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# Evaluation of Techniques To Reduce Assayable Tannin and Cyanide in Cassava Leaves

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Condensed tannins and cyanide are two antinutritional factors in cassava leaves that reduce the nutritional quality of the leaf meal. The effects of certain physical and chemical treatments in reducing the levels of these in cassava leaves were studied. Drying at 60 °C could reduce the assayable tannin content a considerable extent. Wilting the whole branches under shade for 16 h followed by drying the detached leaves at 60 °C was even more advantageous in reducing the levels of these toxic principles. Chopping of the wilted leaves retained a higher percentage of cyanide on drying compared with the drying of wilted whole leaf blades. Highly significant reduction in assayable tannin levels could be brought about when the leaves were sprayed with either sodium hydroxide or ammonia. Since the residual alkalinity of the sodium hydroxide treated leaves was high, ammoniation seems to be the best practical method for reducing the assayable tannins from cassava leaves.

Cassava (*Manihot esculenta* Crantz) is a common tropical root crop that finds extensive use as human food. Being rich in carbohydrates, the tubers offer vast potential as animal feed. The low protein content of the tubers however necessitates proper supplementation of the diet with high-protein sources. Cassava leaf with its high protein content has wide scope as a feed source for poultry and livestock (Ross and Enriquez, 1969; Mahendranathan, 1971). The presence of two antinutritional factors in cassava leaves limits incorporation of leaf meal in animal rations. The toxicity of cassava has been attributed recently to the cyanogenic glucosides (Montgomery, 1969; Ermans et al., 1980). However, recent research work shows that condensed tannins present in cassava tubers (Rickard, 1986) and cassava leaves (Reed et al., 1982) are a potential hazard for the use of cassava in animal feed.

Tannins present in forages have been reported to adversely affect the quality of the feed (Kumar and Singh, 1984). Reduction in voluntary feed intake resulting from reduced palatability due to precipitation of salivary proteins is a major effect of tannins (Mc Leod, 1974; Harborne, 1976). Tannins reduce digestibility by inhibiting digestive enzymes and by altering the permeability of the gut wall (Feeney, 1969; Milic et al., 1972; Griffith, 1979). Tannins have been reported to cause low milk yield, reduction in sulfur availability, and toxic degenerative changes in many organs (Karim et al., 1978; Mohapatra et al., 1977; Singh and Arora, 1980). Condensed tannins in horsebean (*Vicia faba* L.) seeds have been found to affect the laying rate and egg weight of poultry (Tanguy et al., 1977). Tannins in rapeseed meal caused tainting of eggs (Fenwick et al., 1981; Butler et al., 1982). Depressed growth and high mortality have been reported in chicks fed tannic acid (Kaushal and Bhatia, 1982).

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Many workers have reported various chemical treatments for the removal of tannins from sorghum grains, sal (*Shorea robusta* Roxb.), seed meal, etc. (Gandhi et al., 1975; Price et al., 1979). Tannins undergo irreversible changes during heating. Oven drying at 60 °C has been reported to reduce the tannin content of leucaena leaf meal (D'Mello and Taplin, 1978). Processing techniques such as drying or cooking reduce the cyanide content of cassava leaves (Fukuba et al., 1984). The effect of oven drying of cassava chips at 60, 105, and 165 °C was studied by Bourdoux et al. (1980), and they found that maximum cyanide elimination occurred at 60 °C. Cooke and Maduagwu (1978) also reported loss of about one-third of bound cyanide by drying the chips at 47 and 60 °C. Cyanide has been reported to be retained to a greater extent by drying at 70 °C as compared to 50 °C (Nambisan and Sundaresan, 1985). However, the effect of processing techniques on cyanide elimination from cassava leaves has not been as extensively studied as for chips.

Although rich in proteins, the true protein digestibility of cassava leaf meal is low (Eggum, 1970). Condensed tannins in cassava leaves have been implicated in low protein digestibility (Reed et al., 1982). To enhance the biological availability of cassava leaf proteins, attempts were made to reduce the soluble tannin content of cassava leaves by certain physical and chemical treatments. The effect of these treatments on the extent of cyanide elimination was also studied. The results of these studies have been communicated in this paper.

## MATERIALS AND METHODS

Five varieties of cassava of uniform maturity grown under similar conditions in the Institute Farm were used for the study.

**Experiment I. Evaluation of Tannin and Cyanide Content of Varieties.** Ten tender (youngest fully expanded) leaves and ten fully mature (but not senescent) leaves were collected at random from six plants of each variety. The leaf blades were separated by hand, chopped to pieces of 1-cm length, and mixed well by hand, and representative samples of 500 mg were taken

 

**Table I. Tannin Content[a] of Cassava Leaves**

| variety | tannin content,[b] g tannic acid/100 g DM | |
|---|---|---|
| | tender leaves | mature leaves |
| M-4 | 3.98 ± 0.32 | 3.47 ± 0.48 |
| H-1687 | 3.68 ± 0.56 | 3.37 ± 0.44 |
| H-165 | 3.07 ± 0.84 | 3.78 ± 0.31 |
| H-226 | 2.96 ± 0.18 | 3.09 ± 0.38 |
| H-2304 | 2.91 ± 0.53 | 2.93 ± 0.50 |

[a] Mean value from four observations ± SE. [b] DM = dry matter.

from the wet pooled samples of each variety. Four replicates were maintained for the mature and tender leaves of each variety. The samples were homogenized by grinding in a mortar and extracted with 10 mL of absolute methanol at 4 °C. The homogenate was centrifuged at 3000*g* for 20 min and the supernatant collected. The residue was reextracted with two 5-mL portions of methanol. The supernatants were pooled, and an aliquot from this was used for the assay of tannins by the protein precipitation method of Hagerman and Butler (1978). The tannin content was calculated from a standard curve prepared with tannic acid (Sigma Chemical Co.).

The total and free cyanide contents of the leaves were determined by the method of Nambisan and Sundaresan (1984) using pyridine–barbiturate reagent with the modification that the exogenous linamarase used in the study was made by preparing the acetone dry powder from cassava rind. The homogenate of cassava rind was prepared in a Waring blender with chilled acetate buffer (0.1 M, pH 5.5, 1:2 w/v). The enzyme was reconstituted by dissolving the powder in acetate buffer (0.1 M, pH 5.5).

**Experiment II. Effect of Drying Temperatures on Tannin and Cyanide Reduction in Cassava Leaves.** Since the tender leaves had much higher cyanide content with little change in tannin content compared to the mature leaves, only the mature leaves were selected for further studies. Mature leaves were collected as in Experiment I, and the leaf blades were subjected to different drying temperatures (45, 60, 75 °C) in a thermostatically controlled oven for 24 h. The dried leaves from each variety were powdered to form a homogeneous mixture, and aliquots from this were used for the determination of tannins and cyanide (total and free) as described previously.

**Experiment III. Effect of Wilting and Chopping on Tannin and Cyanide Reduction in Cassava Leaves.** Mature leaves of the five varieties were wilted under shade for 16 h without detaching the leaves from the main stem. The wilted leaves were then detached, and petioles were separated by hand. The leaf blades were divided into two lots: One lot from each variety was dried as such at 60 °C for 24 h, and the second lot was chopped into pieces of approximately 1-cm length and dried at 60 °C. The tannin and cyanide contents of the dried leaf meal were determined as described in Experiment I.

**Experiment IV. Effect of Chemical Treatments on Tannin and Cyanide Reduction in Cassava Leaves.** Mature leaf blades were detached from the petioles and sprayed with various chemicals reported to have tannin lowering effects at the rate 40 mL/kg of fresh leaves. The sprayed leaves were stored in closed containers for 24 h after which they were dried at 60 °C for 24 h. The dried leaves were powdered, and aliquots were used for the estimation of tannins and cyanide as described earlier. The various chemicals tested were sodium hydroxide (1 and 6 M), ammonia (2.5 M and concentrated), formalin (0.3%), urea (1 M), poly(vinylpyrollidone) (PVP; 1%), and wood ash alkali. Wood ash alkali was obtained by soaking 130 g of hardwood ashes overnight in 350 mL of water and filtering it (Price et al., 1979).

Of the various chemicals tested, only sodium hydroxide (1 and 6 M) and ammonia (2.5 M and concentrated) had significant tannin lowering/inactivating effects with cassava leaves. However, in these leaves, a slightly higher retention of total cyanide was noted. In order to eliminate this problem, the effect of a two-step drying process was studied wherein the leaves were first dried at 60 °C for 24 h and the dry leaves were then sprayed with alkalies. After exposure to the alkalies for 24 h, the leaves were brought back to 60 °C for 24 h. The dried leaves were powdered, and aliquots were used for the assay of tannins and cyanide.

The residual alkalinity in the alkali-treated leaves was ascertained by soaking 1 g of the dried leaves in 100 mL of water overnight and measuring the pH in an Elico pH meter (Model LI- IOT).

## RESULTS AND DISCUSSION

**Experiment I.** No significant difference could be noted between the tannin contents of tender and mature leaves of cassava (Table I). The tannin content of tender leaves fell in the range 2.91–3.98 while that of mature leaves fell between 2.93 and 3.78 g of tannic acid/100 g of dry matter.

The total and free cyanide contents of the tender leaves were much higher than those of the mature leaves of each variety (Table II). The cyanide content was higher in the tender and mature leaves of varieties H-165, H-226, and H-2304 compared to M-4 and H-1687. De Bruijn (1973) also reported lower contents of cyanide in the older leaves compared with the young leaves.

**Experiment II.** Drying at 45 °C was found to slightly elevate the tannin content of cassava leaf meal (CLM). A progressive decrease in tannin content was noticed in the leaves dried at 60 and 75 °C for all varieties tested (Table III). Heat treatment causes irreversible changes in tannin structure. Oven drying at 60 °C has been reported to reduce the tannin content of leucaena leaf meal (D'Mello and Taplin, 1978). Heat treatment may inactivate the tannins of cassava leaf meal, which therefore lacks chemical sensitivity in the assay system.

Retention of cyanide was minimal in the leaves dried at 60 °C (Table IV). The effect of various drying temperatures on the loss of cyanide from cassava chips has been studied by several workers (Cooke and Maduagwu, 1978; Bourdoux et al., 1980; Nambisan and Sundaresan, 1985). At higher drying temperatures, the linamarase activity present in the leaves may be inactivated, accounting for a higher retention of cyanide in the leaves dried at 75 °C. Higher temperatures also lead to a rapid loss of water from the leaves, thereby reducing the linamarase action.

**Experiment III.** The effect of wilting on tannin and cyanide reduction in cassava leaves was studied. It was found that wilting had a pronounced effect on reducing the assayable tannin content of cassava leaves (Figure 1). Compared with the leaves directly dried at 60 °C, the wilted and dried leaves had significantly low assayable tannin levels. Chopping the wilted leaves prior to drying had only negligible tannin lowering action (Figure 1). It

**Table II. Cyanide Content[a] of Cassava Leaves**

| variety | cyanide content, μg/g DM | | | |
|---|---|---|---|---|
| | tender leaves | | mature leaves | |
| | total | free | total | free |
| M-4 | 1237.97 ± 22.8 | 412.66 ± 13.7 | 436.52 ± 20.1 | 254.64 ± 6.5 |
| H-1687 | 1277.65 ± 17.5 | 361.08 ± 7.4 | 542.26 ± 32.5 | 299.18 ± 9.8 |
| H-165 | 1931.34 ± 37.7 | 1117.00 ± 28.6 | 729.24 ± 19.6 | 373.97 ± 12.5 |
| H-226 | 2237.61 ± 16.5 | 761.83 ± 11.8 | 736.22 ± 12.5 | 370.52 ± 7.3 |
| H-2304 | 1620.10 ± 32.4 | 1080.06 ± 17.9 | 709.14 ± 17.6 | 443.21 ± 8.8 |

[a] Mean value from four observations ± SE.

**Table III. Effect of Drying Temperatures on the Tannin Content[a] of Cassava Leaves**

| | tannin content, g tannic acid/100 g DM | | | |
|---|---|---|---|---|
| variety | fresh leaves | 45 °C | 60 °C | 75 °C |
| M-4 | 3.47 ± 0.48 | 3.80 ± 0.31 | 1.89 ± 0.12 | 1.40 ± 0.11 |
| H-1687 | 3.37 ± 0.44 | 3.54 ± 0.28 | 2.23 ± 0.20 | 1.69 ± 0.18 |
| H-165 | 3.78 ± 0.31 | 3.91 ± 0.16 | 2.97 ± 0.17 | 1.26 ± 0.23 |
| H-226 | 3.09 ± 0.38 | 3.37 ± 0.42 | 2.47 ± 0.09 | 1.32 ± 0.34 |
| H-2304 | 2.93 ± 0.50 | 3.00 ± 0.37 | 2.75 ± 0.13 | 1.34 ± 0.36 |

[a] Mean value from four observation ± SE.

may be assumed that under the conditions provided for wilting (16 h under shade at ambient temperature, 30 ± 1 °C, and mean relative humidity, 65–75%) the hydrolytic oxidative reactions of tannins may be accelerated in the leaves leading to low assayable tannin levels in the wilted dried leaf meal.

The cyanide content (total and free) of the wilted and dried leaves was significantly low as compared with the leaves directly dried at 60 °C (Table V). Wilting provided adequate time for the linamarase to act on the cyanoglucosides of cassava leaves, thereby removing an appreciable amount of cyanide from it. Chopping the wilted leaves tended to elevate the cyanide content (Table V). Dehydration was faster from chopped leaves as compared with the whole leaf blades, and this might have slowed the linamarase activity. Thicker cassava chips have been reported to retain less cyanide than thinner chips (Nambisan and Sundaresan, 1985). To achieve a simultaneous reduction of assayable tannins and cyanide, wilting followed by drying at 60 °C seems to be the appropriate method.

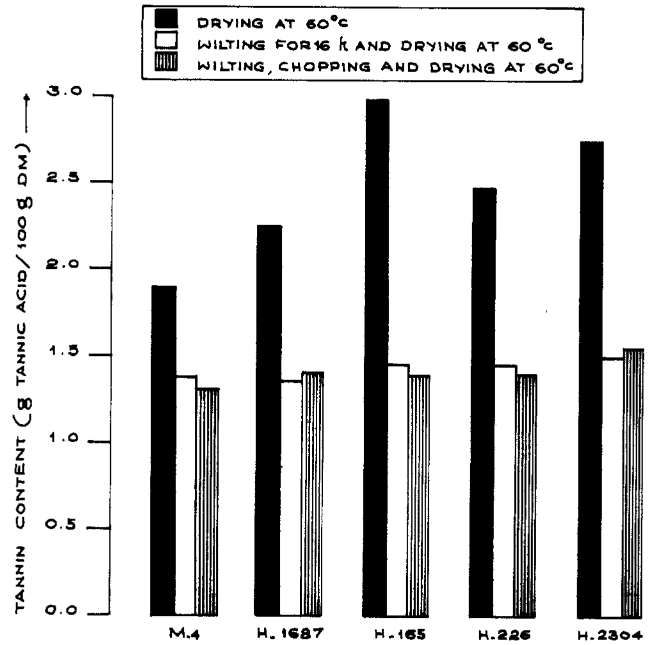


**Figure 1.** Effect of wilting and chopping on assayable tannin reduction in cassava leaves.

**Experiment IV.** Since significant reduction in the assayable tannin levels could be achieved only with alkalis, the results of these alone are reported. Sodium hydroxide (1 and 6 M) was highly effective in reducing the assayable tannin levels in cassava leaves (Table VI). Earlier studies

**Table IV. Effect of Drying Temperatures on the Cyanide Content[a] of Cassava Leaves**

| | cyanide content, μg/g DM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | fresh leaves | | 45 °C | | 60 °C | | 75 °C | |
| variety | total | free | total | free | total | free | total | free |
| M-4 | 436.52 ± 20.1 | 254.64 ± 6.5 | 86.65 ± 6.5 | 66.60 ± 3.4 | 77.34 ± 3.4 | 50.60 ± 2.8 | 138.00 ± 7.4 | 90.65 ± 3.5 |
| H-1687 | 542.26 ± 32.5 | 299.18 ± 9.8 | 113.32 ± 9.8 | 66.40 ± 7.60 | 76.00 ± 6.5 | 46.54 ± 2.4 | 141.33 ± 8.1 | 102.00 ± 6.5 |
| H-165 | 729.24 ± 19.6 | 373.97 ± 12.5 | 186.65 ± 11.1 | 86.56 ± 5.4 | 153.32 ± 9.7 | 60.00 ± 3.9 | 233.31 ± 13.2 | 113.30 ± 4.3 |
| H-226 | 736.22 ± 12.5 | 370.52 ± 7.3 | 166.56 ± 13.2 | 100.60 ± 6.1 | 113.32 ± 10.0 | 59.94 ± 4.6 | 233.35 ± 10.5 | 140.00 ± 5.1 |
| H-2304 | 709.14 ± 17.6 | 443.21 ± 8.8 | 183.98 ± 9.8 | 99.98 ± 2.8 | 150.65 ± 11.3 | 80.00 ± 7.8 | 253.30 ± 15.10 | 146.65 ± 2.9 |

[a] Mean value from four observations ± SE.

**Table V. Effect of Wilting and Chopping on the Cyanide Content[a] of Cassava Leaves**

| | cyanide content, μg/g DM | | | | | |
|---|---|---|---|---|---|---|
| | fresh leaves | | wilting, drying | | wilting, chopping, drying | |
| variety | total | free | total | free | total | free |
| M-4 | 436.52 ± 20.1 | 254.64 ± 6.5 | 55.3 ± 2.5 | 33.34 ± 2.1 | 98.95 ± 6.5 | 39.90 ± 4.3 |
| H-1687 | 542.26 ± 32.5 | 299.18 ± 9.8 | 60.10 ± 3.4 | 39.80 ± 1.8 | 106.65 ± 9.3 | 46.67 ± 2.0 |
| H-165 | 729.24 ± 19.6 | 373.97 ± 12.5 | 83.32 ± 7.3 | 53.16 ± 3.4 | 98.60 ± 3.4 | 60.48 ± 1.9 |
| H-226 | 736.22 ± 12.5 | 370.52 ± 7.3 | 80.25 ± 2.8 | 46.65 ± 4.6 | 93.32 ± 6.8 | 66.50 ± 3.4 |
| H-2304 | 709.14 ± 17.6 | 443.21 ± 8.8 | 58.30 ± 3.5 | 26.65 ± 1.2 | 133.30 ± 12.1 | 70.00 ± 2.8 |

[a] Mean value from four observations ± SE.

**Table VI. Effect of Chemical Treatments on the Tannin Content[a] of Cassava Leaves**

| | tannin content, g tannic acid/100 g DM | | | | |
|---|---|---|---|---|---|
| treatment | M-4 | H-1687 | H-165 | H-226 | H-2304 |
| a. no pretreatment | 3.47 ± 0.48 | 3.47 ± 0.44 | 3.78 ± 0.31 | 3.09 ± 0.38 | 2.93 ± 0.50 |
| b. spraying on fresh leaf blades followed by drying at 60 °C | | | | | |
| T1 1 M sodium hydroxide | 0.81 ± 0.13 | 0.72 ± 0.21 | 1.14 ± 0.23 | 1.02 ± 0.08 | 1.13 ± 0.20 |
| T2 6 M sodium hydroxide | 0.114 ± 0.06 | 0.183 ± 0.003 | 0.10 ± 0.05 | 0.13 ± 0.09 | 0.074 ± 0.002 |
| T3 2.5 M ammonia | 0.70 ± 0.18 | 0.92 ± 0.24 | 1.20 ± 0.05 | 1.06 ± 0.13 | 1.20 ± 0.21 |
| T4 concentrated ammonia | 0.17 ± 0.03 | 0.46 ± 0.07 | 0.80 ± 0.11 | 0.66 ± 0.008 | 0.57 ± 0.02 |
| c. spraying on dry leaves followed by a second drying at 60 °C | | | | | |
| T1 | 0.92 ± 0.32 | 0.86 ± 0.21 | 1.20 ± 0.10 | 1.11 ± 0.20 | 1.23 ± 0.18 |
| T2 | 0.29 ± 0.08 | 0.29 ± 0.10 | 0.57 ± 0.06 | 0.46 ± 0.03 | 0.40 ± 0.05 |
| T3 | 0.86 ± 0.38 | 0.84 ± 0.16 | 1.43 ± 0.09 | 1.40 ± 0.05 | 1.25 ± 0.04 |
| T4 | 0.36 ± 0.10 | 0.51 ± 0.08 | 1.26 ± 0.02 | 1.06 ± 0.02 | 0.86 ± 0.01 |

[a] Mean value from four observations ± SE.

**Table VII. Effect of Chemical Treatments on the Total Cyanide Content[a] of Cassava Leaves**

| treatment | cyanide content, μg/g DM | | | | |
|---|---|---|---|---|---|
| | M-4 | H-1687 | H-165 | H-226 | H-2304 |
| a. no pretreatment | 436.52 ± 20.1 | 542.26 ± 32.5 | 729.24 ± 19.6 | 736.22 ± 12.5 | 709.14 ± 17.6 |
| b. spraying on fresh leaf blades followed by drying at 60 °C | | | | | |
| T1 | 144.0 ± 6.8 | 137.33 ± 12.3 | 123.30 ± 7.1 | 150.60 ± 6.5 | 113.32 ± 9.7 |
| T2 | 192.32 ± 12.4 | 140.67 ± 10.8 | 173.32 ± 6.5 | 186.65 ± 9.6 | 126.65 ± 2.4 |
| T3 | 93.32 ± 6.5 | 73.30 ± 9.10 | 126.65 ± 4.5 | 206.65 ± 7.5 | 146.65 ± 12.3 |
| T4 | 139.98 ± 6.9 | 80.00 ± 2.3 | 140.00 ± 9.6 | 219.98 ± 6.3 | 206.65 ± 11.00 |
| c. spraying on dry leaf blades followed by second drying at 60 °C | | | | | |
| T1 | 84.56 ± 5.4 | 93.30 ± 7.2 | 93.70 ± 2.1 | 106.60 ± 6.7 | 110.50 ± 9.4 |
| T2 | 92.10 ± 6.1 | 120.00 ± 10.6 | 108.50 ± 3.8 | 119.90 ± 5.4 | 116.40 ± 3.2 |
| T3 | 86.90 ± 9.2 | 66.60 ± 4.3 | 96.50 ± 7.4 | 80.00 ± 4.3 | 108.53 ± 6.5 |
| T4 | 72.15 ± 3.8 | 53.32 ± 7.6 | 85.40 ± 6.3 | 52.14 ± 6.3 | 66.70 ± 5.8 |

[a] Mean value from four observation ± SE.

conducted in high-tannin sorghum grains and sal seed meal revealed the tannin-lowering action of sodium hydroxide (Wah et al., 1977; Price et al., 1979; Reichert et al., 1980). Approximately 99% reduction in tannin was noticed in sorghum grains treated with 6 M sodium hydroxide. Decreases of 95–98% were observed in the leaves from various cassava varieties after treatment with 6 M sodium hydroxide (Table VI). A dry matter loss (20–70%) was reported after alkali treatment (Wah et al., 1977; Singh and Arora, 1978), which likely leaches out much of the soluble nutrients. This difficulty was overcome in the present study, which led to no DM loss. Concentrated ammonia reduced the assayable tannin levels by 79–95% (Table VI).

Ammoniation had the specific advantage that excess ammonia could be completely removed during the drying process. The residual pH values of the sodium hydroxide treated leaves were in the ranges 9.3–9.9 and 10.1–10.4 for the varieties treated with 1 and 6 M sodium hydroxide, respectively. The residual pH of the ammonia-treated leaves was however in the range 6.3–6.8 and was near the pH of the fresh leaves (5.9–6.1). Hence, the practical value of the sodium hydroxide treatment is limited although neutralization is a possibility. Therefore, ammoniation seems to be the best method for tannin reduction/inactivation in cassava leaves. Ammoniation has also been reported to elevate the nonprotein nitrogen content of sorghum grains (Price et al., 1979), which is of utmost significance to ruminants. Increased growth performance of rats and chicks was observed by feeding the ammoniated sorghum grains (Price et al., 1979). Ammonia was found to depolymerize the tannins present in sal seed meal, and the processed meal was nontoxic and palatable (Gandhi et al., 1975). It is also possible that tannins may become unreactive both chemically and nutritionally during alkali treatment due to the formation of phlobaphenes (Swain, 1979).

Chemical treatment had been found to retain a greater amount of cyanide as compared to direct drying of leaf blades at 60 °C (Table VII). The optimum pH of cassava linamarase is ca. 6.0 (Cooke and Coursey, 1981), and hence under the alkaline pH range prevailing in the treated leaves, the linamarase activity may be reduced. This difficulty was however partially overcome by the two-step drying process (Table VII).

The low level of assayable tannin in chemically treated leaf meal may elevate the true protein digestibility, thereby ensuring better utilization of dietary proteins. Bird-resistant sorghum containing high levels of tannins was reported to increase the methionine requirement of chicks (Armanious et al., 1973; Armstrong et al., 1974). This has been due principally to the binding of condensed tannins with methionine, thereby making it unavailable (Ford and Hewitt, 1974). Cassava leaf meal is deficient in methionine, which is essential for the detoxification of cyanide. It is likely that the condensed tannins in cassava leaf meal may complex with methionine in dietary proteins, ultimately leading to methionine deficiency in animals. The ammoniated leaf meal may offer better scope for poultry and livestock development without adverse effects.


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**Registry No.** Cyanide, 57-12-5; sodium hydroxide, 1310-73-2; ammonia, 7664-41-7.

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