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Nutrient Composition and Processing Effects on Cassava Leaf (*Manihot esculenta*, Crantz) Antinutrients

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Abstract: Leaves of 3 genetically improved varieties of cassava plants were harvested and subjected to different processing methods including sun-drying (SND), oven-drying (OVD), steaming (STM), shredding (SHD) and steeping (STP) and a combination of these methods to deliberately reduce the high level of cyanogenic glucosides present in the leaves. A combination of SHD and SND (SHD+SND) seemed to be the most effective technique of reducing the cyanide content. Proximate/mineral composition and gross energy were determined. Particular attention was paid into the determination of hydrocyanic acid (HCN), polyphenols (tannic acid) and phytic acid as they constitute the major anti-nutrients militating against the utilization of cassava leaf in animal nutrition. The leaves contained: crude protein 348.0gkg^{-1} DM (range: 332.0 - 363.0gkg^{-1} DM); crude fibre 121.0gkg^{-1} DM (range: 115.0 - 127.0gkg^{-1} DM); ether extract 70.0gkg^{-1} DM (range: 63.0 - 75.0gkg^{-1} DM); ash 69.0gkg^{-1} DM (range: 63.0 - 78.0gkg^{-1} DM) and gross energy 47.0MJkg^{-1} (range: 46.5 - 47.2MJkg^{-1}). The CLM protein content was high and comparable with some rich conventional protein sources of plant and animal origins used in monogastric feed formulation. The mineral content was high particularly Ca, Zn, Ni and K. The "cyanide scare" associated with acute intoxification when food substances rich in cyanide is ingested at high levels seemed to be obviated by a combination of processing methods (SHD+SND).

Key words: Cassava leaf, animal feed, conventional protein sources

Introduction

Cassava (*Manihot esculenta*, Crantz) as an all - season crop as food in several parts of Africa (Nigeria inclusive), Asia and Latin America is well documented (Longe, 1980; Rosling, 1987; Bradbury *et al*., 1991). Nigeria alone, currently produces over 14m tonnes annually, representing about 25% of sub-saharan Africa's output. Although it is the third most important food source in the tropical world after rice and maize, and provides calories for over 160m people in Africa (Polsen and Spencer, 1991) its food value is greatly compromised by the endogenous presence of cyanogenic glucosides. These glucosides, typified by linamarin [2-($-D-glucopyranosyloxy) isobutyronitrile] and lotaustralin [2-($-D-glucopyronosyloxy) methylbutyronitrile] are hydrolyzed to hydrocyanic acid (HCN) by endogenous linamarase. (EC. 3.1.1.21, linamarin, $-D-glucoside glucohydrolase) when cassava tissues are disrupted by cutting, grating, bruising or other mechanical means (Conn, 1969; Bradbury *et al*., 1991).

Cassava leaves, a byproduct of cassava root harvest is (depending on the varieties) rich in protein (14 - 40% Dry Matter), minerals, Vitamin B1, B2, C and carotenes (Eggum, 1970; Adewusi and Bradbury, 1993). Available literature clearly suggest, that apart from lower methionine, lysine and perhaps isoleucine content, the amino acid profile of cassava leaf protein compares favourably with those of milk, cheese, soyabean, fish and egg. In spite of these qualities, the nutritional potentials of cassava leaf meal and cassava protein concentrates remain currently under-researched. The major drawback to the widespread use of cassava leaves as food in Nigeria is "cyanide scare" as its content of cyanogenic glucosides could, depending on the variety, be 6 times higher than in the roots. Apart from cyanide, tannin and possibly phytin (Reeds *et al*., 1982) may limit the nutritional value of cassava leaves.

While various cassava processing techniques may generally lead to substantial cassava detoxification, conditions, such as famine, drought and failure of other less - well adapted root crops generally lead to increased demands for cassava roots and leaves during which the traditional processing methods may be compromised. Apart from the risk of acute cyanide intoxification and death, chronic exposure to sub-lethal levels increases the incidence of goitre, tropical neuropathy, glucose intolerance (Oshuntokun, 1972; Akanji and Famuyiwa, 1993) and Konzo (spastic paraparesis) (Howlett *et al*., 1990).

It is evident from the foregoing that, for the full nutritional potentials of cassava roots and leaves to be realized, current research efforts must focus more on the development of simple, low - cost but efficient techniques that would rid them of cyanide as well as other anti - quality constituents such as tannin and phytin in the leaves. The present study therefore provides analytical information on the nutrient composition of the leaves of come local and genetically improved cassava varieties as well as the processing effects on some of their inherent antinutrients. We ultimately hope to

reconcile the efficacy of such processing techniques with controlled nutritional studies to permit credible local health education programmes with regard to cassava leaf processing and use for human and, or animal feeding.

Materials and Methods

Sample collection: The leaves analyzed were harvested from a local variety - Ege Oda and 3 genetically improved varieties - Tropical Manihot serves (TMS) 6, 30555 and 30572. The local variety was obtained from Oda, and Akure village, while the improved varieties were obtained from the Federal College of Agriculture, Akure. All samples were cultivated in the humid tropical rain forest zone where the rains which fall between March and September/October, average between 1150 and 2000mm annually. Utisols are the predominant soil types with pH ranging between 5.0 and 6.0. All samples processed and analyzed were a composite of expanding leaves, fully expanded and mature leaves harvested on the north side of the plant. About 4kg materials collected from each of the varieties were thoroughly mixed, had their stalk removed, rinsed with distilled water before subjecting them to the different processing methods.

Processing techniques: About 500g each of the cassava leaf varieties were subjected to the following processing techniques:

i. Sun - drying (SND) for 2-3 days with constant turning over to avert fungal growth;
ii. Oven - drying (OVD) on aluminum trays at 80-90^{o}C for 24 hours;
iii. Steaming (STM) of the leaf samples over a wire gauze placed on top of a boiling water for 30 minutes;
iv. Shredding (SHD) of the leaves using Moulinex blender or a chopping knife to cut the leaves into fine pieces;
v. Steeping (STP) of the leaf sample in 5 parts/weight of water for 24 hours;
vi. Steaming and Sundrying (STM + SND) in which the steamed leaf sample was further sundried for 2-3 days with adequate turning over to avert fungal growth;
vii. Shredding and Sundrying (SHD + SND) in which the Moulinex blended samples were now sundried for 2-3 days with adequate turning to avert mouldiness.

All the processed samples were packed into tightly sealed nylon bags and analyses commenced immediately with minimum delay to forestall a further change in quality of samples. Where the analyses could not be completed on the same day, samples were kept frozen at a temperature of -4^{o}C.

Proximate/mineral composition and gross energy: The proximate constituents of the air-dried materials were determined by the method of the Association of Official Analytical Chemist (AOAC, 1980). The sodium and potassium contents were determined by flame photometry, and phosphorus was determined by the vanodo - molybdate method (AOAC, 1980). The other mineral elements were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acid, using Atomic Absorption Spectrophotometer (AAS Model SP9). Gross energy of the dried material was determined against thermocouple grade henzoic acid using a Gallenkamp ballistic bomb calorimeter (Model CBB - 330 - 0104L).

Determination of anti-nutrients:

Hydrocyanic acid (HCN): The cyanogenic potential of the fresh and differently processed cassava leaf varieties was determined (after an initial extraction for 2-3 min of 5-8g material in 0.1m H_3PO_4 by a 2m H_2SO_4 (100^{o}C for 50mins) hydrolysis followed by reaction with chloramine-T pyridine barbituric acid (Konig Reaction) as developed by Bradbury *et al.* (1991). KCN dried over concentrated H_2SO_4 was used to calibrate the standard curve from a stock solution containing 75mgKCN/100ml.

Polyphenols (Tannic acid): The leaf samples, finely milled (250mg in 10ml of 70% aqueous acetone) were extracted for 2hr at 30^{o}C using Gallenkamp orbital shaker (Survey, UK). Pigments and fats were first removed from the leaves by extracting with diethyl ether containing 1% acetic acid. Thereafter, the total polyphenols (as tannic equivalent) were determined in 0.05, 0.2 or 0.5ml aliquot using Folin Cocalteu (Sigma) and standard tannic acid (0.5mg/ml) as described by Makkar & Goodchild (1996).

Phytic acid: The extractions and precipitation of phytin in the fresh and processed leaf materials were done by the method of Wheeler and Ferrel (1971) while iron in the precipitate was determined as described by Makower (1970). Using a 4:6 Fe/P ratio to calculate phytin phosphorus by 3.55 (Young and Greaves, 1940). Where contracts were deeply coloured, they were decolourized with activated charcoal.

Statistical analysis: All values were means for duplicate determinations. Mean values for all parameters within the cassava varieties were assigned coefficients of variation (Steel & Torrie, 1960).

Results and Discussion

The proximate composition and gross energy values of the leaf varieties are presented in Table 1 while the mineral content are presented in Table 2.

The crude protein (CP) ranged from 332.0gkg^{-1} DM in TMS 30555 to 363.0gkg^{-1} in the local variety and a coefficient of variation of 3.7%. The mean crude fibre (CF) content was 121.0±0.5gkg^{-1} DM with a range of 115.0gkg^{-1} DM in the local variety to 127.0gkg^{-1} DM in TMS 30572 with a CV of 4.1%. Ether extract (EE) and ash averaged 70.0 ± 0.5gkg^{-1} and 69.0 ± 0.6gkg^{-1} DM

Table 1: Proximate composition (gkg^{-1} DM) and gross energy values ($MJkg^{-1}$) of cassava leaves (Means, n = 2)

Cassava varieties	Crude protein	Crudefibre	Ether extract	Ash	N-free extract	Gross energy
MS6	35.4	11.8	7.3	7.8	37.8	469.5
TMS30555	33.2	12.3	6.3	6.3	41.6	464.7
TMS 30572	34.3	12.7	7.5	7.2	38.3	470.8
Ege Oda (Local Variety)	36.3	11.5	7	6.9	38.2	472.2
Mean	34.8	12.1	7	6.9	39	469.3
Std dev.	1.3	0.5	0.5	0.6	1.8	3.3
Coefficient of variation (%)	3.7	4.1	7.1	6.5	4.6	0.7

Table 2: Mineral contents of cassava leaves

Cassava varieties	Ca	Mg	Zn	Ni	Na	K	P	Fe
	<---------- (gkg^{-1} DM) ---------->						<---($mgkg^{-1}$)--->	
MS6	2.9	0.24	3.4	3.1	0.52	1.4	600	100
TMS 30555	2.2	0.21	2.6	1.8	0.69	1.4	500	113
TMS 30572	1.2	0.11	2.2	1.2	0.76	2	700	102
Ege Oda (Local Variety)	2	0.48	2.1	2	0.65	1.5	600	105
Mean	2.1	0.26	2.6	2	0.65	1.6	600	105
Std dev.	0.69	0.15	0.57	0.79	0.1	0.3	82	6
Coefficient of variation (%)	32.9	57.7	9.6	39.5	15.4	18.8	14	6

Table 3: Varietal Distribution of Hydrocyanic Acid (HCN), Polyphenols (Tannic Acid) and Phytin in Cassava Leaves (Means, n = 2)

Cassava Varieties	HCN (Mg/100g)	Tannin (g/100g)	Phytin (mg/100g)
MS 6	56.5	7.5	249.1
TMS 30555	40.2	15	197.8
TMS 30572	54.1	9.2	213.8
Ege Oda (Local Variety)	60.6	6.9	107.3
Mean	52.9	9.7	192
SD	8.9	3.6	60.4
CV (%)	16.8	37.1	31.3

respectively. Gross energy averaged 47.0±3.3$MJkg^{-1}$ DM with a range of 46.5$MJkg^{-1}$ in TMS 30555 to 47.2$MJkg^{-1}$ in the local variety. The mineral composition of the cassava leaf varieties as presented in Table 2 indicates that Zn, Ca and Ni were the most abundant minerals with average values of 26.0, 21.0 and 20.0gkg^{-1} DM, respectively while Fe and P were the least, being 105 and 600$mgkg^{-1}$ respectively. The varieties differed markedly in Ca, Mg and Ni content as indicated by the high CV of 32.9, 57.7 and 39.5% respectively.

The hydrocyanic acid (HCN), polyphenols (tannic acid) and phytin contents of the different varieties of cassava leaves are presented in Table 3.

The cyanogenic potential (Table 3) averaged 52.9±8.9 mg HCN/100g with a range of 40.2mgHCN/100g in TMS 30555 to 60.6mg HCN/100g in the local variety and a CV of 16.8%. The total polyphenols (as tannic acid equivalent) averaged 9.7±3.6g Tannin/100g ranging from 6.9% Tannin/100g in the local variety to 15.0g Tannin/100g in TMS 30555 with a CV of 37.1%. The phytin content ranged from 107.3mg/100g in the local variety to 239.1mg/100g in MS6 with an average of 192.0 ± 60.4mg/100g and a CV of 31.3%.

The processing effects on the cassava leaf cyanogenic potential, tannin and phytin are presented in Table 4, 5 and 6, respectively.

Shredded + sundried (SHD + SND) leaves (Table 4) retained the least amount of cyanogen (i.e. 3.7 and 4.1% HCN retention, respectively) while steeping or OVD were least efficient (i.e. 69.1 and 61.6% HCN retention, respectively). Oven-dried (OVD) leaves (Table 5) retained the least amount of tannin (48%) while SND, STM + SND or SHD + SND retained tannin to about the same extent (62 - 64%). Similarly, SND, STM + SND or SHD + SND (Table 6) retained phytin to about the same extent (41-42%) while STP or STM were ineffective.

The data on the proximate energy and mineral content of the cassava leaf varieties clearly indicate their potential as food or feed resource. For example, their content of crude protein (348.0±1.3gkg^{-1} DM), crude fat (70.0+0.5gkg^{-1} DM) and ash (69.0+0.5gkg^{-1} DM) compare favourably with and in certain cases, surpass those reported for most legumes (except groundnut and soyabean) grown in West Africa (FAO, 1973; Ologhobo, 1980; Aletor and Aladejimi, 1989). Also, their protein contents were generally higher than those reported for other cassava leaf varieties (Eggum, 1970; Ravindran *et al.*, 1987): and higher than reported for several leguminous browse plants (Aletor and Omodara, 1994) and several tropical leafy vegetables (Aletor and Adeogun, 1995). The leaf varieties did not differ markedly in their proximate constituents as evidenced in the low coefficients of variation (CV). The high content of mineral

Table 4: Hydrocyanic acid content (mg/100g) in differently processed cassava Leaves (Means, n = 2)

Processing Variables	Cassava Varieties				
	(MS 6)	(TMS 30555)	(TMS 30572)	(Local)	HCN retained (%)
SND	1.8 (3)	1.7 (4)	1.8 (3)	4.1 (6)	4.1±1.1
OVD	41.5 (74)	24.9 (62)	24.9 (46)	39.3 (65)	61.6±11
STM	27.3 (48)	18.4 (46)	36.7 (68)	45.2 (75)	59.0±14.3
SHD	26.4 (47)	19.6 (49)	23.7 (44)	30.8(51)	47.5±3.0
STP	35.7 (63)	27.8 (69)	36.7 (68)	46.1 (76)	69.1±5.3
STM + SND	37.0 (66)	15.3 (38)	16.6 (31)	26.1(43)	44.3±15.0
SHD + SND	1.6 (3)	1.7 (4)	1.7 (3)	2.9 (5)	3.7±0.1
Fresh leaves	56.5 (100)	40.2 (100)	54.1 (100)	60.6(100)	(100)

SND, Sundrying; OVD, Oven-drying; STM, steaming; SHD, Shredding; STP, steeping; values in parenthesis refer to % HCN retained

elements, particularly Ca, Zn, Ni and K in the leaves, when compared with other plants, such as legumes and tubers confirm their importance as rich source of dietary minerals. Unlike the proximate and energy constituents, the mineral content (Table 2) showed distinct variability, particularly in Ca, Ni and Mg content as indicated by the high CV of 32.9, 39.5 and 57.7% respectively. Using these proximate, energy and mineral analytical values as approximate indices of nutritional potential or quality, it would appear that cassava leaves fall between those of most legumes and animal protein. However, their high crude fibre content (121.0+0.5gkg^{-1} DM) are of nutritional concern (especially in non-ruminants) since high dietary fibre can cause intestinal irritation, lower digestibility and overall decreased nutrient utilization (Johnson, 1987).

Because of the risk of acute intoxification associated with the consumption of high cyanide-containing cassava products, most studies on the toxic or potentially toxic constituents of cassava leaves have been skewed rather heavily in favour of the cyanogenic constituents. Consequently, information on their content of other potential toxicants or antinutrients have remained scanty. The present study shows that apart from cyanide (Table 3) which averaged from 52.9+8.9mg HCN/100g, the leaves contained high levels of tannin (9.7+3.6g/100 DM) and phytin (192.0+60.4mg/100g). While the cyanide levels in these varieties were lower than those reported for other varieties (Lancaster and Brooks, 1983, Ravindran *et al*., 1987). The tannin content of the leaves were much higher than found in most grain legumes and cereals. The nutritional significance of dietary cyanide derives from several observations (Tewe *et al*., 1976; Frake and Sharma, 1986; Aletor and Fetuga, 1988, Aletor, 1993a) that cyanide either in synthetic or organic forms can cause marked changes in weight gain, nutrient utilization, liver enzyme activities and thiocyanate concentrations in serum and urine of rats and hamsters. The cyanide detoxification route in man and animals, cyanide - thiocyanate sulphur-transferase (rhodanase) pathway generally requires organic sulphur donors in the form of methionine and cystine thereby precipitating methionine deficiency in an otherwise balanced diet (Maner and Gomez, 1973). Tannin, on the other hand, bring about their nutritional influences (especially in non-ruminants) largely, by binding dietary proteins and digestive enzymes into complexes that are not readily digestible. The poor palatability generally associated with high tannin diets are ascribed to its astringent property which is a consequence of its ability to bind with proteins of saliva and mucosal membranes (Mehansho *et al*., 1987). Paradoxically, there is ample evidence that subject to certain dietary levels, tannin may not always be anti-nutritional in ruminants. For example, condensed tannin in *Lotus pedunculana* while reducing rumen degradation of carbohydrates, may enhance amino acids absorption in the small intestine (Barry and Manley, 1986) via a 'by-pass' process. The presence of phytin in the cassava leaves agree with earlier report (Aletor and Adeogun, 1995) of their widespread occurrence in plants. The anti-nutritional nature of phytin lies in its ability to chelate certain mineral elements, especially Ca, Mg, Fe and Zn (Forbes and Erdman, 1983) thereby rendering them metabolically unavailable. Dietary phytin is of particular importance in non-ruminants (including man) who lack phytase to break down phytin to release phosphorus for metabolism. Phosphorus utilization has become an important current issue on the question of environmental pollution arising from the poor digestibility of P especially in foods/feed of vegetable origin (Huisman, 1991) where a high proportion of the phosphorus may be present as the poorly digestible phytin-P in non-ruminants. In such circumstances, considerable amount of dietary phosphorus may be voided in feaces leading to the pollution of the environment.

The present study, clearly shows that shredding + sundrying or sundrying alone (Table 4) are highly efficient processing techniques for cyanide removal from cassava leaves (i.e. from 56.5mg HCN/100g in the fresh sample to 1.6 or 1.8 mg HCN/100g respectively) while oven drying, steaming and steeping were relatively inefficient. The findings are in consonance with earlier observations (Aletor 1993b; Bokanga, 1994; Nambisan, 1994) that the residual cyanide level in processed cassava products, depends largely on the nature and

Table 5: Polyphenols (as Tannic acid equivalent g/100g DM) in differently processed cassava leaves (Means, n = 2)

Processing Variables	Cassava Varieties				
	(MS 6)	(TMS 30555)	(TMS 30572)	(Local)	Tannin retained (%)
SND	5.8 (77)	8.2 (55)	3.5 (38)	5.9 (86)	63.9±21.6
OVD	3.7 (49)	4.9 (33)	3.9 (42)	4.7 (68)	48.0±15.0
STM	6.5 (87)	5.7 (38)	8.3 (90)	6.7 (97)	78.0±27.0
SHD	7.2 (96)	13.3 (88)	8.3 (90)	6.6 (96)	92.6±3.8
STP	4.2 (56)	11.6 (77)	12.1 (131)	9.3 (134)	99.8±39.4
STM + SND	4.9 (65)	7.9 (53)	4.2 (46)	6.0 (87)	62.6±18.1
SHD + SND	5.0 (67)	8.9 (59)	4.8 (52)	4.9 (71)	62.3±8.3
Fresh leaves	7.5 (100)	15.0 (100)	9.2 (100)	6.9 (100)	(100)

SND, Sundrying; OVD, Oven-drying; STM, steaming; SHD, shredding; STP, steeping; values in parenthesis refer to % Tannin retained

Table 6: Phytic acid content (mg/100g) in differently processed cassava leaves (Mean, n = 2)

Processing Variables	Cassava Varieties				
	(MS 6)	(TMS 30555)	(TMS 30572)	(Local)	Phytin retained (%)
SND	45.7 (18)	37.6 (18)	86.5 (41)	93.1 (87)	41.1±32.0
OVD	184.9 (74)	126.4 (64)	13.0 (64)	81.1 (76)	69.4±6.4
STM	213.9 (86)	238.1 (120)	258.8 (120)	198.5(184)	127.7±41.2
SHD	75.6 (30)	101 (51)	75.1 (35)	71.2 (66)	45.8±16.4
STP	269.8(108)	228.1 (115)	288.9 (135)	136.0(126)	121.3±12.0
STM + SND	98.9 (40)	49.2 (25)	62.6 (29)	78.3 (73)	41.7±21.7
SHD + SND	106.7 (43)	94.8 (48)	69.8 (33)	44.4 (41)	41.1±6.4
Fresh unprocessed leaves	249.1(100)	197.8 (100)	213.8(100)	107 (100)	(100)

SND, Sundrying; OVD, Oven-drying; STM, steaming; SHD, shredding; STP, steeping; values in parenthesis refer to % Phytic Acid retained

duration of the processing technique, It is conceivable that the extremely high linamarase activity in cassava leaves (usually about 25 - 200 times the activity in the roots) coupled with the mechanical damage to the tissues during shredding or wilting during sundrying facilitated linamarin/linamarase union, bringing about hydrolysis of the volatile hydrogen cyanide. The high residual cyanide in oven - dried (80-90^{o}C) and steamed (100^{o}C) leaves may be ascribed to possible denaturation of linamarase activity at temperatures above 55^{o}C (Mpong *et al.*, 1990). Quite unlike the substantial cyanide removal by some of the processing techniques, the mean % tannin and phytin retention remained high (> 41%) implying that residual tannin and perhaps, to a lesser extent, phytin could pose greater problem in processed cassava leaf - based diets.

Conclusion: In conclusion, this study indicates the high potential of cassava leaf as an unconventional protein resource for both humans and animals. However, the principal problems that could undermine this potential are the high fibre content and the presence of antinutrients typified by cyanide, tannin and phytin. While sun-drying and shredding + sundrying reduced cyanide to innocuous levels, the processing techniques were less efficient with regard to tannin and phytin removal. However, the wide range in concentration of tannin and phytin in these leaf varieties is an indication that genetic detoxification (reduction) is feasible. Cassava leaf is therefore tipped as a veritable replacement to the conventional protein sources that are presently of very high cost and also in high competition with human consumption.

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