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Functional Properties of the Proteins of Some Nigerian Oilseeds: Conophor Seeds and Three Varieties of Melon Seeds

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Cucumeropsis edulis has a protein content of 38% and the two varieties of *Citrullus vulgaris* have 34.1% and 30.8%, respectively. *Tetracarpidium conophorum* has the lowest protein content (23.4%). The fat contents of the oilseeds ranged from 43 to 51%. The solubility profiles of the flour proteins showed minimum solubility at two pH values. However, the nitrogen solubility profiles for the protein isolates were more simple in that they showed single isoelectric points. The following functional properties—water-soluble nitrogen, fat absorption capacity, emulsion capacity, and water holding capacity—were determined and found to be comparable to those of soy products. The flours have good foaming stability but poor foaming capacity when compared with soy flour.

As a result of rapid population growth, there is an increasing demand for plant products with aesthetic and organoleptic appeal in the diet. It is desirable to have proteins with relevant functional properties. Some of the functional properties include water holding capacity (Paulsen, 1961; Ziemba, 1966), increase in viscosity (Wood, 1967; Circle et al., 1964), gelling properties (Rakosky, 1970; Frank and Circle, 1959), emulsifying properties (Pearson et al., 1965; Inklaar and Fortuin, 1969), etc.

Proteins have to be highly soluble in order to make it easier to incorporate into food systems. Many workers use the nitrogen solubility index (NSI) or protein dispersibility index (PDI) as a quick test for the functional properties of the proteins (Johnson, 1970).

Soyabean, sunflower, sesame, cotton, and castor are some of the few oilseeds whose protein products, i.e., the grits, flours, concentrates, or isolates, have been extensively used to fortify bakery products (Lemancik and Ziemba, 1962; Ziemba, 1966; Wood, 1967), cereal products (Paulsen, 1961), dairy products (Circle and Smith, 1972), and comminuted processed meat (Rock et al., 1966; Pearson et al., 1965; Inklaar and Fortuin, 1969).

Among the most commonly eaten oilseeds in Nigeria are melon seeds, conophor, and peanuts. Only the functional properties of peanuts have thoroughly been studied. This work is therefore carried out to study the solubility and functional properties of proteins of these oilseeds.

EXPERIMENTAL SECTION

Oilseed Flour Preparation. Conophor seeds (*Tetracarpidium conophorum*) and three melon seeds, viz., *Cucumeropsis edulis* and two varieties of *Citrullus vulgaris*, were purchased locally from the market in Ile-Ife, Oyo State, Nigeria. They were cracked and the hulls removed by windsifting. They were coarsely ground in a hammer mill. The oil in the ground seeds was extracted with petroleum ether 40–60 °C in a Soxhlet apparatus and the resultant flakes desolventized by air-drying. The meal was reground with a small sample mill, and the products were extracted again in the Soxhlet apparatus and air-dried. The final product was stored in polythene bags in the freezer at −20 °C until used.

Oilseed Protein Isolate Preparation. A known weight of the defatted meal was dispersed in an amount of distilled water to give a final meal to liquid ratio of 1:20. The dispersion was gently stirred on a magnetic stirrer for 30 min. The pH of the resultant slurry was adjusted to the point where the protein was most soluble. This had been previously determined in preliminary work, by dropwise addition of 0.01 M NaOH (Figure 1). The extraction was allowed to proceed with gentle stirring for 24 h keeping the pH constant. Nonsolubilized material was removed by centrifugation at 3500 rpm for 30 min. The proteins in the extract were precipitated by dropwise addition of 0.01 N HCl with constant stirring until the pH was equivalent to the point where the protein was least soluble. This was centrifuged at 3500 rpm for 30 min in order to recover the protein. This was then dialyzed against distilled water overnight with stirring on a magnetic stirrer with several changes of water for 48 h at room temperature. The dialysate was freeze-dried and stored in the freezer at −20 °C for further work.

Extractability of the Proteins of Defatted Meal and Isolates as a Function of pH. Two grams of defatted meal and 40 mL distilled water were thoroughly mixed on a magnetic stirrer at room temperature. The pH of slurries prepared from the samples was adjusted to values between 1 and 12 by using either 0.01 N HCl or 0.01 N NaOH.

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Table I. Proximate Composition of the Oilseeds

sample	crude fat, %	crude protein, %	moisture, %	crude fiber, %	NFE, %	ash, %
1, *Tetracarpidium conophorum*	50.6	23.4	2.8	5.7	14.5	3.1
2, *Cucumeropsis edulis*	43.7	38.1	2.1	2.0	5.1	9.0
3, *Citrullus vulgaris* variety 1	47.7	34.1	7.9	7.8	4.3	5.2
4, *Citrullus vulgaris* variety 2	51.1	30.8	5.6	3.2	6.5	2.8

[a] NFE = nitrogen-free extractive.

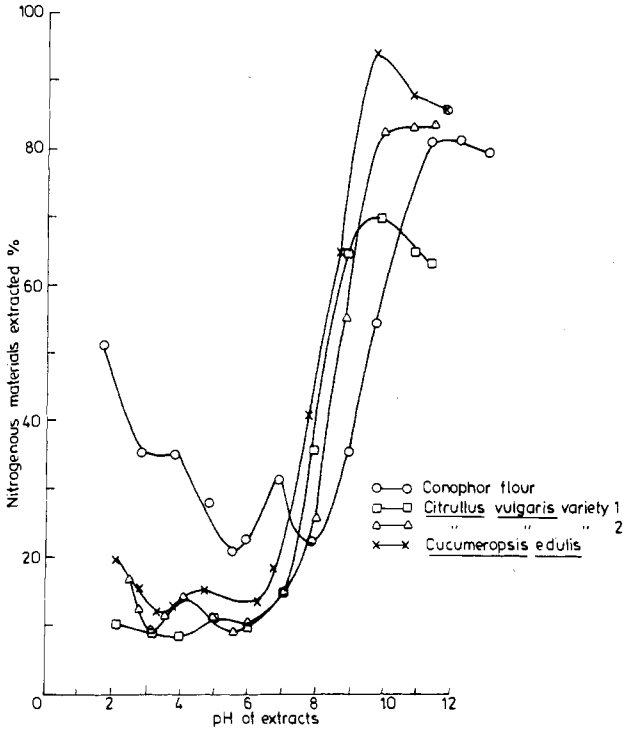

Figure 1. Extractability of conophor flour, *Cucumeropsis edulis* flour, *Cirtullus vulgaris* variety 1 flour, and *Cirtullus vulgaris* variety 2 flour as a function of pH.

Insoluble materials were removed by centrifugation at 3500 rpm for 30 min. The nitrogen contents of the supernatant were determined in duplicate by the American Association of Cereal Chemists micro-Kjeldahl method (1969). The extratibilities of the proteins at optimum pH (10–12) for extraction were *T. conophorum* 85%, *C. edulis* 94%, *C. vulgaris* (variety 1) 83%, and *C. vulgaris* (variety 2) 70%. Analysis of nitrogen was also carried out on a slurry of the defatted meal in distilled water. This is the natural solubility of the protein.

Emulsion Capacity of the Defatted Oilseed Meals and Isolates. Emulsion capacities of the defatted meals and isolates were compared to that of soybean isolate by a modification of the procedure of Inklaar and Fortuin (1969).

Two grams of protein was made into a slurry in 40 mL of water in an Erlenmeyer flask by stirring at 1000 rpm for 15 min with a 1.5-in. magnetic bar. After 10 mL of corn oil was added over a period of 5 min with stirring at 1000 rpm, stirring was continued for an extra minute. The system was transferred to a centrifuge tube, heated in a bath maintained at 85 °C for 15 min with occasional stirring, and then cooled for 15 min in a water bath maintained at 25 °C. The tube was finally centrifuged at 3000 rpm until the volume of oil separated from the emulsion was constant. Results were expressed as percentage of oil that separated from the emulsion layer.

Fat Absorption of the Defatted Meals and Isolates. Fat absorption was determined according to the procedure of Sosulski (1962). A 0.5-g sample and 3.0 mL of corn oil were poured into a 15-mL conical graduated centrifuge tube. The contents were stirred with a glass rod to disperse the sample in the oil. After a holding period of 30 min, the tube was centrifuged at 3200 rpm for 25 min and the volume of separated oil was read. Fat absorption was expressed as percentage of corn oil bound by 100 g of sample.

Water Holding Capacity of the Defatted Meals and Isolates. The Sosulski (1962) procedure was used. Excess water was added to 1.5 g of sample in weighed centrifuge tubes. The suspension was mixed vigorously 4 times with a glass rod, allowing 10-min rest periods between each mixing. The suspension was centrifuged at 3250 rpm for 25 min, the supernatant was decanted, and the tubes were air-dried. The water bound was calculated from the increase in the weight of the sample.

Foaming Capacity and Foaming Stability of the Defatted Meals and Isolates. Modified method of Lin et al. (1974) was used. Two grams of sample was put into a container containing 50 mL of distilled water. The sample was mixed with water by using a food mixer at a speed set for fast beating. The volumes before and after whipping in a 1000-mL graduated cylinder were recorded. The percent volume increase due to whipping was calculated according to the method of Lawhon et al. (1972). The volumes of foams in the standing cylinder for foam stability at $^1/_2$, 1, 10, and 120 min after whipping were recorded.

RESULTS AND DISCUSSION

The proximate composition of the oilseeds are shown in Table I. All the four oilseeds studied have high levels of fat, the lowest being 43.7%. Crude protein is high in all the varieties of melon seed but somewhat lower protein content was found in *T. conophorum*.

This oilseed also contains a very high level of NFE as compared to the melon seeds (14.5% vs. 6%). The protein contents of the defatted melon seeds are much higher than those in *T. conophorum*.

Functional Properties of the Oilseeds. *Solubility in Water.* Water-soluble nitrogen was determined by preparing slurries from the flours in water (1:20). The results are shown in Table II. *Tetracarpidium* and *Cucumeropsis* show the highest solubility.

Solubility in Nitrogen as a Function of pH. (1) Proteins in the Oilseed Flours. The results are depicted in Figure 1. Conophor flour shows more solubility at acid pH. About 50% is soluble at pH 2.0, whereas the other oilseeds show a solubility of lower than 20% at this pH. All the proteins show high solubility at pH 9.0. Minimum solubilities of the N is at pH 5.5 and pH 8.0 for Conophor.

For the melons, however, the minimum solubility is at low pH, about pH 3.0 and 5.5, respectively. That there are two points of minimum solubility suggests that there may be two major proteins in these oilseeds. The higher

Table II. Water-Soluble Nitrogen of the Oilseeds

sample	natural pH of slurry	% N soluble in water
1, *Tetracarpidium conophorum*	6.0	22.9
2, *Cucumeropsis edulis*	6.5	14.9
3, *Citrullus vulgaris* variety 1	5.6	9.2
4, *Citrullus vulgaris* variety 2	6.0	10.6

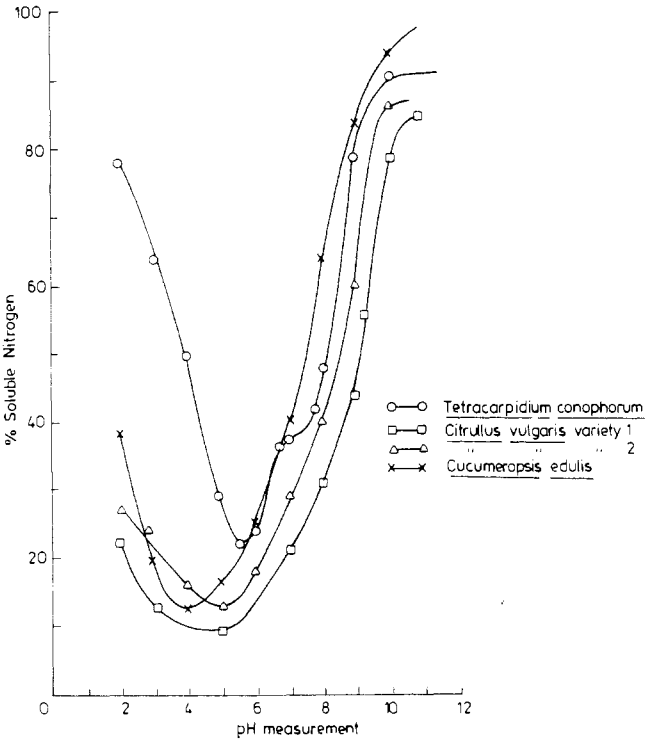

Figure 2. Nitrogen solubility profiles for four oilseed protein isolates.

solubility of conophor at acid pH suggest that the proteins of this nut may be used in acid food, e.g., beverages. To do the same for melon seeds, one may have to hydrolyze the proteins to small peptides—a process that has been known to give rise to bitter peptides.

(2) *Protein Isolates.* The solubility profile of the protein isolates are shown in Figure 2. Here there is a virtual elimination of the double minimum solubilities observed for the flours.

The lower pH (5.5) for minimum solubility for conophor was retained with a slight shoulder at higher solubility at pH 7.5.

The minimum solubilities for *Citrullus vulgaris* varieties 1 and 2 was at pH 4.5 and pH 5.0, respectively, and for *Cucumeropsis* was at a very low pH of 3.5.

Tetracarpidium showed a very high solubility of 80% at pH 2.0 whereas *Cucumeropsis* showed 40% solubility and *Citrullus* showed lower than 25% solubility at this pH. This suggests that the isolate of *Cucumeropsis* will find a very good use in acid beverages and foods. That the double minimum points found in the flours are eliminated in the isolates is probably due to the absence of some interfering substances eliminated during the preparation of the isolates. These substances may affect the charges on the proteins and as such may modify their solubility behavior near the isoelectric points. The solubility properties of isolated proteins differ from those of proteins in their native form (Fontaine et al., 1946).

Table III. Water Holding Capacity of Isolate of Defatted Flours of Oilseeds

sample	flour, %	isolate, %
1, *Tetracarpidium conophorum*	100.0 ± 0.6	141.3 ± 6.4
2, *Cucumeropsis edulis*	200.0 ± 0.6	230.7 ± 6.5
3, *Citrullus vulgaris* variety 1	228.8 ± 0.3	253.9 ± 0.9
4, *Citrullus vulgaris* variety 2	266.6 ± 0.3	398.0 ± 0.7
soy flour	130.0	
soy flour concentrate	227.3	
soy isolate (promine)[a]		416.7

[a] Promine: method of purification.

Table IV. Fat Absorption and Emulsion Capacity of the Oilseeds *Tetracarpidium conophorum*, *Cucumeropsis edulis*, and *Citrullus vulgaris*

sample	fat absorption, %	emulsified, %
1, *Tetracarpidium conophorum* flour	98.5 ± 0.3	2.1 ± 0.2
2, *Tetracarpidium conophorum* isolate	137.0 ± 0.5	18.3 ± 0.3
3, *Cucumeropsis edulis* flour	175.0 ± 0.8	44.9 ± 0.6
4, *Cucumeropsis edulis* isolate	242.1 ± 0.2	63.2 ± 0.2
5, *Citrullus vulgaris* variety 1 flour	219.0 ± 0.4	79.1 ± 0.6
6, *Citrullus vulgaris* variety 1 isolate	252.0 ± 0.6	102.4 ± 0.7
7, *Citrullus vulgaris* variety 2 flour	263.8 ± 0.2	88.1 ± 0.2
8, *Citrullus vulgaris* variety 2 isolate	301.8 ± 0.8	101.6 ± 0.4
9, wheat flour[a]	84.2	7-11
10, soy flour[a]	84.4	18.0
11, soy concentrate Isopro	133.0	2.8
12, soy isolate (promine)	119.2	22.2
13, sunflower[a]	207.8	95.1
14, sunflower isolate (DE-60)	256.7	25.6

[a] Literature source Lin et al. (1974); promine, Isopro, and DE-60 denote method of purification.

Water Holding Capacity (WHC). The WHC's of the isolates and flours are presented in Table III. The results showed that the WHC's of the isolates are higher than those of the flours.

The WHC of *T. conophorum* is a significantly lower than those of the melon seeds. Compared with soy flour isolates, the WHC's of the isolates of the seeds studied are low with the exception of *C. vulgaris* variety 2, which has a WHC comparable to that of soybean.

Fat Absorption and Emulsion Capacity. Fat absorption is as shown in Table IV. Fat absorption in *T. conophorum* is comparable to that in soybean (Lin et al., 1974), but the melon seeds have much higher fat absorption than either *T. conophorum* or soybean. They are comparable to that of sunflower shown in Table V (Lin et al., 1974).

Emulsification capacity also follows the same trend, being very high in the *C. vulgaris* varieties. An important property of protein intended as a meat additive is the ability to bind fat.

The high values shown by the oilseeds under study indicated that they may be useful in meat additives and as meat extenders.

Foaming Capacity and Foaming Stability of *T. conophorum*, *Cucumeropsis edulis*, and *C. vulgaris*.

Table V. Foaming Capacity and Foaming Stability of the Oilseeds *Tetracarpidium conophorum*, *Cucumeropsis edulis*, and *Citrullus vulgaris* (Mean Values of Duplicate Samples)

	vol of foam after whipping, mL			
	foaming capacity		foaming stability	
sample	1/2 min	1 min	10 min	2 h
1, *Tetracarpidium conophorum* flour	66		14.9	14.6
2, *Cucumeropsis edulis* flour	144.8		97.2	93.8
3, *Citrullus vulgaris* variety 1 flour	141.7		90.3	82.8
4, *Citrullus vulgaris* variety 2 flour	146.2		93.0	90.0
5, soy flour[a]		160	131	14.6
6, soy concentrate (Isopro)[a]		400	28	93.8
7, soy isolate[a]		660	603	82.8
8, sunflower[a]		600	522	9.0

[a] Literature source Lin et al. (1974). Isopro denotes method of purification.

The foaming capacity and the foaming stability of the flours are shown in Table V. The foaming capacity of the flours are inferior to that of soy flour (shown in the table for comparison). The isolate of the studied oilseeds showed little or no foaming power. However, *Citrullus* and *Cucumeropsis* showed very good foam stability comparable to that of soy flour and isolate after 2 h.

Tetracarpidium showed very little foaming capacity and stability.

Summary. The solubility profile of the oilseeds showed that proteins of *T. conophorum* are highly soluble at a low pH, a factor that it may be of use in beverages.

Citrullus and *Cucumeropsis* have potential as human food because of the good functional properties—fat holding capacity, emulsion capacity, and water holding capacity. Their foaming capacity is very low, but the stability of the foam in *Citrullus* and *Cucumeropsis* is good after 2 h.

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Amylase Activities in Fungal Rennets and Whey Protein Concentrate Powder

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Amylase activities were characterized in fungal rennets and in whey protein concentrate powder. Starch hydrolysis was detected by the release of radioactive low molecular weight compounds from starch. Hydrolysis patterns indicative of α-amylase or glucoamylase attack on the starch substrate were observed. Among the fungal rennets tested, those derived from *Endothia parasitica* cultures contained amylolytic activities that were more heat sensitive than those derived from *Mucor* cultures. Time-dependent reductions in yield value were observed when such whey concentrate powders were added to starch-containing foods.

Fungal protease preparations are commonly used as substitutes for calf rennets in cheese making (Ernstrom and Wong, 1974). They are less expensive and readily available unlike the calf rennets, which are subject to the uncertainties of the agricultural industry. Fungal rennets may be derived by the fermentation of media containing wheat bran, corn meal, soybean meal, casein, and/or dried milk (Arima and Iwasaki, 1965; Sardinas, 1966; Charles et al., 1970). Simple purification steps may then be used to produce a partially purified preparation enriched in milk clotting activity. Three species, *Mucor pusillus* var. *Lindt*, *Mucor miehei*, and *Endothia parasitica* are the source of the fungal rennets used in the United States. These fungi also readily produce amylase activity when grown in culture (Barnett and Fergus, 1971; Chapman et al., 1975; Fergus, 1969) on many types of media (Adams and Deploey, 1976). The occurrence of amylase activities in commercial fungal protease preparations used as rennet substitutes in cheese making is, therefore, not surprising.

The possibility of amylase contamination in these crude protease preparations has received scant attention, although de Koning et al. (1969) were aware of the presence of amylase activities in bacterial milk clotting preparations. In fact, they suggested that the detection of these enzymatic activities in calf rennets would provide a useful test for their adulteration with the less expensive bacterial

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