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Cyanide Contents of Some Nigerian Legumes and the Effect of Simple Processing*

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ABSTRACT

The cyanide contents of seeds of varieties of Phaseolus aureus, Vigna unguculata, Cajanus cajan *and* Canavalia gladiatus *were studied. Results obtained show total cyanide contents of 381–1093, 285–1223, and 208–953 mg kg*$^{-1}$ *dry weight of intact seed, testa and cotyledons, respectively, with testa having 3–5 times higher HCN than cotyledons in some cases. Soaking of seeds in water for 24 h led to appreciable losses in HCN, while boiling for 3 h caused drastic reduction (49–95%) in HCN. After boiling for 3 h, innocuous HCN levels were obtained with seeds previously soaked in water for 24 h and divested of testa. Thus soaking of bean seeds in water and subsequent removal of testa prior to boiling will decrease HCN in bean meals.*

INTRODUCTION

The occurrence of cyanogenic glycosides in crop plants such as cassava, *Manihot esculenta* Crantz (Nartey, 1968; 1978) and some cereals (Erb *et al.*, 1981) is well known. Cassava contains the cyanogenic glucosides linamarin and lotaustralin, while the main cyanogenic principle in cereals is dhurrin. The consumption of foods containing these toxic cyanogens could result in acute or chronic cyanide toxicity. The former is fatal, resulting in a high rate of mortality and morbidity, while the latter has been associated with goitre

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(Epechi, 1967) and Tropical Ataxic Neuropathy (Osuntokun, 1973). Extensive investigations have been carried out on cyanide contents of legume seeds grown elsewhere (Montgomery, 1964; Butler, 1965; Jansz & Pieris, 1978; Vanderborght, 1979; Ermes, 1981), whereas reports on Nigerian legumes have been scanty, and so far restricted to varieties of *Phaseolus lunatus* (Jansz & Pieris, 1978; Vanderborght, 1979). Since varieties of *Vigna unguculata* and *Cajanus cajan* represent the more frequently consumed legume seeds in Nigeria, there is need for evaluation of their toxicity in relation to HCN content. Jansz & Pieris (1978) and Cooke & Maduagwu (1978) have reported the effects of simple processing on the cyanide contents of legumes and cassava chips, respectively. This paper reports the cyanide contents of legume seeds frequently consumed in Nigeria, and suggests simple processing procedures to minimise HCN in bean-based meals.

MATERIALS AND METHODS

Sources of reagents

Anhydrous picric acid was a product of British Drug House (BDH) Chemicals Ltd., Poole, UK. *p*-nitrophenyl β-D-glucoside (PNP-glucoside) was from Merck, Darmstadt. Other reagents and organic solvents were of the best analytical grade.

Source of legume seeds

All legume seeds were obtained from retail shops in Benin City, Nigeria.

Pre-treatment of legume seeds

After screening to remove unhealthy and wounded seeds, a portion of each legume seed type was split into testa and cotyledons whilst dry. Triplicate 6·0 g samples of each seed were soaked in 150 ml of distilled water in open 100 ml beakers for 24 h, after which testa, cotyledons and soak-water were separated. The testa and cotyledon fractions were rinsed with water and air-dried over Whatman filter papers. In another treatment, 6·0 g of each intact legume seed type was weighed into each of six 500-ml beakers containing 200 ml of distilled water. The contents of three of the beakers were boiled for $1\frac{1}{2}$ h, while those of the remaining three were boiled for 3 h. The procedure was repeated with soaked and unsoaked cotyledon fractions. The boiled intact seeds and cotyledons were separately rinsed with distilled water and air-dried on Whatman filter papers. Samples not immediately in use were stored in the refrigerator at about 9°C.

Extraction of cyanide

The extractions were carried out on the intact legume seeds, testa, cotyledons, soaked testa, soaked cotyledons, boiled intact bean, and boiled cotyledon. Two grams of material was thoroughly ground in a hand mortar and extracted with 30 ml of 0·1M HCl for 10 min. The homogenate was centrifuged for 15 min at 1000 *g*, and the pH of the clear supernatant was adjusted to 6·8 with base. It was then clarified by centrifugation at 1000 *g* for 5 min. The extract thus obtained was kept on the bench for 24 h at ~28°C, and was found to be stable and turbidity-free. All extractions were done in triplicate.

Analysis of legume extracts for cyanide

The extracts and soak water were analysed for total HCN by measuring the HCN released on hydrolysis of endogenous cyanogenic glucosides with partially purified cassava peel linamarase according to the procedure described by Ikediobi *et al.* (1980). In this method, the HCN released by enzymic hydrolysis is estimated in a colorimetric reaction with alkaline picric acid. 0·5 ml of extract was incubated for 10 min at room temperature (~28°C) with 1·0 ml of linamarase and 0·5 ml of 0·2M Na phosphate buffer, pH 6·8, in a stoppered quick-fit tube. An extract blank containing 0·5 ml extract and 1·5 ml of 0·2M Na phosphate buffer, pH 6·8, was also set up in order to eliminate interference with the picric acid assay (Zitnak, 1973). At the end of incubation, 4·0 ml of alkaline picric acid was added to each tube and the tubes were then warmed at 95°C in a water-bath for 5 min. On cooling, the contents of the tubes were clarified by centrifugation at 2000 *g* for 5 min. Thereafter the absorbance of the orange colour was read at 495 nm in a Pye-Unicam UV Spectrophotometer. Corresponding cyanide levels were extrapolated from a standard KCN curve, and expressed in mg kg^{-1} dry matter.

RESULTS AND DISCUSSION

The choice of the alkaline picrate method of Ikediobi *et al.* (1980) for analysis of legume extracts arose from the need to evaluate total, rather than bound, cyanide. It has been shown that this method is most suitable for determination of total cyanide (Izokun-Etiobhio & Ugochukwu, 1984). The total HCN content of intact legume seeds studied ranges from 381 to 1093 mg kg^{-1} dry matter (Table 1). Jansz & Pieris (1978) and Vanderborght (1979) have reported HCN values of 32–999 ppm for varieties of *Phaseolus*

TABLE 1
HCN Content of Intact Legume Seeds before Soaking in Water

Species	*Local name*	*mg kg^{-1} HCN in dry matter*
Phaseolus aureus var.	'Odudu'	458 ± 10
Vigna unguculata var.	'Ife-brown'	381 ± 5
Cajanus cajan var.	'Kafanchan'	393 ± 13
Vigna unguculata var.	—	402 ± 7
Vigna unguculata var.	'Akidi'	557 ± 3
Phaseolus aureus var.	'Odudu-iba'	458 ± 6
Vigna unguculata var.	Black-eyed bean	419 ± 4
Cajanus cajan var.	'Osisi'	854 ± 7
Canavalia gladiatus var.	—	1093 ± 12

Values are mean ± SEM of three separate determinations.

lunatus (lima bean) grown in Nigeria. Studies on legume seeds grown elsewhere indicate a wide variability in HCN levels between different legume species, and also between varieties of the same species (Montgomery, 1964; Jansz & Pieris, 1978; Vanderborght, 1979). Thus while the variety of *Canavalia gladiatus* studied has a cyanide content of 1093 mg kg^{-1} dry matter, Jansz & Pieris (1978) did not detect any traces of HCN in another variety grown in Sri Lanka. Our results on Table 1 are consistent with these variabilities, which may be attributed to differences in some factors of growth environment; for example, soil nitrogen content and use of nitrogen fertilizers. The latter factor has been shown to increase the level of cyanide in *Sorghum almum* during growth (Kriedman, 1964). Table 2 shows the levels of HCN in testa and cotyledons before soaking in water. The results show a higher content of HCN in testa than cotyledons for most of the seeds studied. Usually, the outer layer of many cyanogenic tissues contains more HCN than the edible inner layer (Bourdoux *et al.*, 1980). However, a reversal of this trend was found in *Canavalia gladiatus*, where the cotyledons have three times as much HCN as the testa. After seeds were soaked in water for 24 h there were appreciable reductions in cotyledon HCN, which may well account for the HCN detected in the soak water (Table 3).

As would be expected, HCN losses during boiling were higher after 3 h than 1½ h in all cases (Table 4). Although boiling of the intact legume seeds for 3 h led to large reductions in HCN (49–95%), some of the seeds still retained appreciable levels of residual HCN. Since the average domestic cooking time for beans in Nigeria does not usually exceed 3 h, it follows that most bean meals prepared from intact seeds might contain harmful levels of residual HCN. The most desirable results were obtained with cotyledons soaked for 24 h before boiling. After boiling for 3 h, these cotyledons gave

TABLE 2
HCN Levels in Testa and Cotyledons before Soaking in Water

Legume	*Local name*	*mg kg^{-1} HCN in dry matter*	
		Testa	*Cotyledon*
P. aureus var.	'Odudu'	1 006 ± 4	338 ± 9
V. unguculata var.	'Ife brown'	939 ± 17	225 ± 4
C. cajan var.	'Kafanchan'	1 001 ± 8	297 ± 4
V. unguculata var.	—	×	×
V. unguculata var.	'Akidi'	×	×
P. aureus var.	'Odudu-iba'	1 223 ± 5	234 ± 5
V. unguculata var.	Black-eyed bean	815 ± 6	208 ± 5
C. cajan var.	'Osisi'	×	×
C. gladiatus var.	—	285 ± 4	953 ± 10

Values are mean ± SEM of three separate determinations.
×, Not determined. Testa could not be separated from cotyledons whilst dry.

higher HCN losses (89–94%) compared with cotyledons unsoaked before boiling (59–85%). Moreover, the residual HCN of the cotyledons soaked before boiling were reduced to very low levels, except in *Canavalia gladiatus*. Cyanide losses of up to 99% have been obtained with seeds of *Phaseolus lunatus* boiled after soaking and removal of testa (Jansz & Pieris, 1978). However, processing methods do not result in total elimination of HCN from foods, although only very low levels remain (Wood, 1965; Cooke & Maduagwu, 1978; Jansz & Pieris 1978; Ikediobi *et al.*, 1980). Montgomery

TABLE 3
HCN Content of Testa, Cotyledons and Soak Water after Soaking Legume Seeds in Water for 24 h

Legume	*Local name*	*mg kg^{-1} in dry matter*		*mg litre^{-1} in soak water*
		Testa	*Cotyledon*	
P. aureus var.	'Odudu'	922 ± 7	293 ± 8	182 ± 5
V. unguculata var.	'Ife brown'	737 ± 4	178 ± 4	179 ± 8
C. cajan var.	'Kafanchan'	635 ± 4	144 ± 9	118 ± 10
V. unguculata var.	—	736 ± 8	201 ± 3	83 ± 7
V. unguculata var.	'Akidi'	874 ± 5	165 ± 3	201 ± 11
P. aureus var.	'Odudu-iba'	999 ± 8	191 ± 6	182 ± 4
V. unguculata var.	Black-eyed bean	641 ± 7	183 ± 8	95 ± 3
C. cajan var.	'Osisi"	768 ± 11	129 ± 5	168 ± 5
C. gladiatus var.	—	195 ± 3	738 ± 5	150 ± 6

Values are expressed as mean ± SEM of three separate determinations.

TABLE 4

Residual HCN of Intact Legume Seeds, Soaked Cotyledons and Unsoaked Cotyledons Boiled in Water for $1\frac{1}{2}$ h and 3 h (mg kg^{-1} d.m.)

Legume	*Intact legume*		*Cotyledons unsoaked before boiling*		*Cotyledons soaked 24 h before boiling*	
	$1\frac{1}{2}$ h	*3 h*	*$1\frac{1}{2}$ h*	*3 h*	*$1\frac{1}{2}$ h*	*3 h*
P. aureus var.	285 ± 4 (38)	104 ± 10 (77)	174 ± 6 (48)	85 ± 4 (75)	90 ± 11 (73)	31 ± 3 (91)
V. unguculata var.	240 ± 4 (37)	85 ± 5 (78)	123 ± 5 (45)	50 ± 4 (78)	76 ± 6 (66)	23 ± 4 (90)
C. cajan var.	191 ± 7 (51)	68 ± 3 (83)	111 ± 4 (63)	39 ± 5 (87)	63 ± 3 (79)	19 ± 3 (94)
V. unguculata var.	175 ± 3 (56)	77 ± 2 (80)	×	×	81 ± 4	25 ± 5
V. unguculata var. (Akidi)	152 ± 2 (73)	41 ± 7 (93)	×	×	72 ± 4	31 ± 5
P. aureus var.	182 ± 3 (60)	110 ± 7 (76)	100 ± 4 (57)	44 ± 2 (81)	58 ± 7 (75)	26 ± 3 (89)
V. unguculata var.	163 ± 5 (61)	92 ± 3 (78)	85 ± 3 (59)	31 ± 7 (85)	37 ± 3 (82)	22 ± 2 (89)
C. cajan var.	130 ± 5 (84)	38 ± 3 (95)	×	×	61 ± 4	35 ± 4
C. gladiatus var.	712 ± 6 (35)	553 ± 5 (49)	731 ± 7 (23)	394 ± 3 (59)	339 ± 4 (64)	118 ± 3 (89)

The values are mean ± SEM of three separate determinations.

× = not determined. Values in brackets indicate % decreases in HCN when compared with corresponding results before soaking and/or boiling in water.

(1964) and Vanderborght (1979) have reported the stability of cyanogenic glucosides of seeds of *Phaseolus lunatus* to cooking. This correlates with the high levels of residual HCN in seeds of *Canavalia gladiatus* irrespective of processing procedure. The toxicity of the seeds of this legume may well be responsible for the frequent complaints of food poisoning in areas where they are cultivated as a subsistence crop. Jansz & Pieris (1978) have reported various processing methods for minimising HCN in seeds of *Phaseolus lunatus*. These include prolonged boiling of germinated seeds whose testa have been removed, and a procedure whereby damaged seeds are soaked, boiled, divested of testa and boiled again in that order. These methods, while resulting in drastic reduction of residual HCN, are rather tedious, and may compromise the nutritional quality of the meal as well as its palatability. In addition, such operations may not be easily performed on a large scale. It is therefore suggested that soaking of bean seeds in water and subsequent removal of testa before prolonged boiling would make for reduced HCN in bean-based meals.

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