of the amino acids in the NaOH extracts while there is a general loss in the $H_2O$ extract.

The chemical scores, compared to whole hen's egg, are presented in Table 4. Only Thr., Ile. and His. can be adjudged to have adequate chemical scores in all the extracts. The chemical scores for the sulphur amino acids seem sufficiently high except in the water extract. The total aromatic amino acids scored lower than egg except in NaCl extract where there appears to be an enrichment. The limiting amino acids in the defatted seed meal are Ile., followed by Lys, and the aromatic amino acids; in the NaCl extract it was Ile., followed by Lys. and Val., while in the water extract it was the sulphur amino acids, followed by Lys. and Ile. Considering

**TABLE 4**
Chemical Scores of the Papaya Seed Protein Extracts

| *Amino acid* | *Chemical score*[a] | | | |
|---|---|---|---|---|
| | *Defatted seed meal* | *5% NaCl seed extract (pH 8·0)* | *Water extract (pH 7·0)* | *0·25% NaOH extract (pH 10·0)* |
| Thr. | 244 | 165 | 129 | 252 |
| Val. | 71·9 | 73·5 | 191 | 55·6 |
| Met. + Cys. | 166 | 113 | 12·2 | 129 |
| Ile. | 33·8 | 29·6 | 44·8 | 41·1 |
| Leu. | 143 | 146 | 173 | 125 |
| Phe. + Tyr. | 51·4 | 119 | 88·5 | 86·1 |
| His. | 98·7 | 98·1 | 154 | 111 |
| Lys. | 43·8 | 45·3 | 18·1 | 45·4 |

[a] The chemical score was calculated as described earlier (Afolabi & Oke, 1981).

the adequacy of the amino acids, it appears there are too many limiting amino acids.

## SDS–Gel Electrophoresis characterisation

The SDS–polyacrylamide gel electrophoresis of papaya proteins isolated in water, NaCl and NaOH solutions at pH 7·0 and pH 10·0, respectively, showed that protein isolate in water contained one band, the salt isolate in water contained one band, the salt isolate contained five bands while the NaOH isolate contained six bands. The single band found in the water isolate had a molecular mass of $70{\cdot}7 \times 10^3$ daltons. The subunits of the salt-soluble fraction had a molecular mass ranging between $37{\cdot}6 \times 10^3$ and $105{\cdot}9 \times 10^3$ daltons while the NaOH-soluble fraction subunits had molecular masses ranging between $18{\cdot}2 \times 10^3$ and $104{\cdot}0 \times 10^3$ daltons. The electrophoretic data show that the protein bands are discrete bands except in the water-soluble fraction where some trailing was observed. This finding is consistent with the reports of Permolet *et al.* (1982) which showed that Osborne fractions from oat meal were 'clearly individualized'.

## Bulk density

The papaya seed meal appears less dense than the protein concentrate ($P < 0{\cdot}01$). This may be due to the inclusion of non-protein components (the hull), which have low density, in the seed meal. Compared to the soya bean products, the papaya seed products have lower density ($P < 0{\cdot}01$).

## Water and fat absorption capacity

The water absorption capacity of both the papaya and soya bean protein isolates appeared higher than their corresponding seed meals (Table 5). Similar values were obtained for the soya flour by Dench *et al.* (1981). A statistical comparison showed that only the papaya seed products are significantly different ($P < 0{\cdot}01$) from each other. A suggestion which arises from this result is that the protein isolate has more hydrophilic groups exposed than the meals. Earlier, Bull & Bresse (1968) had observed a linear relationship between the hydrophilic group content of a protein and water-binding capacity.

**TABLE 5**

Some Functional Properties of Seed Meals and Protein Concentrate made from Papaya and Soya Bean. (Values are means of three determinations ± standard deviation of the means)

| *Functional property* | *Seed meal* | | *Protein concentrate* | |
|---|---|---|---|---|
| | *Papaya* | *Soya bean* | *Papaya* | *Soya bean* |
| Bulk density (g $ml^{-1}$) (Apparent) | 0·24 ± 0·02 | 0·45 ± 0·01 | 0·27 ± 0·02 | 0·29 ± 0·01 |
| Water absorption capacity (%) | 104 ± 0·38 | 234 ± 1·53 | 224 ± 1·53 | 314 ± 114 |
| Fat absorption capacity (%) | 118 ± 1·73 | 218 ± 2·00 | 205 ± 3·21 | 252 ± 2·65 |
| Emulsification ability in fat (%) | 35·0 ± 1·73 | 40·3 ± 2·08 | 39·7 ± 2·00 | 50·3 ± 1·53 |
| Emulsifying stability (%) | 40·3 ± 0·58 | 44·3 ± 4·51 | 40·0 ± 2·65 | 55·7 ± 1·15 |
| Whipping property (% volume increase) | 5·17 ± 0·76 | 55·7 ± 2·52 | 20·3 ± 0·58 | 221 ± 3·51 |

For the fat absorption capacity, the trend observed was that the protein isolate always had higher values ($P < 0{\cdot}01$). Also, the values for the papaya products were always lower than that of the soya bean ($P < 0{\cdot}01$). This observation may be expected as the fat absorption capacity is both related to ability to physically trap fat (Kinsella, 1976) and the binding of the paraffin chains of the fat to the numerous side chains of the protein. As earlier observed by Dench *et al.* (1981), a high correlation was obtained between fat absorption and bulk density for both papaya and soya bean products ($r = 1{\cdot}0$).

## Emulsification properties

The emulsification ability of the papaya seed meal and its protein concentrate appears to be low when compared with the soya bean products ($P < 0{\cdot}01$). The emulsifying abilities of the papaya seed meal and the

protein concentrate are similar. Compared to the soya products, only the protein concentrate has a significantly higher value ($P < 0{\cdot}01$); the meals have similar values. The emulsifying properties of the papaya seed products, which appeared lower than those from soya bean, suggest that their use as fat emulsifiers in food is limited. It has been observed that papaya seed extracts contained tannins, phytates and thioglucosinolates (unpublished). The presence of these contaminants might have reduced emulsification attributes due to the formation of insoluble protein complexes.

**Whipping properties**

The whipping properties estimated by the foaming capacity have been found to be very low when compared with similar soya bean products ($P < 0{\cdot}01$). The foam of the papaya seed products collapsed within 30 min at room temperature. Graham & Philips (1976) observed that flexible protein molecules such as $\beta$-casein, which can rapidly reduce surface tension, give good foamability, whereas a highly ordered globular molecule such as lysozyme gives a low foamability. The low whipping ability of the papaya seed protein may be due to the high proportion of globulins ($53{\cdot}9 \pm 0{\cdot}89$, Table 2) of its protein component.

In conclusion, this study on the proteins of papaya seed tends to suggest limited use of the seeds both as food and in the food industry. However, if antinutritive factors, as well as protein complexing agents such as the phytates, thioglucosinolates and tannins, are removed, their functional properties may be enhanced.

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