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Assessment of Cyanogenic Potential, Nitrate and Nitrite Contents, and Trypsin Inibitor Activity of Some Nigerian Legumes

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Edible seeds of seven varieties of legumes commonly consumed by Nigerians in large quantities were evaluated for total protein, cyanogens, nitrate and nitrite contents, and trypsin inhibitor activity using chemical, biochemical, enzymatic, and spectrophotometric methods. All analyzed samples contained cyanogen and nitrate with levels ranging from 5.88 ± 0.26 to 28.55 ± 1.32 mg/100 g of DM and from 49.64 ± 4.60 to 239.42 ± 7.20 mg/100 g of DM, respectively. Only three of the varieties contained detectable levels of nitrite, which varied from 0.54 ± 0.01 to 3.19 ± 0.2 mg/100 g of DM. Trypsin inhibitor activity was detected in all of the samples, ranging from 7.75 to 100.75 μmol/min/mg of protein. Total protein content of the legumes ranged from 17.8 to 28% on a dry weight basis. There was a positive correlation ($r = 0.71$) between the cyanogenic potential and the nitrate content of the dry seeds. Processing reduced about 78.6−88.8% and 71.0−89.5% of total cyanogen and nitrate contents of the seeds, respectively. Following administration of 5.0−15 mg of NO_3 to rats by stomach intubation, analysis of their 24, 48, and 72 h urine showed that only 40% of the administered nitrate appeared in the urine unmetabolized. Processing was shown to drastically reduce these antinutritional factors to very low levels. The health implications of these findings are discussed.

KEYWORDS: **Cyanogens; nitrate; nitrite; trypsin inhibitor activity; legumes**

INTRODUCTION

The seeds from several species of legumes are important sources of protein in the diets of millions of people in the tropics, where many depend on plant protein as the major source of their dietary protein. In some parts of eastern Nigeria, some of the native legumes, *Vigna unguiculata* Mensensis (local name, Akidi), are used as nutritional therapy for children recovering from severe illnesses. One of the factors that can limit the protein availability of plant sources is an antinutritional factor such as cyanogenic glycosides and their hydrolytic products, trypsin inhibitor, and, to some extent, nitrates and nitrites. The cyanogenic potentials of intact seeds for a variety of legumes (*1*, *2*) as well as some other toxicants (*3*−*5*) have been reported.

However, some of the local varieties of legumes, particularly *Vi. unguiculata* Mensensis (Akidi and Odudu), usually consumed in large quantities by eastern Nigerians have not been investigated for their cyanogenic contents. Also, the levels of some other antinutritional factors need to be assessed because very little documented information exists on them. In addition to limiting the availability of the protein from plant sources, some of these antinutritional factors constitute health problems. The acute and chronic toxic effects of cyanogenic glycosides and their hydrolytic products are well documented (*6*−*9*). Nitrite, a naturally occurring metabolite of nitrate biotransformation, has been reported to be a precursor of carcinogenic *N*-nitrosamines (*10*, *11*), and *N*-nitroso compounds have been implicated in the causation of cancer of the urinary bladder and stomach (*12*). There is now increasing evidence that thiocyanate, the main cyanide metabolite, can lead to the promotion of *N*-nitrosamine formation in vivo, which can enhance carcinogenesis (*13*, *14*). Thus, the grave risk posed by the cyanide of cassava with potential increases from other sources such as legumes increasingly justifies efforts to measure the cyanogenic potential of other food materials. This study is therefore centered on the assessment of the cyanogenic potential, nitrate and nitrite contents, and trypsin inhibitor activity of some varieties of Nigerian legumes.

MATERIALS AND METHODS

Material. The seeds of seven varieties of edible legumes, mainly *Vigna* species, were investigated for their antinutritional factors and total protein content. They include *Vi. unguiculata* Mensensis (two varieties of Akidi and Odudu each), cowpea or black-eyed pea (Kano white and Ife brown), and *Voandzenia subterranean* (Bambara groundnut).

Sample Preparation. Good and healthy looking seeds were separated from insect-infested ones by hand picking. Known amounts

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Table 1. Antinutritional Factors and Protein Contents of Some Nigerian Legumes in Their Processed and Unprocessed Forms

material	total cyanogens[a] (mg/100 g of DM)		NO_3[a] (mg/100 g of DM)		NO_2[a] (mg/100 g of DM)		trypsin inhibitor activity (μmol/min/mg of protein)		total protein (%)
	processed	unprocessed	processed	unprocessed	processed	unprocessed	processed	unprocessed	unprocessed
Vi. uniguiculata Mensensis									
black Akidi	2.78 ± 0.4	17.4 ± 1.20	15.12 ± 2.0	63.68 ± 5.0	ND[b]	0.54 ± 0.01		70.75	23
brown Akidi	1.12 ± 0.2	10.01 ± 0.5	15.81 ± 1.8	54.51 ± 6.2	ND	ND		100.75	21
red Odudu	3.47 ± 1.1	28.55 ± 1.32	42.03 ± 4.6	239.42 ± 7.2	ND	3.19 ± 0.4		41.25	28
brown Odudu	2.95 ± 0.7	21.53 ± 2.0	29.52 ± 3.2	164.25 ± 3.1	0.62 ± 0.1	1.82 ± 0.3		30.75	24
Vo. subterranean									
cowpea (beans)		18.24 ± 2.1		49.64 ± 4.6		ND		81.25	18
Kano white	1.02 ± 0.5	5.88 ± 1.3	4.7 ± 0.6	49.66 ± 3.7	ND	ND		7.75	24
Ife brown	1.32 ± 0.5	6.17 ± 0.8	5.3 ± 0.4	52.65 ± 6.1	ND	ND		91.75	25.3

[a] For total cyanogens, NO_3, and NO_2, each value is $X \pm$ SD calculated from four determinations. [b] ND, not detected/not determined.

were weighed and ground into flour using a kitchen blender and stored in airtight containers.

Sample Extraction for Cyanide Analysis. Three grams of each sample flour was extracted into 15 mL of 0.1 M orthophosphoric acid using a gallenpark shaker. This was followed by filtration using Whatman no. 4 filter paper. The filtrate was then centrifuged at 10000*g* for 15 min to get a very clear supernatant.

Determination of Cyanide Content of Samples. The determination of the total cyanogen content of samples was according to the alkaline picrate method of Ikediobi et al. (*15*) using exogenous linamarase enzyme.

Sample Extraction for Nitrate and Nitrite Contents. Three grasm of each sample flour was extracted in 15 mL of double-distilled water using a gallenpark shaker. After filtration, the supernatant (or filtrate) was centrifuged as described above.

Nitrite and Nitrate Determinations. The nitrite content of the samples was determined according to the method of Follett and Ratcliff (*16*). Nitrate was determined after reduction to nitrite using a cadmium column. Cadmium was prepared according to the method of Follett and Ratcliff (*16*). Prior to the determination, the column was washed with 25 mL of diluted HCl (0.1 M), followed by 50 mL of distilled water, and finally with 25 mL of diluted ammonia buffer.

Ammonia Buffer Solution (pH 9.6−9.7). Twenty milliliters of concentrated HCl was added to 500 mL of water, and then 50 mL of 0.88 ammonia was added, and the solution was diluted to 1 L.

Diluted Ammonia Buffer. Ten milliliters of ammonia buffer was diluted to 100 mL with water.

Trypsin Inhibitor and Total Protein Content. The trypsin inhibitor activity (TIA) was determined by using the spectrophotometric method described by Arntfield et al. (*17*), whereas the crude protein was determined by microKjeldahl estimation of nitrogen using a factor of N × 6.25 (*18*).

Processing of Local Legumes into Foods. Traditionally, cowpeas or black-eyed peas (Ife brown and Kano white) are prepared by boiling for ∼1.5 h. Akidi and Odudu are prepared by boiling for longer times until they are softened, and their cooking water is thrown away after cooking. Bambara groundnuts in some instances are ground into flour using machines. The flour is then mixed with other ingredients and cooked.

Animal Experiment. Eighteen male albino Wistar rats (average weight = 100 g) previously maintained for 7 days on nitrate- and nitrite-free diet were grouped into three groups, each group consisting of six rats. Each of the rats in group 1 was given 5−15 mg of NO_3 equivalent as $NaNO_3$ and group 2 received 5−15 mg of NO_2 equivalent as $NaNO_2$ orally by stomach intubation.

Group 3 was used as control and received nitrate- and nitrite-free diet and drink the same as those in groups 1 and 2. The rats were then placed in Technoplast metabolic cages, and their urine was collected after 24, 48, and 72 h. The appearance of nitrite and nitrate in urine as one of the means of assessing the in vivo metabolism of these compounds was determined.

Human Experiment. Urine samples from 20 healthy volunteers (all women) who are also nonsmokers from a community in Awgu, Enugu State, Nigeria, where large quantities of these local legumes and cassava are consumed, were collected and analyzed for thiocyanate (SCN^-) according to the method of Haque and Bradbury (*19*). This is to assess their cyanide over load resulting from consumption of legumes and cassava.

RESULTS AND DISCUSSION

The results of this study show that all seven varieties of legumes analyzed contained appreciable quantities of nitrate and cyanide (see **Table 1**). Varieties that had particularly high nitrate contents tended to have high cyanide content. A positive correlation ($r = 0.71$) was found between the nitrate and cyanide contents of these legumes. One of the obvious implications of the high levels of cyanide measured in some of these legumes is that they may contribute to the chronic cyanide toxicity of dietary origin if they are not well processed. The potential cyanide intake from the processed legumes is above the Codex Alimentarius (*20*) safe level of 10 mg of HCN equivalent/kg for cassava products. However, only plants that accumulate >200 mg of HCN equivalent/100 mg of fresh weight are considered to be dangerous. The presence of cyanogens in diets could result in depletion of sulfur-containing amino acids of the body, because these are needed to detoxify the CN, converting it to thiocyanate (SCN). Thiocyanate formed could in turn act as a catalyst for the nitrosation of the residual nitrite and secondary amines endogenous in food to form *N*-nitroso compounds. There is now increasing evidence that SCN, the main cyanide metabolite in the body, can lead to the production of *N*-nitrosamines in vivo, which can enhance carcinogenesis (*13*, *14*). The SCN measured in the urine of subjects from one of the communities where the seeds of these legumes are consumed frequently and in large quantities ranged from 3.2 to 5.7 ppm (∼55.04−98.04 μmol/L), although cassava consumption is also high in this area. Thiocyanate excretion is a relatively good indicator of cyanide intake (*21*, *22*) but not an accurate indicator of biological toxic dose of cyanide.

The levels of nitrate and nitrite measured in these legumes are shown in **Table 1**. Although the nitrate levels of some are quite high, the nitrite levels may be considered very low. The values of nitrite and nitrate in the processed legumes fall below the WHO (*23*) recommended acceptable daily intake (ADI) for nitrite (0.2 mg/kg of body weight) and for nitrate (5 mg/kg of body weight) for children weighing <10 kg.

Nitrates are normally considered to be harmless to humans. However, their conversion to nitrite or other nitroso compounds may produce toxic or carcinogenic substances (*24*). Nitrite, on the other hand, is known to be the precursor of *N*-nitrosamines (*10*) and induces cancer in experimental animals (*25*). After ingestion, residual nitrite in foods and drinks forms traces of

Table 2. Urinary Excretion of Nitrate and Nitrite in Rats

animals	time 24 h	48 h	72 h
group I			
NO_3 administered (mg)	5	10	15
nitrate excreted (mg)	1.9	4.2	5.95
group II			
NO_2 administered (mg)	5	10	15
NO_2 excreted (mg)	ND	0.05	0.03
group III			
NO_3 or NO_2 administered			
NO_2 excreted (mg)	ND	ND	ND
NO_3 excreted (mg)	ND	ND	0.02

certain nitroso compounds in the stomach, where the pH is <7 on reacting with secondary amines, which might also be present in the foods (*11*). Similarly, Von Oettingen (*26*) stated that ingestion of small doses of nitrate over a certain period may cause cyanosis because of the reduction of nitrate to nitrite in the gastrointestinal tract. The risks to humans with respect to conversion of nitrate in the oral cavity and stomach leading to the possible formation of nitrosanines are well discussed in the work of Galler (*27*). Statistical analysis showed a good positive correlation ($r = 0.71$) between cyanide content and nitrate accumulation in the plants, indicating that cyanide content strongly affects nitrogen biotransformation in the legumes.

The ADI for nitrate is 0.2 mg/kg of body weight (*23*), and ingestion of 8−15 g of nitrate (which can be converted to nitrite) is known to cause severe gastroenteritis, with abdominal pains, blood in the stool and urine, weakness, and collapse (*28*, *29*). Oral administration of 5−15 mg of both nitrate and nitrite separately to rats by stomach incubation and analysis of their 24, 48, and 72 h urine showed that only ∼40% of the nitrate appeared in the urine (see **Table 2**). This indicates that the remaining 60% has been metabolized, presumably with some converted to nitrite.

The total protein content of these legumes ranged from 17.8 to 28%, justifying their use as a source of dietary protein. In this context, *Vi. unguiculata* Walp. has been reported to contain fairly well balanced protein of the essential amino acids (*30*). TIA was also detected in all of the varieties of legumes and ranged from 7.75 to 100.75 μmol/min/mg of protein. Trypsin inhibitors are known to reduce the protein availability of plant proteins.

The various processing techniques to reduce cyanide and the antinutritional factors of these food materials have been employed by the consumers of these legumes. Such techniques include cooking the food overnight and throwing away the water, as employed in processing Odudu, and boiling for a long time, as employed in the preparation of *Vi. unguiculata* Mensensis (Akidi). The effect of processing on the antinutritional factor contents of these seeds is shown in **Table 1**. The processes reduced the level of cyanogens by 78.6−88.8% and that of nitrate by 76.26−90.5%.

It is important that the seeds of these legumes, particularly Odudu and *Vi. unguiculata* Mensensis, should not be given to children as a protein supplement without adequate processing to reduce these antinutritional factors to negligible levels. This is because many enzymes involved in the detoxification of toxicants present in foods are not produced adequately in infants. As a result, infants have limited capacity to metabolize these inevitable constituents of food and hence are highly vulnerable to their harmful effects, such as methoemoglobinaemia.

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