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Aspects of Melon Seed Protein Characteristics

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ABSTRACT

Protein from Nigerian melon seed types Colocynthis citrullus *Linn) was studied by means of electrophoresis, chromatography on Sephadex, sedimentation and amino acid analysis. The major protein, a globulin, was separated into three fractions by gel filtration and ultra-centrifugation. The major fraction had a molecular weight of about 300 000 by gel filtration. SDS-PAGE fractionated the globulin into six subunits. The major subunits have molecular weights of 33 700, 29 600 and 21 600. The water-soluble protein contained only one main band of molecular weight 12 000. The total salt-extractable protein was characterised by a low level of lysine and relatively large amounts of glutamic acid, aspartic acid and arginine. The last three amino acids accounted for over a third of the total amino acid content. No marked differences were observed in the amino acid profile, electrophoretic or gel chromatographic properties of protein from different seed types.*

INTRODUCTION

The egusi melon (*Colocynthis citrullus*) (Oyolu, 1977) is one of the members of the Cucurbitacea or gourd family which is widely cultivated

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in Nigeria and others parts of West Africa for its seeds. The protein-rich oil meal obtained from the ground seed kernel is used to thicken and emulsify soups or is fermented to produce a flavouring agent.

In spite of its nutritional and functional importance, the proteinaceous component of the seed has been little studied. Oyenuga & Fetuga (1975) studied the amino acid composition and nutritive value of the protein in melon seed meals. Stafford & Oke (1977) investigated the feasibility of extracting melon seed proteins for supplementing weaning foods.

The attractive aspects of melon seeds include a high concentration of protein and the absence of any known anti-nutritional factors. However, any programme for effectively and efficiently utilizing melon seed protein products on a large scale should be based on a knowledge of the physico-chemical properties of the protein in melon seed. Onuora & King (1983) reported on the factors affecting the extractability of melon seed proteins and showed that the major protein is a globulin. Proteins from five melon seed types were studied by chromatography on Sephadex, electrophoresis, sedimentation and amino acid analysis.

MATERIALS AND METHODS

Melon seeds

Samples of melon seeds types 1 to 5 (Oyolu, 1977) were supplied by Dr C. Oyolu of the Department of Crop Science, University of Nigeria at Nsukka, Nigeria. Sun-dried melon seeds, corresponding to Oyolu's type 5 and designated 'market sample' in this study, were obtained from a wholesale dealer in Enugu, Nigeria.

Melon seed flour

Unheated solvent-defatted flours were prepared as previously reported (Onuora & King, 1983).

Protein solubility fractions

Protein fractions based on solubility according to the classical Osborne method were obtained as described earlier (Onuora & King, 1983). The extracts, with each solvent corresponding to the water-soluble protein

(WSP), globulin (GLOB), prolamin (PROL) and glutelin (GLUT) fractions, respectively, were pooled and dialysed at 2·2 °C in cellophane tubing against distilled water. Dialysis was stopped after 48 h when the globulin fraction was free of chloride ions. The dialysed extracts and the residue (RES) were freeze-dried and stored at 2·2 °C until required.

Total salt-extractable protein

Total salt-extractable protein (TSSP) was prepared by extracting a 1-g sample of flour with three 20-ml aliquots of 0·5M sodium chloride solution at 18 °C for 1 h each time. The salt solution was buffered at pH 7 with 0·01M phosphate buffer containing sodium azide (0·02%, w/v). The extracts from each sample were combined, centrifuged, dialysed and freeze-dried as described above.

Total protein

Total extractable melon seed protein (TP) was prepared by extracting melon seed flour with the treatment buffer used for SDS–PAGE. Detergent (SDS) solution has been shown to extract all melon seed nitrogen (Onuora & King, 1983).

Protein isolates

The dilute alkali isolate (PA) was prepared by extracting melon seed flour with dilute sodium hydroxide solution (pH 10·2). The salt isolate (PS) was prepared by extracting with 0·5M sodium chloride solution, pH 7. Each extract was acidified to pH 4 with 1M hydrochloric acid solution in order to precipitate the protein curd. The curd was washed with water and neutralized with a few millilitres of 1M sodium hydroxide solution, bringing the pH to 7. The neutralized suspension was freeze-dried. Using an alternative procedure, two isolates were prepared from the one sample of melon seed flour by successively extracting with two solvents. In one case the solvents were distilled water (pH 7) followed by dilute sodium hydroxide (pH 10·2). The proteins in the water extract (IIW) and alkali extract (IIWA) were recovered by acid precipitation, neutralization and freeze drying. An extract (IICA) was similarly prepared by extracting with dilute hydrochloric acid (pH 2) and dilute sodium hydroxide (pH 10·2).

Electrophoresis

Samples of melon seed flour were extracted for 1 h with a treatment buffer. A small aliquot of the extract was diluted with distilled water or treatment buffer, mixed with a few drops of glycerol and a drop of tracker dye (bromophenol blue solution). Freeze-dried protein fractions, extracts and isolates were simply dissolved in the treatment buffer. Any undissolved solid was removed by centrifugation. The final solution contained approximately 1·5 mg per millilitre of protein. The treatment buffer for ordinary polyacrylamide gel electrophoresis (PAGE) was a 0·02M glycine/sodium hydroxide buffer (pH 10) containing 2%(w/v) 2-mercaptoethanol to keep the proteins reduced. For SDS–PAGE it was a 0·01M sodium phosphate buffer, pH 7·0, containing 1%(w/v) SDS and 1%(w/v) 2-mercaptoethanol. All samples containing SDS were incubated for 2 h at 37 °C.

Ordinary PAGE was carried out using a Shandon analytical PAGE apparatus Mark 111, whereas SDS–PAGE was performed in a Shandon vertical slab PAGE apparatus Mark 11 (Shandon Southern Products Ltd, Runcorn, Cheshire, Great Britain). The separating gels were cast using 7·5%(w/v) total acrylamide for PAGE and 10% for SDS–PAGE. The rods were run at a constant current of 3 mA per tube, whilst the slabs were run at a constant voltage of 150 V.

Gels were fixed in 12·5%(w/v) trichloroacetic acid solution before staining with 0·25%(w/v) PAGE Blue 83 (BDH Ltd, Poole, Great Britain). Bands were located by viewing with transmitted light from an evenly illuminated translucent screen.

Gel filtration

Samples of unheated melon seed flour (MSF) prepared from all of the seed types were extracted with eluant solution, 0·01M sodium phosphate buffer, pH 7, containing 0·5M sodium chloride and 0·02% sodium azide. A 4-ml aliquot of each extract was applied directly to the gel column. Alternatively, the extract was dialysed, freeze-dried for storage and resolubilized in eluant before chromatography.

The proteins were fractioned on a column (2·6 × 85 cm) of Sephadex G-200 using upward elution at the rate of 10 ml/h. The absorbance at 280 nm of the 3 ml fractions was plotted against the elution volume.

The void volume of the column was determined with blue dextran

(molecular weight = 2 000 000). For the estimation of molecular weights, the column was calibrated by determining the elution volumes of five standard proteins: ribonuclease A, chymotrypsinogen A, ovalbumin, aldolase and apoferritin.

Ultracentrifugation

Analytical ultracentrifugation of protein solutions was performed using a MSE Centriscan 75 ultracentrifuge. A 0·5-ml sample was analysed under vacuum at 15 °C or 20 °C at a fixed speed. Samples of protein were dissolved in 1·00M or 0·5M sodium chloride in phosphate buffer (pH 7·0).

Amino acid analysis

The amino acid composition of melon seed salt-extractable protein was determined with an autoanalyser (model JLC-6AH Jeol (UK) Ltd) (Hamilton, 1963). An accurately weighed sample of about 100 mg was hydrolysed in about 20 ml of 6M hydrochloric acid solution under nitrogen at 110 °C for 22 h. The hydrolysate was filtered and the filtrate diluted to a known volume with distilled water. An aliquot was evaporated under vacuum and the residue was dissolved in sodium citrate buffer.

RESULTS AND DISCUSSION

Electrophoresis

The PAGE patterns of glycine/sodium hydroxide buffer (pH 10) extracts of flours prepared from seed types 1, 2, 3, 4 and 5 and the market sample in each case revealed eight bands with relative mobilities of 0·06, 0·18, 0·28, 0·36, 0·44, 0·54, 0·64 and 0·80. The major band had a relatively low mobility (0·16–0·18). Since over ninety per cent of melon seed nitrogen is soluble at pH 10 (Onuora & King, 1983) the extracts contained most of the protein of the melon seed types.

It is possible that the high pH of the extraction buffer caused depolymerization by partial hydrolysis of protein molecules, accounting for some of the bands. However, a high pH was necessary because of the low solubility of the major fraction of melon seed protein at less alkaline

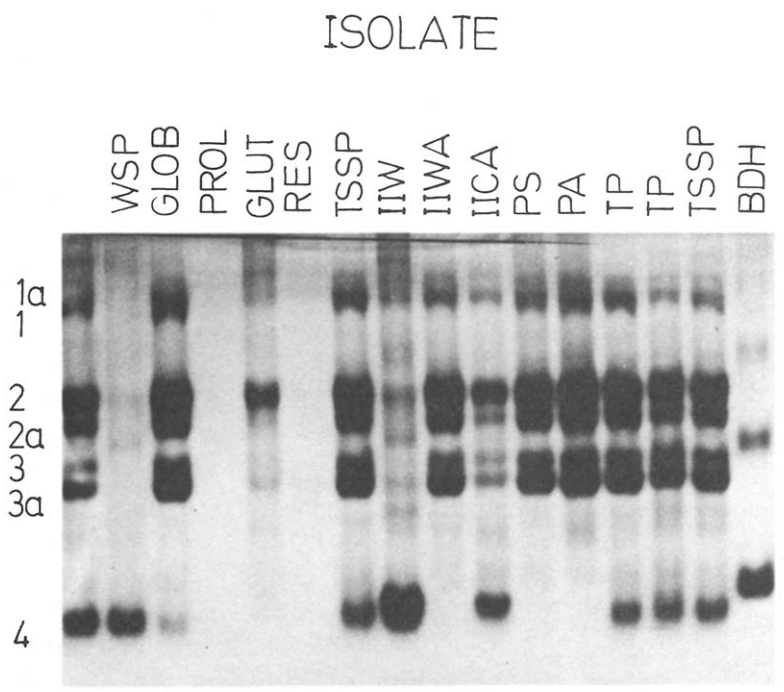

Fig. 1. SDS-PAGE of melon seed protein fractions and isolates. WSP, water-soluble protein fraction; GLOB, globulin; PROL, prolamin fraction; GLUT, glutelin fraction; TSSP, total salt-extractable protein; IIW, isolate (after acid extraction); PS, salt isolate; PA, alkali isolate; TP, total protein of melon seed flour; TSSP, total salt-extractable protein; BDH, molecular weight markers (14 300–71 500); RES, residue.

pH values (Onuora & King, 1983). The addition of sodium chloride to the buffer was not considered because the concentration required to appreciably increase the solubility at neutral pH would be impractical for electrophoresis.

A better resolution of melon seed protein components was achieved using SDS–PAGE (Fig. 1). Molecular weight markers were included as a means of estimating the subunit molecular weight (Table 1). A difference in the number and electrochemical properties could be observed. The electropherograms of the crude buffer extracts and that of the dialysed extracts were indistinguishable. No advantage in resolution is gained by dialysing melon seed protein extracts before SDS–PAGE.

The total protein extract and the total salt-extractable protein show a characteristic SDS–PAGE pattern of three pairs of bands and a single

TABLE 1
Subunit Molecular Weight in Melon Seed Total Salt-Extractable Protein

Band No.	*Relative mobility (%)*	*Molecular weight*	*Relative intensity*
1	14·5	58 800	Weak
1a	19·1	53 000	Medium
2	39·2	33 700	Very strong
2a	44·9	29 600	Strong
3	53·1	24 600	Medium
3a	58·8	21 600	Strong
4	84·6	12 100	Medium

broad fast moving band: 1, 1a; 2, 2a; 3, 3a and 4 (Fig. 1). The water-soluble protein fraction and the water isolate have identical patterns consisting mainly of band 4 and traces of other bands. The globulin and all the isolates except the alkali extract (IICA) have similar electropherograms. Each electropherogram contains bands of the total protein extract except band 4. The glutelin fraction shows one major band, band

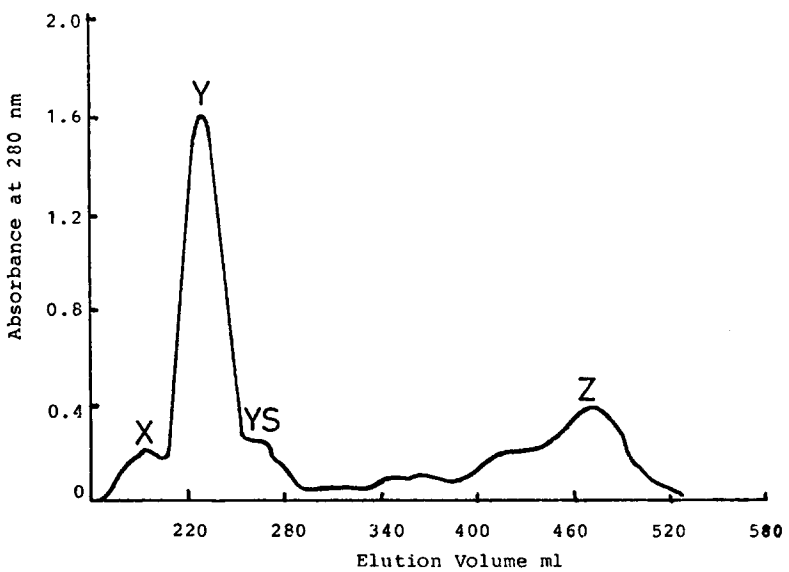

Fig. 2. Gel filtration pattern of salt-extractable protein of melon seed, market sample.

2, and traces of the others except 4. The prolamin fraction and the residue show no bands, implying that these fractions were devoid of protein. Isolate IICA contains all the typical melon seed protein bands. However, bands 2a, 3 and 3a have diminished intensities. The subunit molecular weights determined for the various melon seed protein materials are shown in Table 1. The estimated molecular weights ranged from 12 000 for band 4 to 59 000 for band 1. Stafford & Oke (1977) reported only two major bands with molecular weights of 21 200 and 32 000; these probably correspond to bands 3a and 2.

The water-soluble protein of melon seed prepared by dialysis of the water extract of melon seed flour and the water isolate precipitated with acid have identical SDS electropherograms. The major component in these protein fractions is the fast moving band 4. The extracts GLOB, PA, PS and IIWA (Fig. 1) contain the same protein components—the six larger subunits with molecular weights of 21 000, 24 600, 29 600, 33 700, 53 000 and 58 800. These components constitute the major protein of melon seed. The absence of the 12 000 molecular weight band from the electropherograms was not expected as the salt and alkali would extract the water-soluble protein fraction in addition to the other fractions. That this is indeed the case is proved by the presence of band 4 in the band pattern of the total salt-extractable protein (TSSP). The absence of this band in the salt and alkali isolate patterns suggests that this protein fraction is more difficult to precipitate and tends to remain soluble during the precipitation stage of the isolate preparation.

Gel filtration

The elution patterns of the salt-extractable protein of seed types 2–5 and of the market sample on Sephadex G-200 were found to be identical (Fig. 2). In each case there is one main peak (Y) of relatively high molecular weight and a broad diffuse band (Z) of lower molecular weight. The main peak has two shoulders, one eluting in advance (X) and the other eluting behind (YS). Fractions Y and YS had a high 280/260 nm absorption ratio of 1·57, indicating that they may be pure protein whereas the corresponding ratios for fractions X and Z were 1·07 and 1·05 respectively, suggesting that they may be contaminated with nucleic acid. The estimated molecular weights of these fractions are presented in Table 2. Since the separations were not sharp, the molecular weights determined must be regarded as estimates. No estimate was made for the very diffuse band (Z). The fact that dialysis

TABLE 2
Molecular Weight of Salt-Extractable Melon Seed Protein Fractions Separated on a Sephadex G-200 Column

Seed type	*Protein fraction*	*Elution volume (ml)*	*Estimated molecular weight ($\times 10^5$)*
2	X	194	5·79
	Y	236	2·79
	YS	262–272	1·77–1·49
3	X	195	5·69
	Y	230	3·09
	YS	254–262	2·03–1·77
4	X	195	5·69
	Y	230	3·09
	YS	260–269	1·83–1·57
5	X	193	5·89
	Y	228	3·20
	YS	260–269	1·83–1·57
Market sample	X	194	5·79
	Y	230	3·09
	YS	260–266	1·83–1·65
Market sample (dialysed)	X	194	5·79
	Y	230	3·09
	YS	263–266	1·74–1·65

of the protein extract prior to gel filtration significantly reduced the size of this band means that this fraction in the crude extracts contains uv-absorbing non-protein molecules.

The molecular weights of the protein fractions are much larger than the subunit molecular weights determined by SDS–PAGE. The major gel filtration fraction has a molecular weight of about 300 000, whereas the largest subunit is about 59 000. The melon seed protein must therefore be highly polymerized.

SDS–PAGE of the eluted fractions was carried out in order to rationalize gel filtration and electrophoresis data. Since peak Y and the shoulder YS were not clearly separated, they were recovered for electrophoresis as a mixture; the results are summarized in Table 3.

The high MW shoulder, X, generally contained bands 2 and 3a equivalent to subunit MW 34 000 and 22 000, respectively. The major gel filtration peak and shoulder (Y + YS) contained all the melon seed subunits except band 4. Band 4 was present only in peak Z. It is clear from

this that the low molecular weight fraction, Z, in all the seed types is equivalent to the water-soluble protein because it contains only the SDS–PAGE band 4, as do extracts WSP and IIW. Since the salt extract eluted on the gel contained water-soluble protein and globulin, the high MW fractions X and Y + YS are globulin. This is confirmed by the presence of the characteristic globulin bands in the SDS–PAGE pattern

TABLE 3
SDS–PAGE Bands of Salt-Extractable Melon Seed Protein Fractions Separated on a Sephadex G-200 Column

Seed type	*Protein fraction*	*Bands present*
2	X	2 3a
	Y + YS	1,1a 2,2a 3,3a
	Z	4
3	X	2 3a
	Y + YS	1,1a 2,2a 3,3a
	Z	4
4	X	2 3a
	Y + YS	1,1a 2,2a 3,3a
	Z	4
5	X	2 3a
	Y + YS	1a 2,2a 3,3a
	Z	4
Market sample (dialysed)	X	1a 2 3a
	Y + YS	1a 2,2a 3,3a
	Z	4
Market sample (crude extract)	Y + YS	1a 2,2a 3,3a
5	TSSP	1,1a 2,2a 3,3a 4
Market sample	TSSP	1,1a 2,2a 3,3a 4

of these fractions. It is therefore concluded that melon seed globulin consists of three components with molecular weights of 570 000–590 000, 310 000 and 160 000–200 000. There is no report in the literature known to the authors on gel chromatography of protein in Nigerian melon seeds. However, Mourgne *et al.* (1969) separated the globulin of another cucurbit (*Cucurbita maxima*) into three components on DEAE-Sephadex. The predominant component had a molecular weight of 340 000.

Sedimentation

The sedimentation characteristics of salt-extractable melon seed protein samples in sodium chloride solution are summarized in Table 4. The sample designated as 'major globulin' is equivalent to fraction Y + YS separated by gel filtration.

In 1M sodium chloride TSSP and GLOB yielded three components. The middle component was in greatest concentration in both samples and had sedimentation constants (S) of 10·3 and 8·7, respectively. The other components were present at too low a concentration to permit the calculation of sedimentation constants. In 0·5M NaCl phosphate buffer, pH 7, the eluants used for gel filtration, TSSP and GLOB each yielded three components as in 1M sodium chloride solution. The middle component (S = 10·9) was again dominant. The extract WSP did not sediment even at 116 000 *g*.

TABLE 4
Sedimentation Characteristics of Melon Seed Protein in Sodium Chloride Solution

Protein sample	*Centrifugation conditions*			*Number of components*	*Sedimentation constant of major component*
	NaCl conc. (M)	*RCF* (*g*)	*Temp.* (°C)		
TSSP	1·0	116 000	15	3	10·3
TSSP	0·5	180 000	20	3	10·9 6·5[a]
GLOB	1·0	116 000	15	3	8·7
GLOB	0·5	262 000	15	3	10·9
Major GLOB	1·0	116 000	15	2	9·0

[a] Minor component.

Amino acid profile

The amino acid compositions of salt-extractable protein of melon seed types and of the major globulin are given in Table 5. These data indicated that the protein of melon seeds, like oil seeds in general, was rich in arginine, aspartic and glutamic acid which together accounted for over a third of the total amino acids. In a survey of the amino acid compositions of meals and seed globulin of several cucurbits, Jacks *et al.* (1972) observed that arginine, aspartic and glutamic acid were the most abundant amino acids. The low level of lysine agrees with previous

TABLE 5
Amino Acid Compositions of Salt-Extractable Protein of Melon Seed Types (g Amino Acid/16 g N)

Amino acid	*Seed type*						*Major globulin*
	1	*2*	*3*	*4*	*5*	*Market sample*	
Essential							
Iso-leucine	3·1	3·6	3·6	3·6	3·4	3·0	4·1
Leucine	5·4	5·8	6·0	6·0	5·7	5·1	6·3
Lysine	3·3	2·5	2·5	2·7	2·6	2·4	2·9
Methionine	3·6	3·4	2·7	2·7	3·1	3·3	1·7
Phenylalanine	4·3	4·8	4·8	4·8	4·5	4·0	5·9
Threonine	2·7	3·1	2·9	2·9	2·9	2·6	3·4
Valine	3·3	3·8	3·9	3·9	3·7	3·3	4·7
Non-essential							
Alanine	3·7	4·0	4·2	4·0	4·0	3·8	4·6
Arginine	13·3	13·6	14·8	15·2	13·6	13·0	14·4
Aspartic acid	7·3	7·9	8·0	8·0	7·9	7·0	9·1
Glutamic acid	15·0	16·0	16·7	16·0	16·2	14·7	15·9
Glycine	3·8	4·1	4·3	4·3	4·3	4·0	4·3
Histidine	2·0	1·7	2·1	2·2	2·1	2·0	2·1
Proline	3·6	3·4	4·8	3·0	4·0	3·4	3·3
Serine	3·9	4·2	4·3	4·2	4·3	3·7	4·7
Tyrosine	2·5	2·6	2·7	2·9	2·7	2·5	2·9

reports (Oyenuga & Fetuga, 1975; Stafford & Oke, 1977). Differences between the melon seed types were relatively small. The salt-extractable protein of seed type 1 and the market sample generally contained slightly lower levels of amino acids than the rest. The main difference between the major globulin component and the total salt-soluble protein of the seeds was that the former contained a lower concentration of methionine and a higher concentration of many other amino acids, particularly the essential ones.

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