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Effect of Sodium Chloride, Calcium Chloride and Sodium Hydroxide on *Denolix regia* Protein Solubility

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ABSTRACT

Extraction of the flamboyant seed protein was carried out using various concentrations of NaCl, NaOH, $CaCl_2$ solutions. Protein extractability increased with increase in salt concentration. Protein was more soluble in NaOH than in NaCl and $CaCl_2$. Solubility in NaCl and $CaCl_2$ increased with increase in pH. Minimum solubility was around pH 3·0–4·5 with about 20–30% of protein remaining in solution.

Protein isolates were analyzed for their proximate composition, their solubility over a pH 1–11 range and amino acid content. Fractionation and characterization of the protein isolates by gel electrophoresis gave rise to six protein fractions with molecular weights ranging from $11{\cdot}4 \times 10^3$ to $85{\cdot}1 \times 10^3$.

INTRODUCTION

Flamboyant (*Denolix regia*) seeds are not used for consumption in West Africa. The plant is primarily grown as an ornamental and shade providing tree. The trees, however, bear large quantities of fruits, each containing about 40 seeds. These seeds have been found to contain about 24·6% crude protein (Marfo *et al.*, (in press)). Preliminary studies on the quality of the

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protein in our laboratory show that the cotyledon contains fairly good protein.

However, much information is not available on the nature of the proteins of flamboyant seed. In the present investigation, total proteins have been isolated using sodium chloride, sodium hydroxide and calcium chloride and analysed using various physico-chemical methods.

MATERIALS AND METHODS

The flamboyant seeds were collected from the University of Ife, Ile Ife, Nigeria campus. Some of the seeds were soaked overnight and manually separated into three fractions, exocarp, endosperm and cotyledon. The endosperm and the cotyledon (1 kg each) were dried at 50°C for 72 h and ground into a fine powder. The exocarp was discarded as the preliminary analysis for protein yielded a very low result. One kilogram of seeds was ground into fine powder (i.e. whole seed flour) with a hammer mill. Each flour was sieved with a 40 mesh sieve to ensure fairly uniform particle sizes. The sieved flours were defatted with petroleum ether (40–60°C). The defatted flours were used for the subsequent analyses described below.

Protein extractability

Proteins were extracted by suspending 5 g of defatted flamboyant seed flour in 50 ml of the solvent (water pH 7·0, 7% NaCl pH 8·0, 7% $CaCl_2$ pH 8·0 and 0·25% NaOH pH 10·0); the pH was adjusted to the desired value by adding either 2M HCl or 2M NaOH. The suspension was mechanically shaken for 30 min at room temperature. The slurry formed was centrifuged at 3500 *g* for 15 min using a Gallenkamp centrifuge. The pH of the supernatant was determined with a pH meter. Aliquots (5 ml) were used for nitrogen determination by the Kjeldahl method (AOAC, 1980). The protein was then calculated by multiplying N by 6·25. The influence of salts (NaCl, $CaCl_2$ and NaOH) and salt concentrations on protein leaching was investigated.

Protein concentrate

Protein concentrate was obtained by the method of Rhee *et al.* (1972). Defatted seed flour (50 g) was extracted with 500 ml sodium chloride solution (7%), pH 8·0. The sample was centrifuged for 15 min at 3500 *g* using a Gallenkamp centrifuge. The process was repeated on the same sample and

the supernatants were pooled together. The concentrate was obtained from the supernatant by adjusting the pH to 4·5 by adding either HCl or NaOH. The solution was centrifuged and the precipitate was dissolved in 3·0% NaCl solution and dialysed against cold distilled water at 4°C for 48 h. The protein concentrate was then centrifuged and dried in an oven at 50°C to constant weight. The protein content of the concentrate was determined by the Kjeldahl method (AOAC, 1980). The protein as a percentage of total protein in the seed was also calculated.

Protein solubility

Using the Samson *et al.* (1971) method, the solubility of the extracted protein was determined. A set of centrifuge tubes was arranged in a test-tube rack. A suspension (5% w/v) of the protein in water was adjusted to various pH values (pH 1·0 to pH 11·0) with either 1·0M HCl or 1·0M NaOH solution. The suspension was stirred for 45 min at room temperature (27°C), centrifuged at 3500 *g* for 15 min and the supernatant was analysed for protein (N × 6·25) by the Kjeldahl method (AOAC, 1980).

Amino acid pattern

The amino acid pattern of the protein was determined using the method of Moore & Stein (1963) and Spackman *et al.* (1958). Using 6·0M HCl, the protein was hydrolysed under vacuum for 24 h at 110°C. Amino acids were calculated and reported as gram per 16 g N.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was carried out using the Weber and Osborne (1969) method. Apparent molecular weight was determined using authentic markers (bovine serum albumin, aldolase, cytochrome C and γ-globulin).

Analysis of isolated protein

Proximate analysis of isolated proteins was carried out. Moisture, protein (N × 6·25), fat, and ash were determined by the AOAC (1980) method. Phytate and tannin in the isolated proteins were determined according to Wheeler & Ferrel (1971) and Price *et al.* (1978), respectively.

RESULTS AND DISCUSSION

Protein leaching

Table 1 shows the solubility of flamboyant seed protein in water (pH 7·0), $CaCl_2$ and NaCl solutions (pH 8·0). The extracted proteins were expressed as percentage of the total protein in the seed or the fraction of the seed. The higher solubility of the protein in salt solutions than in water indicates that flamboyant seeds contain more globular proteins than albuminous protein (Gheyasuddin *et al.*, 1970).

Effect of various concentrations of NaCl, NaOH and $CaCl_2$ on protein leaching

The effect of concentrations of NaCl, $CaCl_2$ and NaOH on the extractability of flamboyant bean protein is shown in Figs 1, 2 and 3. The relationship between solubility and concentration was eventually the same for chloride and calcium chloride solutions but different for sodium hydroxide solution. The extractability profiles for the $CaCl_2$ and NaCl indicate that the cations in the chlorides had little effect on extractability of the protein. Extractability was greatest in the NaOH solution, but beyond 1% (w/v) NaOH concentration, the slurry became gelatinous and difficult to centrifuge at 3500 *g*, most especially in the case of the endosperm. Raising the concentration of NaCl and $CaCl_2$ initially increased protein extractability but at higher concentrations extractability decreased with maximum extractability (75·3 ± 5·4%) at 7% (w/v), pH 8·0, in each case; the use of high concentrations of NaOH led to an increase in extractability, browning of the protein, discharging of nauseating odour and gelatination of the sample (Figs. 2 and 3). As a result of these observations the leaching solutions used in protein concentrate preparation, where maximum protein solubility was required (Rhee *et al.*, 1973), contained 7% (w/v) NaCl and had pH 8·0. Plant & Tulsiani (1969) reported that addition of NaCl (2% w/v) at pH 10, raised

TABLE 1
Solubility of Flamboyant Protein in Presence of Water, $CaCl_2$ and NaCl

Salt and concentration	*Percentage protein*		
	Whole seed	*Endosperm*	*Cotyledon*
Water, pH 7·0	14·5 ± 2·6	14·1 ± 2·1	14·5 ± 2·6
7% $CaCl_2$, pH 8·0	69·2 ± 4·4	66·9 ± 2·1	75·1 ± 3·9
7% NaCl, pH 8·0	69·9 ± 6·5	66·2 ± 4·3	75·3 ± 5·4

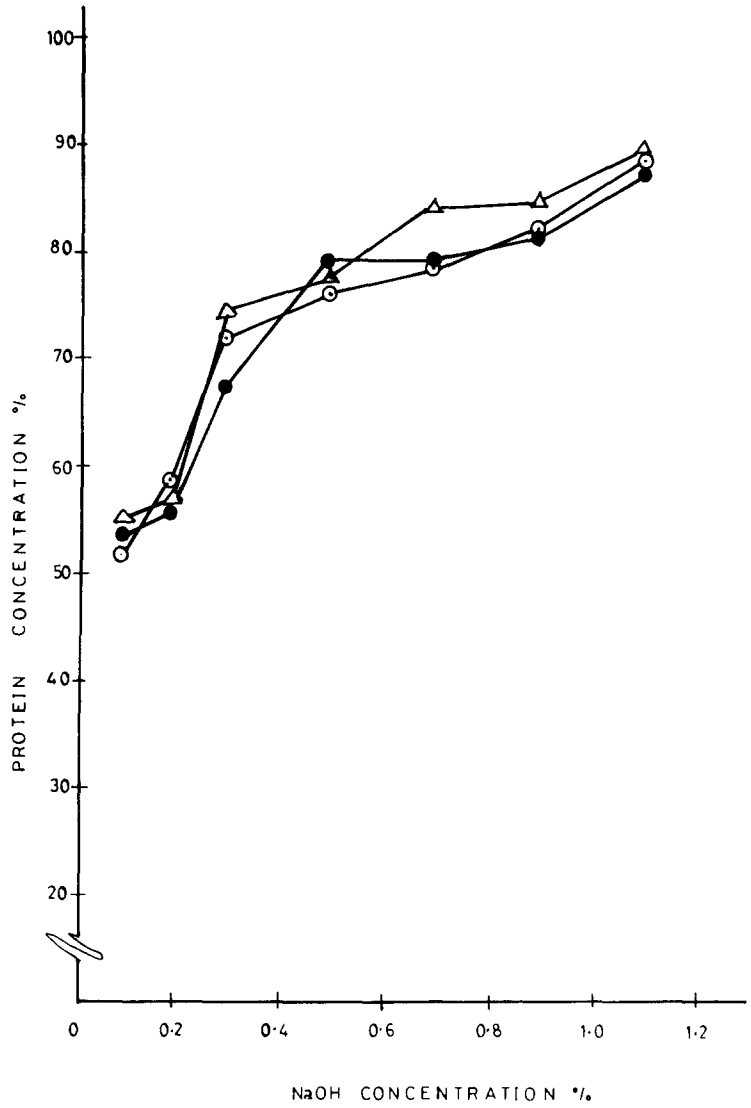

Fig. 1. Effect of NaOH concentration on protein concentration (w/v) in extract of whole seed (⊙), cotyledon (△) and endosperm (●) of seeds of *Delonix regia*.

protein solubility to approximately 74% and Del Valle *et al.* (1983) reported a solubility increase of mesquite protein from 65% to 86% by addition of 1% NaCl. The addition of 7% (w/v) NaCl raised the solubility from 14·5 to about 69·9 ± 6·5% in the whole seed, 66·2 ± 4·3% in the endosperm and 75·27 ± 5·42% in the cotyledon (Table 1).

Figure 4 shows the solubility of flamboyant seed protein in 3% sodium chloride solution at different pHs. About 42% of the protein was soluble at

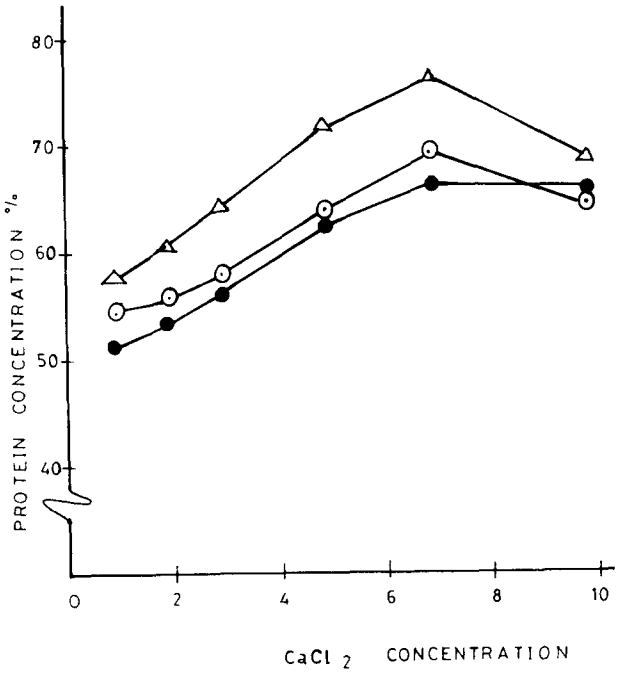

Fig. 2. Effect of $CaCl_2$ concentration on protein concentration (w/v) in extracts of whole seed (⊙), cotyledon (△) and endosperm (●) of seeds of *Delonix regia*.

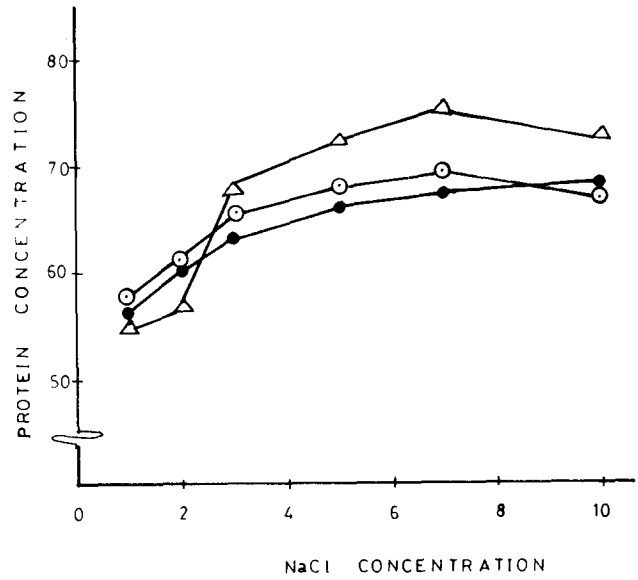

Fig. 3. Effect of NaCl on protein concentration (w/v) in extracts of whole seeds (⊙), cotyledon (△) and endosperm (●) of seeds of *Delonix regia*.

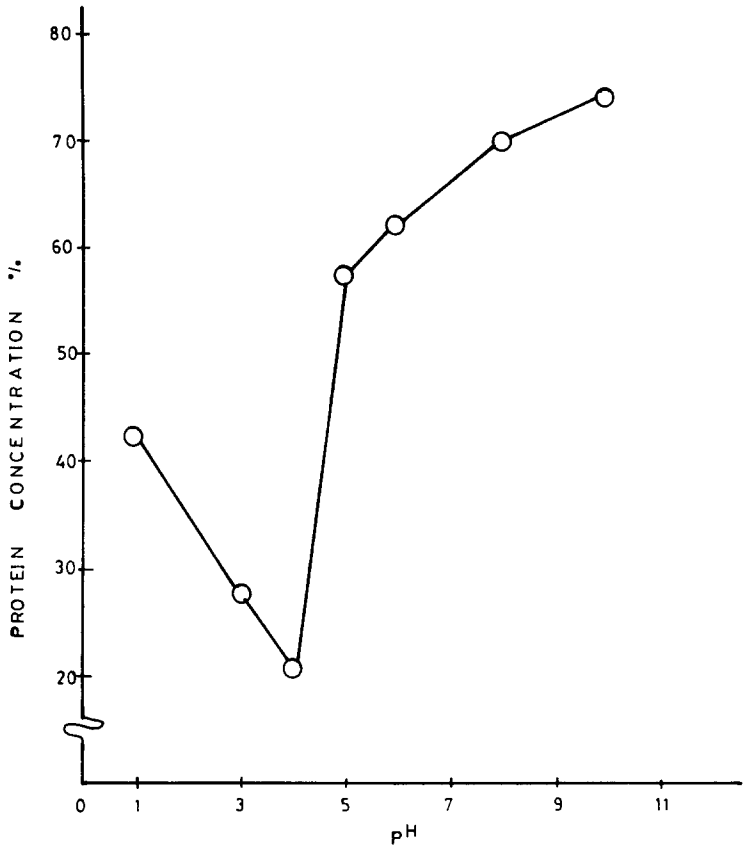

Fig. 4. Effect of pH on protein concentration (w/v) in extracts of whole seed of *Delonix regia.*

pH 1·0 but the solubility decreased sharply to about 21% at about pH 4·0. The solubility of the flamboyant seed protein in 3% NaCl, at various pHs, showed a profile of V-shape with the minimum solubility in the range pH 3–4·5. Solubility increased sharply from pH 5·0 to pH 8·0 and gradually from pH 8·0 to pH 11. At pH 10 the solution became very slimy and difficult to centrifuge at 3500 *g*.

The solubilities of the water-isolated, NaOH-isolated and NaCl-isolated proteins are shown in Fig. 5. The curves (Fig. 5) show that, at the isoelectric point, some proteins were still soluble irrespective of the solvent used to extract the protein. Comparing the solubility of the water, NaCl, and NaOH isolated proteins the water-isolated protein was most soluble in the pH range 6–11. The water-isolated was least soluble at pHs 1 and 2. The water and alkali isolates showed a small range of minimum solubility at pH 3–5 while the salt isolate showed a relatively wide range of minimum solubility at pH 3–6. These observations for salt- and water-isolated proteins were similar to observations made by Kabirulla & Wills

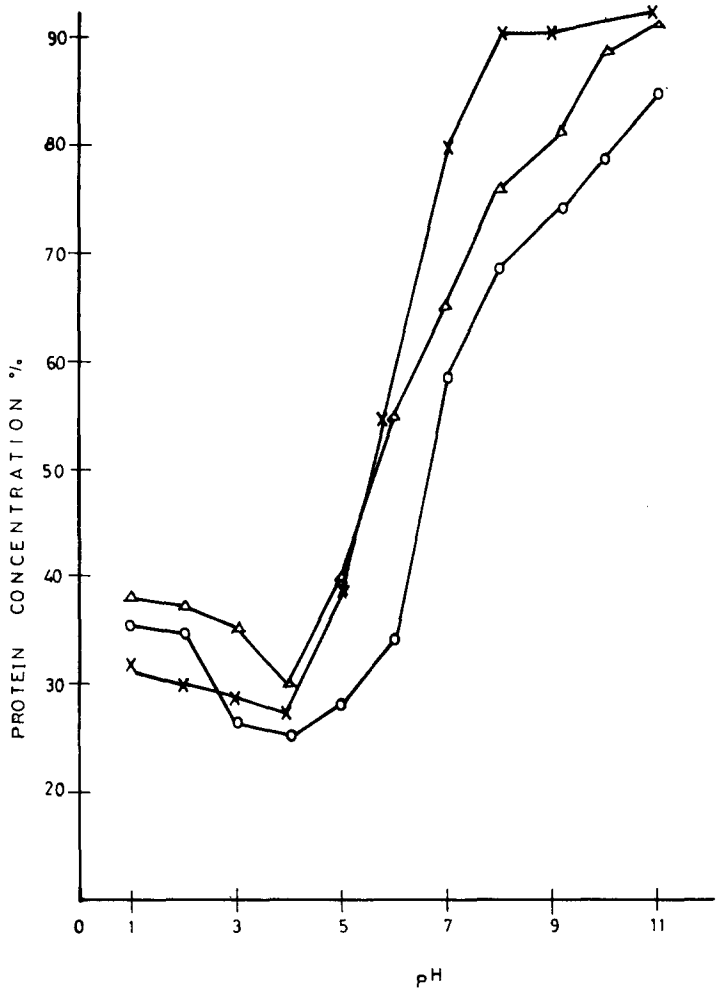

Fig. 5. Solubility of *D. regia* seed protein isolated from NaOH solution (△), NaCl solution (○) and water (×) at different pH.

(1983) in sunflower protein. The isoelectric point was about pH 4·5. Wolf (1977) observed that isolated proteins often have their isoelectric points around pH 5·0. Around the isoelectric point, solubility of flamboyant seed protein was about 26–30% of the total protein. A similar observation was made by Hang *et al.* (1970) for leguminous seed proteins.

The composition of the protein concentrate prepared from the NaCl extract of the cotyledon is shown in Table 2. The concentrate contained 78·8% protein (dry weight basis) or approximately twice as much protein as in the cotyledon flour. A similar result was reported by Wolf (1977). The protein content of the concentrate was 80·5% for the alkali isolate. Nilo

TABLE 2
Composition of the Cotyledon and the Protein Isolated from the Cotyledon of Seeds of Flamboyant Tree (*Denolix regia*)

	Colour (%)	H_20 (%)	*Protein* (%)	*Fat* (%)	*Ash* (%)	*Phytate* (%)	*Tannin* (%)
Cotyledon flour protein	Greyish	9·7 ± 0·5	35·8 ± 0·5	9·7 ± 0·4	5·9 ± 0·2	2·1 ± 0·3	1·2 ± 0·1
NaCl-isolated protein	White	3·2 ± 0·2	78·8 ± 1·8	0·3	3·8 ± 0·2	0·1[a]	0·3 ± 0·1[a]
NaOH-isolated protein	Cream	3·7 ± 0·4	80·5 ± 1·6	0·2 ± 0·1[a]	2·8 ± 0·1	0·1[a]	0·3 ± 0·1[a]

[a] Approximated to the nearest decimal place.

Rivas *et al.* (1981) obtained 95% protein for a sesame concentrate. Wolf (1977) reported that different raw materials yielded concentrates which usually contained about 80% protein. The concentrate contained 0·3 ± 0·2% tannin, about 0·1% phytate and about 0·3% ether extract. Phytate in food complexes some mineral ions such as Zn, Ca, Mg, etc., and decreases their bioavailability (O'Dell, 1969). The presence of phytate and tannin in the protein is therefore disadvantageous to the consumer.

Table 3 shows the results of the gel electrophoresis. The gel electrophoresis of the NaOH-isolated protein, NaCl-isolated protein and $CaCl_2$-isolated protein gave six bands for each. The band with the highest molecular weight ($85·1 \times 10^3$) was found in the $CaCl_2$-isolate and the band with the least molecular weight ($11·4 \times 10^3$) was found in NaOH-isolated protein. The water-isolate, on the order hand, gave a single band with a molecular weight of 69 000 daltons on gel electrophoresis.

The low molecular weight (Table 3) of the NaOH-isolated protein was probably due to hydrolysis of the protein by the alkali during extraction.

TABLE 3
Determination of Molecular Weight by Acrylamide SDS Gel Electrophoresis

NaOH	*NaCl*	*$CaCl_2$*	*H_2O*
81 300	83 200	85 100	
57 500	71 600	70 900	69 900
45 700	61 000	61 900	
26 700	42 200	41 700	
13 800	31 300	31 600	
11 000	23 700	24 000	

TABLE 4
Amino Acid Pattern of NaCl-Isolated Protein

Amino acid	*Sample (g/16 g N)*	*FAO/WHO*	*Chemical score*
Aspartic	10·3		
Tyrosine	3·5		
Serine	4·6		
Glutamic	15·1		
Proline	8·7		
Glycine	4·9		
Alanine	3·9		
Valine	5·3	4·2	126
Methionine	1·9	2·2	86
Isoleucine	3·7	4·2	88
Leucine	7·1	4·8	148
Threonine	3·2	2·6	123
Phenylalanine	4·3	2·8	154
Lysine	3·8	4·2	90
Histidine	2·2	2·4	92
Arginine	11·5		
Cysteine	1·2		

Amino acid pattern

Table 4 shows the amino acid pattern in the NaCl-isolated protein. The amino acid pattern of NaCl-isolated protein from the flamboyant seeds showed that the protein was deficient in lysine compared with FAO/WHO (1973) reference protein. It is, however, rich in glutamic acid, aspartic, proline and leucine. The levels of leucine, phenylalanine, threonine and isoleucine were higher but methionine was lower than the FAO/WHO (1973) reference protein. The sulphur-containing amino acids in the isolated protein constituted about 3·1 g/16 g N. The methionine content was slightly higher than that of common legume proteins.

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