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Original article

Evaluation of the distribution of cyanide in the peels, pulps and leaves of three new yellow cassava (*Manihot esculenta* Crantz) varieties for potential livestock feeds

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Summary. The total and free cyanide levels in fresh peels, pulps and leaves of three cassava (*Manihot esculenta* Crantz) varieties: TMS 01/1368 (UMUCASS 36), TMS 01/1412 (UMUCASS 37) and TMS 01/1371 (UMUCASS 38) were determined using standard enzymatic methods. Results showed that the total cyanide levels in the peels, pulps and leaves of these cassava varieties were lower than the reference range for fresh cassava peels, pulps and leaves which were given as 364.2 to 814.70 for the peels, 34.3 to 301.30 for the pulps and 200 to 800 $mgkg^{-1}$ CN^{-} for the leaves respectively. The cyanide levels in the peels, pulps and leaves of TMS 01/1412 were found to be lower than that of TMS 01/1368, TMS 01/1371 and the reference range. Furthermore, the peels had the highest levels of total and free cyanide followed by the leaves and lastly the pulp. Results obtained from this study indicate the usefulness of the peels, pulps and leaves of these cassava varieties as potential livestock feeds. Finally, results obtained suggest that these cassava byproducts may require simpler processing compared with byproducts of high cyanide cassava varieties before being converted into livestock feeds.

Key words: cassava byproducts, animal nutrition, cyanide, yellow Cassava varieties

Introduction

Cassava (*Manihot esculenta*) is one of the most important food crops in the tropical countries with Nigeria being the largest producing country in the world (1). A major problem experienced by agro-based industries in developing countries is the management of wastes. Agro wastes in Nigeria are mostly subjected to open air burning with its attendant environmental implications (2). Inefficient and improper disposal of solid wastes creates serious hazards to public health, including pollution of air and water resources and increases in rodent and insect vectors of disease create public nuisances, otherwise interfere with community life and development (3). The failure or inability to salvage and re-use such materials economically results in the unnecessary waste and depletion of natural resources (3). To date, emphasis is on biological conversion of plant wastes, especially agricultural wastes into value added products.

Three major wastes of cassava in Nigeria are the leaves, pulps and peels (generated as a result of mechanical removal of the two outer coverings of cassava roots subsequent to its processing). Furthermore, the leaves, peels and pulp constitute more than 25% of the whole cassava plant (4).

In recent times, the by-products of cassava have been found to be potential sources of livestock feed ingredients (4). Hence, the trend has been the inclusion of cassava products that meet minimum requirements for incorporation into commercial livestock feed production. This is especially important as it would reduce the pressure on demand for available cereal grains.

However, the use of cassava and its by-products in livestock feeding has been limited due to a number of factors one of which is the presence of toxic cyanogenic glucosides in high quantities in them that can lead to reduced animal performance (5) and in extreme cases, death.

Recently, National Root Crops Research Institute, Umudike, Nigeria through a joint breeding programme with the Institute of Tropical Agriculture, Ibadan, Nigeria, developed some yellow pro-vitamin A cassava varieties in an effort to curb the scourge of vitamin A deficiency and resultant diseases in the tropics. Previous studies on the fresh roots of these cassava varieties showed that they contained low levels of cyanide (6). However, the cyanide levels in their leaves, pulps and peels have not been investigated. Since the current trend is the biological conversion of plant wastes into value added products, there was the need to investigate the cyanide levels in these cassava by-products to see their usefulness in livestock nutrition and this led to this study.

Methods

Plant materials

Fresh leaves and tubers of the cassava varieties (TMS 01/1368 or UMUCASS 36, TMS 01/1371 or UMUCASS 38, TMS 01/1412 or UMUCASS 37) used for this experiment were collected from National Root Crops Research Institute, Umudike immediately after harvest in 2014.

Processing of the plant materials

The leaves and tubers were thoroughly washed and the tubers were peeled, re-washed, cut into small pieces and analyses were carried out on the cut samples on fresh weight basis.

Preparation of cassava extracts

Fifty gram of the pulps or peels of each variety was homogenized with a blender in 200 mL of 0.1M orthophosphoric acid buffer medium using a warring blender while 10 g of the leaves of each variety was homogenized in 50 mL of 0.1M orthophosphoric acid buffer medium using a warring blender. The homogenates (pulp, peel or leaves) were each filtered and the filtrates were kept in a freezer.

Extraction and purification of linamarase

Cassava peels (200 g) were weighed and homogenized using 350 mL of acetate buffer (0.1 M) pH 5.5 in warring blender. The homogenate was filtered using muslin cloth to obtain the filtrate. The filtrate was centrifuged at 10,000 g for 5 min to obtain the supernatant. A measured quantity (200 mL) of the supernatant was collected and brought to 60% of ammonium sulphate saturation and this was kept overnight in a refrigerator in order to obtain the precipitate which was collected and centrifuged at 10,000 g for 5 min. The precipitate obtained after centrifugation was dissolved in 50 mL of 0.1M phosphate buffer pH 6.0 and dialyzed in the same buffer with 3 changes of the buffer every 8 h (7). The partially purified enzyme was used for the determination of the total cyanide concentrations of the samples.

Determination of total cyanide and free cyanide

The total and free cyanide in the peels, pulps and leaves were determined using the method of Esser and co-workers (8).

Statistical analysis

Data generated was analyzed using the Statistical Package for Social Sciences Version 17.0. Results are presented as means ± standard deviations (SD). One way analysis of variance (ANOVA) was used for comparison of means. Differences between means were considered to be significant when $p<0.05$.

Results and Discussion

One of the characteristics of cassava plant is the presence of linamarin, a glycoside that is hydrolysable and releases cyanide in a process known as cyanogenesis. The use of cassava products for feed or consumption is strongly affected by the levels of this toxic cyanogenic glycoside (6). It is well recognized that plants containing cyanogenic glucosides also contain enzymes capable of degrading them (9). Plant glucosidases hydrolyzing the cyanogenic glucosides were found to have high specificity towards the cyanogenic glucosides. These enzymes (β-glucosidases) breakdown cyanogenic glucosides liberating hydrocyanic acid in a reaction that involves the cleavage of the sugar moiety in the glucosides (10) as shown in Figure 1.

The reference range for total cyanide in fresh cassava tubers, peels, leaves and pulps were given as 88.30- 416.30, 34.3- 301.30, 364.2- 814.70 and 200-800 mg/kg respectively (5, 11).

The peels of TMS 01/1368 contained significantly higher ($p<0.05$) total and free cyanide levels compared with the pulps and leaves respectively while the pulp had the least total cyanide level but its free cyanide levels did not significantly differ ($p>0.05$) from that of the leaves (Table 1). The total cyanide levels in the peels and leaves of this cassava variety were found to be lower than the reference range for fresh cassava peels and leaves respectively.

As shown in Table 2, the peels of TMS 01/1371 contained significantly higher ($p<0.05$) total and free cyanide levels compared with the pulps and leaves while the pulp had the least ($p<0.05$). The total cyanide levels in the peels and leaves of this cassava variety were also found to be lower than the reference range for fresh cassava peels and leaves respectively.

The peels of TMS 01/1412 contained significantly higher ($p<0.05$) amounts of total and free cyanide compared with the pulps and leaves respectively while the pulp contained the least ($p<0.05$) (Table 3). The cyanide levels in the peels, pulps and leaves of this cassava variety were found to be lower than that of other cassava varieties (TMS 01/1368 and TMS 01/1371) investigated. In addition, the cyanide levels in the peels, pulps and leaves of this cassava variety were lower than the reference range for fresh cassava peels, pulps and leaves respectively.

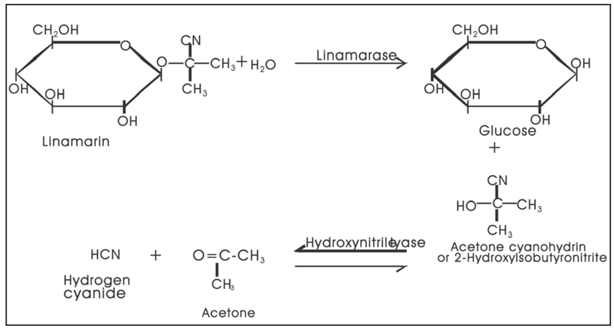

Figure 1. Breakdown of cyanogenic glucosides (10)

Cassava leaf is the major site of synthesis of cyanogenic glucosides by the plant (12). Therefore, results obtained with respect to the cyanide levels in the cassava varieties investigated and which levels were lower than the reference standard suggest that these cassava varieties are low cyanide varieties. Results obtained further suggest that these cassava byproducts may require simpler processing compared with

Table 1. Total cyanide and free cyanide concentrations in fresh cassava peels, pulps and leaves of TMS 01/1368

Cassava variety TMS 01/1368	Conc. of total cyanide ($mgkg^{-1}$ CN^-)	Conc. of free cyanide ($mgkg^{-1}$ CN^-)	Reference range total cyanide ($mgkg^{-1}$ CN^-)
Peels	$80.67±0.46^c$	$34.71±0.50^b$	364.2- 814.70
Pulps	$35.06±0.87^a$	$16.74±0.28^a$	34.3- 301.30
Leaves	$49.80±0.38^a$	$19.95±0.13^a$	200- 800

Values in the table are the means ± SD of three determinations. $^{a-c}$Means with different superscript along the columns are significantly different (p<0.05). Conc- Concentration.

Table 2. Total cyanide and free cyanide concentrations in fresh cassava peels, pulps and leaves of TMS 01/1371

Cassava variety TMS 01/1371	Conc. of total cyanide ($mgkg^{-1}$ CN^-)	Conc. of free cyanide ($mgkg^{-1}$ CN^-)	Reference range total cyanide ($mgkg^{-1}$ CN^-)
Peels	$98.33±0.69^c$	$46.12±0.14^c$	364.2- 814.70
Pulps	$40.82±0.27^a$	$19.47±0.55^a$	34.3- 301.30
Leaves	$56.72±0.41^b$	$26.28±0.79^b$	200- 800

Values in the table are expressed as means ± SD of three determinations. $^{a-c}$Means with different superscript along the columns are significantly different (p<0.05). Conc- Concentration.

Table 3. Total cyanide and free cyanide concentrations in fresh cassava peels, pulps and leaves of TMS 01/1412

Cassava variety TMS 01/1371	Conc. of total cyanide ($mgkg^{-1}$ CN^{-})	Conc. of free cyanide ($mgkg^{-1}$ CN^{-})	Reference range total cyanide ($mgkg^{-1}$ CN^{-})
Peels	79.13 ± 1.76[c]	31.91 ± 0.14[c]	364.2- 814.70
Pulps	26.72 ± 0.51[a]	12.22 ± 0.45[a]	34.3- 301.30
Leaves	46.06 ± 0.29[b]	16.62 ± 0.36[b]	200- 800

Values in the table are expressed as means ± SD of three determinations. [a-c]Means with different superscript along the columns are significantly different (p<0.05). Conc- Concentration.

byproducts of high cyanide cassava varieties before being converted into biologically useful products and this is an important finding in this study.

Cassava roots were classified according to their potential toxicity to humans as: non-toxic (less than 50 mg HCN kg^{-1} of fresh roots), moderately toxic (50-100 mg HCN kg^{-1} of fresh roots) and dangerously toxic (above 100 mg HCN kg^{-1} of fresh roots) (13-15). Further classification by Hahn and Keyser (16) indicated that the range of total cyanide in the pulp of edible cassava varieties is considered low when it is less than 100 mg HCN per kg (fresh weight) while the lethal dose of cyanide in most animals is about 2 mg/kg body weight.

Following the reference range of total cyanide in fresh cassava peels, pulps and leaves and which values as obtained for the fresh peels, pulps and leaves of these cassava varieties were below this reference range, suggest these cassava varieties to be low cyanide varieties. This finding thus makes them promising candidates for livestock feeds.

In all these cultivars, their peels consistently had the highest levels of total and free cyanide followed by the leaves and lastly the pulp.

Finally, results obtained suggest that these cassava byproducts may require simpler processing compared with byproducts of high cyanide cassava varieties before they could be used for livestock nutrition.

Conclusion

The study indicated the low cyanide levels in the fresh leaves, pulps and peels of the cassava varieties (TMS 01/1368, TMS 01/1371 and TMS 01/1412) indicating their potential usefulness as livestock feeds.

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