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Research Note

Trypsin Inhibitors in Some Lesser Known Seeds in Nigeria

ABSTRACT

Trypsin inhibitors (TIs) were extracted and determined spectrophotometrically from seed meals of African breadfruit (Treculia africana), *African walnuts* (Coula edulis *Bail*), *Calabash* (Lagenaria sicerania) *and Castor* (Ricinus communis). *The TI activities were for Castor 1319* $\times 10^{-4}$, *African breadfruit 620* $\times 10^{-4}$, *and Calabash 28·4* $\times 10^{-4}$ *and for African walnuts only a trace of TI units per milligram protein of the extract. The total protein content and* in vitro *protein digestibility for the four seed meals were also determined and observed to be quite high for Calabash seeds.*

INTRODUCTION

The fast growing population of Nigeria means full utilization of all existing crops. The lesser known seeds like African breadfruit (ABS), African walnuts (AFW), Calabash (CBS) and Castor (COS) seeds, which could serve as cheaper protein-rich foods for use as weaning foods and in animal feeds, are being examined. However plant foods may contain antinutritional factors, and protease inhibitors have been found in some leguminous seeds (Kunitz, 1947; Warsy *et al.*, 1974; Liener & Kakade, 1980; Elkowicz & Sosuslki, 1982). Little information on the protease inhibitors in these lesser known seeds listed above is available, hence it was thought worthwhile to do this study.

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MATERIALS AND METHODS

Source of samples

The seeds of *Treculia africana, Coula edulis, Lagenaria sicerania* and *Ricinus communis* were purchased in 1985 at Ikot Ekpene market in Cross River State of Nigeria. All the seeds were from the 1985 harvest but the exact harvest month was not known. Seeds purchased from different stalls were pooled together and analysed.

Reagents

Trypsin (Sigma, USA) solution was prepared by dissolving 0·02 g trypsin in 200 cm^3 of $2{\cdot}5 \times 10^{-3}$ M HCl. Other reagents used were of analytical grade.

Sample preparation

After preliminary cleaning, the seeds were dehulled by hand and ground in a mortar to a meal passing through a 0·2 mm sieve. The meal was defatted with *n*-hexane at room temperature. The lipid-free meal was then stored in airtight bottles at 4°C for later analysis.

Assay of trypsin activity and expression of tryptic unit

The trypsin activity was assayed spectrophotometrically using casein as the substrate by the method of Kunitz (1947) as modified by Warsy (1974). A tryptic unit (TU) is expressed as the amount of trypsin which gives rise to an increase of one unit of absorbance at 280 nm/min at 37°C and pH 7·6 (Kunitz, 1947).

Extraction of trypsin inhibitors

The TIs were extracted from 5 g defatted meal using 2·5% trichloroacetic acid (TCA) according to the method of Warsy *et al.* (1974). Five independent extractions were performed for each seed type and the corresponding TI activity of each extract determined.

Assay of trypsin inhibitor activity and expression of TI unit

The effect of TIs on the trypsin activity was determined by the caseinolytic method outlined by Warsy (1974). Controls were run simultaneously. The TI unit (TIU) is expressed as the amount of the inhibitor which reduces trypsin

activity by one tryptic unit (Kunitz, 1947). Values are the mean of five duplicate determinations and the standard errors of the mean were calculated. The inhibitor specific activity is expressed as the number of TI units per milligram protein of the extract.

Protein determination

Protein content of the extract was determined by the method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as standard.

Nitrogen content (%N) of the seed meal was determined by the micro-Kjeldahl method (AOAC, 1975) and protein content of the seed meal was calculated using the N factor of 6·25.

***In-vitro* protein digestibility determination**

The *in-vitro* protein digestibility of the meal sample was determined by the method of Akeson & Stahmann (1964) as modified by Oke & Umoh (1975). The percentage hydrolysis is expressed as the loss of the original nitrogen content of the sample after enzyme hydrolysis. Results of five duplicate samples were averaged and the standard error of each mean calculated.

RESULTS AND DISCUSSION

The values of TI activity in the seed meals are presented in Table 1. Castor seed with its high ricin content (Jenkins, 1963) and TI activity must be processed properly before consumption. Anosike & Egwuatu (1981) observed that the toxicity of Castor seed can be reduced or even eliminated by fermentation in the production of 'ogiri', a harmless food condiment used by some people in parts of Eastern Nigeria. Calabash seeds have a comparatively low TI activity as observed in this study. The trace TI activity observed in African walnut is probably the reason why no adverse effects are observed in people who consume raw walnuts. Further investigation is still necessary before consumption of walnuts can be given full clearance.

Table 2 shows the values for protein digestibility of the seed meals. Protein digestibility is a more important indicator of protein quality than the amino acid composition. Hence there is a need to investigate other antinutrients in Calabash seed with a high protein digestibility, so that the Calabash seed, which resembles melon seed—'egusi'—, can be used like the latter in native dishes. The poor *in-vitro* protein digestibilities of Castor and African breadfruit seeds may be due to a combination of toxins such as TIs and hemagglutinins (Liener, 1976), coupled with the indigestible nature of many legume proteins.

TABLE 1
Antitryptic Activity and Protein Content in the Raw Seed Meals
(mean of five determinations ± standard errors of the mean)

Sample	*Total protein (determined)*,[a] (%)	*Total protein (FAO, 1968)*, (%)	*Protein content of extract*[b] *(mg/cm³)*	*TIU*[c] *per cm³* ($\times 10^{-4}$)	*Specific*[d] *activity* ($\times 10^{-4}$)
African breadfruit	16·8 ± 0·21	12·6	0·255 ± 0·007	158 ± 1·77	620 ± 3·27
African walnut	8·3 ± 0·14	7·2	0·221 ± 0·002	trace	trace
Calabash	34·9 ± 0·30	28·2	0·669 ± 0·017	19 ± 0·63	28·4 ± 1·11
Castor	15·4 ± 0·17	18·0	0·225 ± 0·003	297 ± 1·16	1 319 ± 8·07

Notes: [a] Total protein in seed meal determined by micro-Kjeldahl method as %N × 6·25.
[b] Protein content of extract determined by method of Lowry *et al.* (1951); extractions were from 5 g defatted meal.
[c] TIU determined by the casein digestion method of Warsy *et al.* (1974) as reduction in tryptic activity. (TIU = control TU–residual TU after adding inhibitor extract.)
[d] Specific activity = TIU per milligram protein of extract.

TABLE 2
Protein Digestibility of the Seed Meals

Sample	*Digestibility with pepsin-trypsin* (%)	*Digestibility with pepsin-pancreatin* (%)
African breadfruit (5)	58·9 ± 1·06	65·0 ± 1·09
African walnut (5)	75·3 ± 0·72	78·1 ± 0·79
Calabash seed (5)	85·4 ± 1·04	89·1 ± 0·67
Castor seed (5)	32·3 ± 0·54	40·1 ± 0·26

Notes: % digestibility = P1–P2/P1 × 100, where P1 = total nitrogen content of sample before enzyme digestion; P2 = total nitrogen content of sample after digestion.
Values in parentheses indicate numbers of samples analyzed in duplicate.
Digestibility values are mean ± standard errors of the mean of five independent determinations.

The values for protein content of the seed meals as found in this study are in good agreement with Eka (1985) and Edet *et al.* (1985) for African breadfruit seeds and with FAO (1968), Dunu (1984) and Itam (1983) for Calabash and Walnut seeds.

Before recommending these lesser known seeds for use in weaning and other foods, further studies must be carried out on the inactivation of the TIs by processing.

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