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Relationship of Tannin Levels and Trypsin Inhibitor Activity with the in Vitro Protein Digestibilities of Raw and Heat-Treated Winged Bean (*Psophocarpus tetragonolobus*)

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Three varieties of winged bean were heat processed in five different ways to determine the relationship among in vitro protein digestibility (IVPD), trypsin inhibitor activity (TIA), and assayable tannin. The IVPD of raw winged bean ranged from 68.8 to 72.9%. Heat treatments increased the IVPD to 76.0–90.7% and reduced both TIA and tannin. In terms of energy costs, 5-min autoclaving and quick cooking were the two most effective methods of improving IVPD. Dry heat (200 °C, 30 min) and direct boiling (20 min, no soaking) were least effective. Reductions in tannins and TIA did not necessarily result in proportional increases in IVPD.

The problem of plant protein digestibility has been extensively studied. Trypsin inhibitors, tannins, and other "antiphysiological factors" have been suggested as factors responsible for the low digestibility of some plant proteins (Jaffe, 1968; Bressani et al., 1975; Featherstone and Rogler, 1975).

No direct evidence, however, has been produced to show which is the reason for the poor digestibility of certain legume proteins. Recently, Elias et al. (1979) suggested that in legumes, tannins might play an important role in the reduction of protein digestibility. Subsequently, in studying the effects of winged bean (*Psophocarpus tetragonolobus*) meals on broiler performance, de Lumen et al. (1982) suggested that the hull of winged bean, with its high tannin content and indigestible fiber, may be the main factor responsible for the lower metabolizable energy of the winged bean diets that led to the poorer response of the broilers. The present work was undertaken to determine the possible relationship of the tannin levels and trypsin inhibitor activity with the in vitro protein digestibilities in several varieties of raw and heat-treated winged beans.

MATERIALS AND METHODS

Winged beans of varieties V1 (yellowish seed coat), 243 (brown seed coat), and C33PB (dark brown seed coat) were grown locally at the Experimental Farm of Agricultural University of Malaysia, Serdang, Malaysia. Winged bean meals were prepared by grinding the mature beans manually with a pestle and mortar, and the fine powder was stored below 0 °C in a glass container before use. All chemicals are of analytical reagent grade and were purchased from Sigma Chemical Co. or Merck.

Preparation of the Heat-Treated Winged Bean Meals. *Dry Heat Treated Winged Bean Meals.* These were prepared by heating 10 g of finely ground winged bean meals, in a beaker at a thickness not exceeding 1 cm, in an oven maintained at 200 ± 2 °C for 30 min.

Autoclaved Winged Bean Meals. The autoclaved winged bean meals were prepared by autoclaving 10 g of the finely ground bean meals in a beaker at a thickness not exceeding 1 cm at 120 °C, 1.05 kg/cm^2 for 5 or 10 min, after the desired temperature had been reached. The autoclave was preheated before use to minimize the time required to reach the desired temperature (approximately 7 min).

Boiled Winged Bean Meals. One gram of whole winged beans was treated by boiling in distilled water (10 mL) for 20 min, their cooking broths were drained off, and the beans were dried in an oven at a temperature of 50 °C for 2 h. The beans were then ground to yield the boiled winged bean meals.

Cooked Winged Bean Meals (Normal Cooking). The cooking of winged beans was carried out as described by Rockland et al. (1979). Ten grams of the whole beans was soaked in 30 mL of distilled water at room temperature (32 °C) for 24 h, followed by cooking in 6 volumes of boiling distilled water for 240 min with a loose cover to minimize water loss. The cooked beans were drained and dried in

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Table I. Effect of Heat Treatments on Assayable Tannins, Trypsin Inhibitor Activity, and in Vitro Protein Digestibilities of Winged Beans[a]

variety	heat treatment[c]	tannin level, mg of D-catechin/g of protein	trypsin inhibitor activity, IU/g of protein	in vitro protein digestibilities,[b] %
243	raw	32.00	85113	71.3
V1	raw	1.04	77177	68.8
C33PB	raw	30.07	86525	72.9
243	5-min autoclaving	12.69	33700	84.4
V1	5-min autoclaving	0.75	36900	83.7
C33PB	5-min autoclaving	13.37	56700	85.5
243	10-min autoclaving	8.22	23600	81.7
V1	10-min autoclaving	0.72	9900	83.7
C33PB	10-min autoclaving	7.62	30100	81.8
243	dry heat	10.03	0	76.0
V1	dry heat	0.60	0	80.0
C33PB	dry heat	11.35	0	81.4
243	20-min boiling	2.59	17800	80.9
V1	20-min boiling	0	25400	78.5
C33PB	20-min boiling	3.19	30500	78.9
243	normal cooking	1.94	0	88.9
V1	normal cooking	0	0	83.5
C33PB	normal cooking	2.13	0	90.7
243	quick cooking	1.94	1300	88.7
V1	quick cooking	0	0	86.4
C33PB	quick cooking	1.06	5300	85.0

[a] Means of triplicate analysis. The protein contents of 243, V1, and C33PB are 30.9%, 33.3%, and 28.2%, respectively, as determined by the Kjeldahl method. Colors of the seed coat are yellowish (V1), brown (243), and dark brown (C33PB). [b] Standard deviation less than 2%. [c] The details of time and temperature of heat treatments are given under Materials and Methods.

an oven at a temperature of 50 °C for 2 h. The beans were then ground to yield the cooked winged bean meals.

Quick-Cooking Winged Bean Meals. The quick-cooking winged bean meals were prepared according to the procedure of Rockland et al. (1979). The raw beans were blanched in boiling water for 2 min and soaked at room temperature in 3 volumes of solution consisting of 2% sodium chloride, 0.75% sodium bicarbonate, and 0.25% sodium carbonate. After 24 h, the hydrated beans were drained and rinsed lightly. The drained beans were cooked in 5 weight-volumes of distilled water for 15 min. Subsequently, the beans were drained and dried in an oven at a temperature of 50 °C for 2 h. The dried beans were then ground to yield the quick-cooking winged bean meals.

Determination of Trypsin Inhibitor Activity. Trypsin inhibitor activity (TIA) of the bean meals was determined by a method modified (Tan and Wong, 1982) from Kakade et al. (1974). *N*-Benzoyl-DL-arginine *p*-nitroanilide was used as the trypsin substrate. One inhibitor unit (IU) of trypsin inhibitor is defined as the amount of inhibitor that inhibits 1 μg of pure trypsin. Total TIA was expressed as IU/g of protein (dry weight).

Determination of the Tannin Content. The method of Burns (1971) as modified by Maxson and Rooney (1972) was used for tannin determination. One gram of winged bean meal was extracted with 10 mL of 1% concentrated HCl in methanol for 24 h at room temperature. After centrifugation at 10000*g* for 5 min, 1 mL of the supernatant was mixed with 5 mL of vanillin–HCl reagent and the absorbance was read at 500 nm after 20 min. Values of tannins were expressed in mg of D-catechin/g of protein (dry weight).

Determination of the in Vitro Protein Digestibilities (IVPD) of Winged Bean Meals. The in vitro protein digestibilities of winged bean meals were determined by the multienzyme method described by Satterlee et al. (1979), a modification of the "Three Enzyme Assay" of Hsu et al. (1977). The enzymes used were porcine pancreatic trypsin (Sigma type IX, T-0134, 14190 BAEE units/mg of protein), bovine pancreatic α-chymotrypsin (Sigma type II, C-4129, 60 units/mg of powder), porcine intestinal peptidase (Sigma Grade III, P-7500, 40 units/g of powder), and bacterial protease (*Streptomyces griseus*, Sigma type XIV, P-5147, 4 units/mg of solid). The IVPD was calculated from the pH of the protein solution after appropriate incubations with the enzymes.

RESULTS AND DISCUSSION

Tannin Levels, Trypsin Inhibitor Activity, and in Vitro Protein Digestibilities of Raw Winged Beans. The tannin levels, TIA, and IVPD of the three varieties of raw winged beans are shown in Table I. TIA ranged from 77177 to 86525 IU/g of protein. Tannin level was lowest (1.04 mg/g of protein) in the yellowish V1 variety and highest (32 mg/g of protein) in the 243 variety, which has a brown seed coat. The IVPD of the raw winged beans ranged from 68.8 to 72.9%. The IVPD of casein determined by the same method was 90%. Ekpenyong and Borchers (1979) also reported low IVPD for raw winged beans.

Effects of Heat Treatments on the Winged Bean Trypsin Inhibitor Activity. It has been established that trypsin inhibitor activity could be reduced by moist heat treatments (Sohonie and Bhandarkar, 1954; Cerny et al., 1971; Ekpenyong and Borchers, 1981; Tan and Wong, 1982).

Dry heat and normal cooking inactivated all the TIA (Table I). Quick cooking destroyed 94–100% while boiling winged beans in distilled water for 20 min inactivated 65–80%. Five minutes of autoclaving was the least effective, inactivating 35–60% of the TIA while 10 min of autoclaving destroyed 65–87%. The effectiveness of autoclaving and 20-min boiling in destroying TIA varied among the three varieties.

Effect of Heat Treatments on the Tannin Levels of Winged Beans. Reduction in tannin contents of winged beans and other legumes by soaking or heating has been reported (Haslam, 1966; Price et al., 1979; Sathe and Salunkhe, 1981; Deshpande and Cheryan, 1983). The effects of various types of heat treatments on the tannin levels of the three varieties of winged beans are also shown in Table I. Autoclaving and dry heat treatment reduced

56–75% of the assayable tannin content. On the other hand, cooking (normal, 20-min boiling and quick cooking) decreased assayable winged bean tannin by 89–100%.

Effect of Heat Treatments on the in Vitro Protein Digestibilities of Winged Beans. Five minutes of autoclaving, normal cooking, and quick cooking were effective in improving winged bean IVPD. These treatments increased the digestibility from 68.8–72.9% (raw winged beans) to 83.5–90.7%. Similar results have been reported by Ekpenyong and Borchers (1980). They also reported that long cooking (soak 18 h, cook 5 h, IVPD 83.6%) was more effective than quick cooking (soak 5 h, cook 1 h) in improving winged bean IVPD. Our results, however, indicate that with presoaking in salt solution, 15-min cooking was sufficient to increase winged bean IVPD to 85–88.7%. It is also interesting to note that the IVPD of 10 min autoclaved winged beans were lower than those of 5 min autoclaved samples, presumably due to the nonenzymatic browning or thermal cross-linking reactions occurring during the autoclaving. Dry heat and direct boiling increased winged bean IVPD to only 76–81.4%.

In terms of saving in energy cost, 5 min of autoclaving and quick cooking were the two most efficient methods for improving winged bean protein digestibility. The quick-cooking method offered an additional advantage: it reduced the level of assayable tannins and TIA more than the 5 min of cooking.

Relationship of Winged Bean Tannin Levels with the in Vitro Protein Digestibilities. Tannins are nonspecific inhibitors of enzymes and may also form complexes with food proteins. It has been suggested that in legumes, tannins might reduce protein digestibility (Elias et al., 1979). A survey of 16 varieties of winged bean levels (Tan et al., 1983), however, indicated that the levels of tannin in winged beans might not be sufficiently high to be of concern in nutrition. On the other hand, de Lumen et al. (1982) showed that inclusion of the winged bean hulls, which contain more than 50% of the total tannin, might have adversely affected the nutritional quality of winged bean meal in chicken. The tannin content of the autoclaved beans used in de Lumen et al.'s work of about 7.0 mg/g of protein is not directly comparable with beans used in the present work due to differences in methods of determination and units.

The various types of heat treatments reduced winged bean tannin contents to different extents. The IVPD of different winged bean samples show no direct correlation with their tannin contents. Thus, the IVPD of the yellowish raw winged bean variety V1, which contains relatively trace amounts of tannins, was lower than those of raw varieties 243 and C33PB, both of which contain 30 times more tannin. Also, the IVPD of 5 min autoclaved winged bean variety V1, which contains very little tannins, was comparable to those of the high-tannin, 5 min autoclaved winged bean varieties 243 and C33PB.

A caution, however, must be exerted in interpreting the nutritional significance of the reduction of winged bean assayable tannin content by heat treatments. It is known that during heat treatment some tannins may bind with proteins; the protein-bound tannins are normally not detectable by the vanillin method, thus leading to a lower estimate of tannins in the heat-treated samples. Price et al. (1979) reported that in sorghum, reduction of tannin by moist and alkaline conditions contributed to overcoming the nutritionally harmful effect of tannin. Subsequently, they reported that while cooking methods also drastically reduced the level of assayable tannins in high-tannin sorghum grain, the treatment simultaneously diminished, rather than improved, the nutritional quality of the grain (Price et al., 1980). Thus, even though our results indicated that in winged beans the assayable tannin levels are not sufficiently high to affect protein digestibility, animal experiments are needed to determine whether the tannins in raw winged beans or the residual (assayable) tannins in heat-treated winged beans are nutritionally harmful.

Relationship between Winged Bean Trypsin Inhibitior Activity and the in Vitro Protein Digestibility. Using the Three Enzyme Assay of IVPD, Ekpenyong and Borchers (1979) suggested that the improvement of the protein digestibility of winged bean by heat treatment was due to the destruction of trypsin inhibitors during the treatment. Our results showed that the increase in the IVPD of winged beans by heat treatment was not exclusively related to the decrease in TIA in the heat-treated samples. For example, while IVPD of the 5 min autoclaved V1 was comparable to those of the normal and quick-cooking V1, their TIA differed considerably. Similarly, the 5 min autoclaved C33PB sample, with a TIA of 56700 IU/g of protein and a tannin content of 13.37 mg/g of protein, had an IVPD comparable to that of quick-cooking C33PB, which had a TIA of only 5300 IU/g of protein and trace amount of tannins. Also, the high-TIA and high-tannin 5 min autoclaved 243 sample had an IVPD comparable to that of the normal cooking VI sample, which had a negligible TIA and tannin content.

The high amount of TIA in C33PB (i.e., 56 700), which also had high IVPD (85.5%), suggests that other factors may be responsible for increased IVPD. One reason may be the increased vulnerability of the protein to proteolytic enzymes due to heat denaturation. Also, it has been reported that raw soybeans or soybean trypsin inhibitor preparations do not depress proteolytic activity in the intestinal tract of rats and mice (Nitsan and Bondi, 1965). Apparently, in rat and mice the quantity of enzymes secreted from the pancreas is sufficient to prevent an inhibition of proteolysis by the trypsin inhibitors. Animal feeding experiments are needed to establish the nutritional significance of the above findings.

In conclusion, our results show that improvement in winged bean IVPD by most heat treatments was not exclusively related to the destruction of TIA or reduction of tannin contents.

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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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