# Ergot Alkaloid and Chlorogenic Acid Content in Different Varieties of Morning-glory (*Ipomoea spp.*) Seeds

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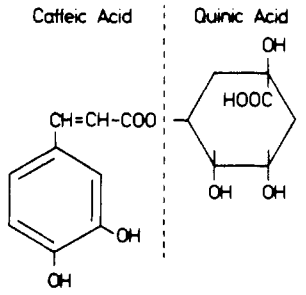
Morning-glory plants (*Ipomoea spp.*) occur widely as weeds among cultivated field crops such as soybeans. Thin-layer chromatographic, ultraviolet spectrophotometric, and colorimetric studies were carried out to determine the ergot alkaloid and chlorogenic acid contents of several varieties of morning-glory seeds. Of a large number of samples collected in the field or obtained from domestic sources, only the Heavenly Blue variety contained ergot alkaloids, measured by a spectrophotometric procedure. The values ranged from 0.01 to about 0.035% of the fresh weight of the seeds. Chlorogenic acid was found in all morning-glory varieties tested. The content ranged from 0.55 to 1.3%, determined by direct ultraviolet spectroscopy of the ethanolic extracts and by a colorimetric procedure. The ultraviolet method appears simpler and more reliable for measuring chlorogenic acid in morning-glory and possibly other toxic weed seeds.

Morning-glory plants (*Ipomoea spp.*) occur widely as nuisance weeds in fields of soybean and other field crops (Baldwin and Evans, 1972; Buchanan et al., 1975; Crowley et al., 1979). Several horticultural varieties of morning-glory seeds are reported to contain psychoactive compounds such as ergot alkaloids (Capper, 1966; Der Marderosian and Youngken, 1966; Taber et al., 1963; Wilkinson et al., 1986, 1987). As these seeds are not readily separated from soybeans and other grains during normal harvesting and cleaning, they may present a problem to the product user.

The psychoactive compounds identified are mainly lysergic acid derivatives and clavine alkaloids, sometimes called ergoline compounds (Der Marderosian, 1965). The literature reports a wide range of values for the ergot alkaloid content of various morning-glory cultivars. The data are difficult to interpret because a variety of extraction and preparation methods are used to estimate the alkaloid content.

One objective of this study was to determine the extent to which the ergot alkaloid content might vary in wild morning-glory seeds found as actual contaminants of grain from different locations in the United States. For comparison, analysis of seeds from some domestic varieties of morning-glory were included.

Chlorogenic acid (3-caffeoylquinic acid), a naturally occurring polyphenolic compound, is reported to act as a




clastogenic (chromosome-damaging) agent in hamster cells (Stich et al., 1981; Stich and Rosin, 1984; MacGregor, 1984) and to participate in enzymatic and nonenzymatic browning reactions in potatoes (Hoover, 1963; Malmberg and Theander, 1985), sunflower seeds (Cater et al., 1972), leaf protein concentrates (Free and Satterlee, 1975), milk proteins (Hurrell and Finot, 1984), and other foods (Deshpande et al., 1984). Therefore, it was of interest to establish its content in morning-glory seeds. This study measured the chlorogenic acid content of a number of seed varieties obtained from different locations. Chlorogenic acid was measured by two independent techniques: ultraviolet spectroscopy and colorimetry.

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## EXPERIMENTAL SECTION

**Materials.** Morning-glory seeds, collected from dockages of soybean grain screenings at various locations in the United States, were provided by the U.S. Federal Grain Inspection Service. Domestic varieties were purchased in a local seed store.

**Analysis for Ergot Alkaloids.** *a. Spectrophotometry.* Alkaloids were extracted and estimated by an adaptation of the procedure of Wilkinson et al. (1986). Two grams of seed was ground in a mortar and immediately extracted for 30 min, with stirring, with 50 mL of acetone–2% aqueous tartaric acid (70:30, v/v). The mixture was centrifuged for 3 min and the residue reextracted twice more for 10 min with 50 mL of the extraction solution. Acetone was removed from the combined supernatants in a rotary evaporator. The aqueous residue was washed three times with 50-mL portions of methylene chloride–isopropyl alcohol (3:1, v/v) and adjusted with saturated sodium bicarbonate solution to pH 7.7–8.2. Total ergot alkaloids were then extracted with three 50-mL portions of the methylene chloride–isopropyl alcohol solution. All extractions were done under subdued light at 23–24 °C. The residue from 5 mL of extract evaporated to dryness in a stream of nitrogen was mixed with 1 mL of 0.1 N sulfuric acid and 2 mL of Van Urk reagent (0.2 g of *p*-(dimethylamino)benzaldehyde and 3 mg of ferric chloride dissolved in 100 mL of 65% sulfuric acid) and left to stand 30 min. Absorbance was then measured at 580 nm in a Cary 14 spectrophotometer against a suitable blank. Total alkaloid content was calculated from a standard curve prepared from ergonovine, and results were expressed as ergonovine equivalents.

*b. Thin-Layer Chromatography.* Preparative thin-layer chromatography was performed on 0.25-mm-thick 20 × 20 cm silica gel 60 precoated plates (Merck, Darmstadt, FRG). The extracted solutions were evaporated on a stream of nitrogen. The residue was dissolved in 1 mL of methyl alcohol, and then 15 μL of this solution was applied as a single band on the thin-layer plate. Comparable amounts of the available standards were placed alongside the unknown extract for direct comparison. The thin-layer plate was developed with chloroform–methanol (8:2, v/v) and then with methylene chloride–isopropyl alcohol (9:1, v/v). After chromatography, the plates were sprayed with the van Urk reagent followed by 1% sodium nitrite solution (Figure 1).

**Analysis for Chlorogenic Acid.** Ground seeds were defatted with petroleum ether in a Soxhlet apparatus for 18 h. Next, 500 mg of defatted material was extracted with 100 mL of 100% ethyl

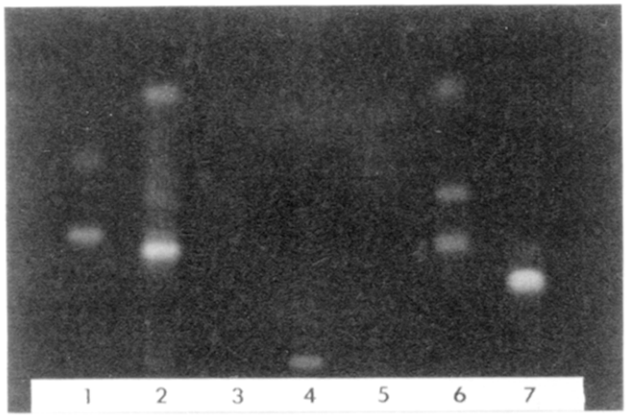


**Figure 1.** One-dimensional thin-layer chromatogram of morning-glory seed extracts and standards photographed under ultraviolet light: 1, ergonovine; 2, Heavenly Blue (Holland) seeds; 3, field seeds (FSY-1011), 4, lysergic acid; 5, Scarlet O'Hara seeds; 6, Heavenly Blue (Germany) seeds; 7, lysergol.

alcohol, also for 18 h. The volume of the ethyl alcohol extract was adjusted to 100 mL.

*a. Thin-Layer Chromatography.* The plates were spotted and developed with ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:27, v/v) until the solvent front reached 13 cm. The air-dried plate was then sprayed with natural product reagent (1% methanolic diphenylboric acid ethylamino ester) followed by 5% ethanolic poly(ethylene glycol) 4000. The visualized bands were collected, dissolved in hot methanol, filtered, and hydrolyzed with 1 mL of 6 N HCl for 1 h under reflux. The hydrolysates were cooled and neutralized with NaOH. The cooled solutions were evaporated to dryness under a stream of nitrogen and the residues taken up in 0.5 mL of methanol. About 25 $\mu$L of each hydrolysate was then spotted on a TLC plate alongside authentic samples of caffeic and quinic acids. The chromatograms were developed as described above. Next, the TLC plates were sprayed with diazotized *p*-nitroaniline reagent (0.5% *p*-nitroaniline in 2 N HCl, 5% $NaNO_2$, and 20% sodium acetate solutions in a ratio of 1:10:30, v/v).

*b. Ultraviolet Spectrophotometry.* The UV spectrum, 220–400 nm, was determined on the ethyl alcohol extract after suitable dilution and the concentration of chlorogenic acid calculated from the absorption maximum at 326–328 nm from a standard curve prepared from authentic chlorogenic acid. The molar extinction coefficient ($\epsilon$) of chlorogenic acid was determined as 19 300 ± 200 ($n$ = 5) compared to a reported value of 19 200 (*Merck Index*, 1976).

*c. Colorimetry.* The quantitative colorimetric assay for chlorogenic acid was based on the Hoepfner reaction in which the phenolic compound reacts with sodium nitrite and acetic acid to produce a yellow color that changes to intense carmine red on addition of alkali (Hoepfner, 1932; Snell and Snell, 1953). The residue from 2 mL of ethanolic extract, after evaporation to dryness in a stream of nitrogen, was dissolved in 2 mL of hot (~60 °C) water and diluted to 10 mL with cold water. Buffer (0.3 mL) consisting of 10% glacial acetic acid and 30% anhydrous sodium acetate in water was added. After the mixture was cooled to 8 °C, 0.3 mL of 40% sodium nitrite was added, followed after 3 min by 0.3 mL of 10% NaOH. The absorbance was read after 15 min at 520 nm against a reagent blank. Total chlorogenic acid was calculated from a standard curve prepared with authentic chlorogenic acid.

## RESULTS AND DISCUSSION

**Estimation of Yield of Ergot Alkaloids.** Figure 1 shows that only Heavenly Blue variety of morning-glory seeds contained a number of van Urk positive (violet-blue) zones. No significant violet-blue spots were noted with the varieties obtained in the field. These results suggest that the nondomestic varieties may contain only traces of ergot alkaloids.

Figure 2 shows a linear calibration curve for the ergonovine standard determined with the van Urk reagent.

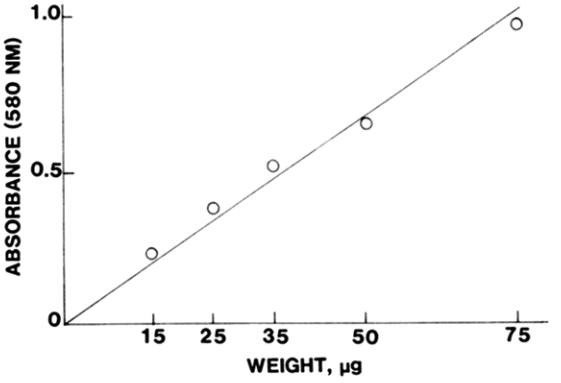


**Figure 2.** Relationship between the amount of standard ergonovine and the absorbance at 580 nm (standard curve by colorimetric method).

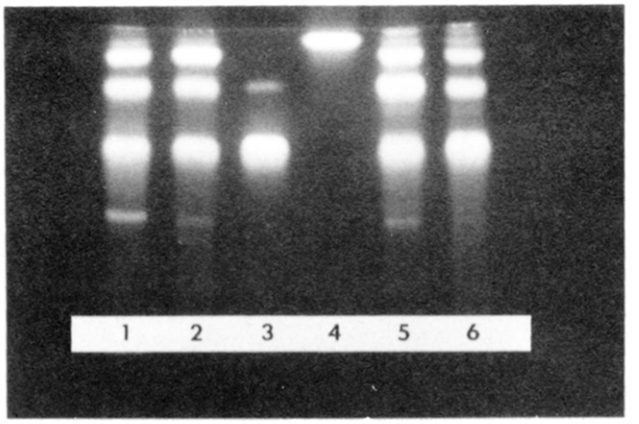


**Figure 3.** One-dimensional thin-layer chromatogram of morning-glory seed extracts and standards photographed under ultraviolet light: 1, Heavenly Blue (Holland) seeds; 2, field seeds (FSY-1011); 3, chlorogenic acid; 4, caffeic acid; 5, field seeds (FSY-1108); 6, field seeds (FSY-1035).

**Table I. Total Ergot Alkaloid Content of Morning-glory Seeds**

| horticultural var. | country of origin | total alkaloid, mg/100 g seed |
|---|---|---|
| domestic | | |
| Heavenly Blue | Holland | 35 |
| Heavenly Blue | Denmark | 22 |
| Heavenly Blue | Germany | 10 |
| Giant, Mixed colors | Denmark | 0 |
| Scarlet O'Hara | U.S. | 0 |
| Early Call | Japan | 0 |
| field | | |
| FSY 959-1126 (21 samples) | U.S. | 0 |

Table I shows the total ergoline alkaloid content in morning-glory seeds determined by colorimetric spectroscopy. Only the three Heavenly Blue cultivars contained detectable amounts of alkaloids, ranging from 10 to 35 mg/100 g of seed fresh weight (Table I). Other authors, using a variety of extraction procedures, have reported ergot alkaloid contents for this cultivar to be 23 (Genest, 1965), 24 (Taber et al., 1963), 28–38 (Witters, 1975), and 52 mg/100 g (Wilkinson et al., 1986). Although we cannot assess the degree to which this apparent compositional variation reflects differences in extraction procedures, significant variations in the ergoline alkaloid content of this cultivar must be assumed. This conclusion is also supported by our results. Obvious factors governing alkaloid biosynthesis are the environment (e.g. soil fertility, climate, or growing season) and genetic variation.

The striking result of the analysis of seeds from three other domesticated morning-glory varieties and 21 samples

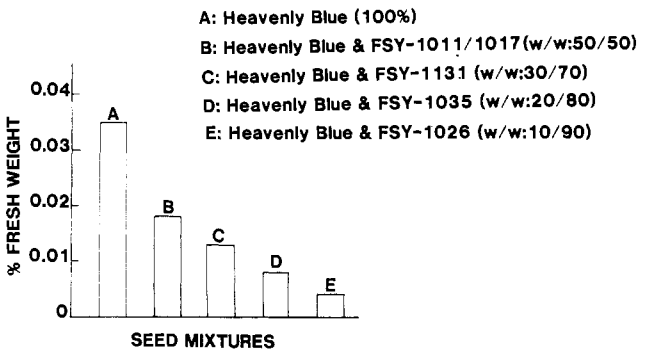


**Figure 4.** Ergot alkaloid content of Heavenly Blue and mixtures of Heavenly Blue with other varieties of morning-glory seeds.

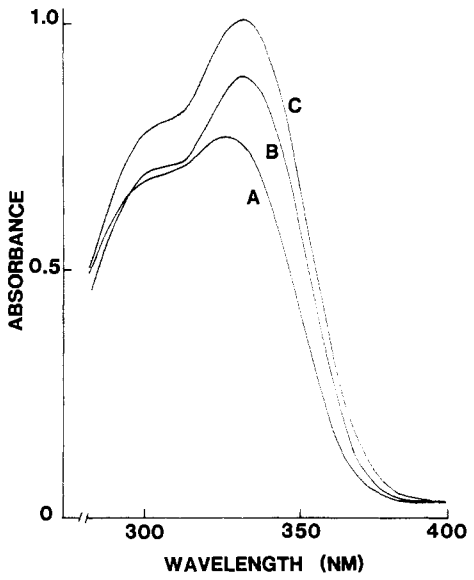


**Figure 5.** Ultraviolet absorption spectral of caffeic acid (A), chlorogenic acid (B), and an ethanol extract of morning-glory seeds (C).

of nondomestic seed obtained from grain screenings was the complete lack of alkaloids. Either alkaloids were not synthesized in these seeds during growth of the plant or the alkaloids were somehow degraded after harvest.

To establish that the lack of ergot alkaloids in these seeds was not due to the analytical procedure, we mixed ground Heavenly Blue (Holland) seed with ground seed showing no ergot alkaloid content. Figure 4 shows the results of these mixing (spiking) experiments. The results clearly show that the concentration of ergot alkaloids increased in direct proportion to the content of the Heavenly Blue sample. Thus, the method of extraction and analysis reported here offers a reliable assay for measuring the ergot alkaloid content of morning-glory seeds.

**Chlorogenic Acid Content.** The chromatograms of all morning-glory seed extracts showed a major band whose $R_f$ value (0.57) was identical with that obtained with authentic chlorogenic acid. Two additional strongly fluorescing bands with $R_f$ values of 0.78 and 0.88 were also present (Figure 3). They were tentatively classified as chlorogenic acid isomers, possibly 4-*o*-caffeoylquinic, 5-*o*-caffeoylquinic, or dicaffeoylquinic acids (Wagner et al., 1984). The three compounds were extracted, hydrolyzed in acid solution, and rechromatographed. The presence of caffeic acid as a brown spot ($R_f$ 0.91) and quinic acid as a light tan spot ($R_f$ 0.69) confirmed that the original bands were chlorogenic acid and two of its isomers.

Figure 2 also shows that the ratio of the three isomers seem to vary among the different seeds. For example, from the observed sizes of the three chromatographic spots, it is estimated that the ratio of concentrations of the three isomers in sample 5 is approximately 2:2:1. The corresponding ratio in sample 6 is 3:1:1.

**Table II. Recovery of Chlorogenic Acid Added to a Sample of Morning-glory Seeds (FSY 1011) before Extraction ($n$ = 2 for Each Concentration of Added Chlorogenic Acid)**

| chlorogenic acid added | | | |
|---|---|---|---|
| % of max | mg | recovery, % | mean, % ± SD |
| 25 | 1.7 | 95.3 | 95.5 ± 0.3 |
| | 1.65 | 95.8 | |
| 50 | 3.2 | 97.5 | 96.6 ± 2.0 |
| | 3.31 | 95.2 | |
| 75 | 5.05 | 96.0 | 95.1 ± 1.3 |
| | 4.85 | 94.2 | |
| 100 | 6.60 | 101.3 | 95.8 ± 7.9 |
| | 6.60 | 90.2 | |

**Table III. Comparison of Ultraviolet Absorption and Colorimetric Methods in the Determination of Chlorogenic Acid Content of Morning-glory Seeds (Mean ± Standard Deviation, $n$ = 3 for Each Determination)**

| | chlorogenic acid, % fresh weight | |
|---|---|---|
| sample | UV absorption method ($OD_{328\ nm}$) | colorimetric method ($OD_{520\ nm}$) |
| domestic | | |
| Heavenly Blue (Holland) | 0.78 ± 0.06 | 0.69 ± 0.03 |
| Heavenly Blue (Denmark) | 0.55 ± 0.14 | 0.44 ± 0.15 |
| Heavenly Blue (Germany) | 0.66 ± 0.04 | 0.54 ± 0.16 |
| field | | |
| FSY-1011 | 1.31 ± 0.03 | 0.90 ± 0.10 |
| FSY-1035 | 0.78 ± 0.02 | 0.54 ± 0.06 |
| FSY-1039 | 1.12 ± 0.04 | 0.90 ± 0.10 |
| FSY-1104 | 0.94 ± 0.09 | 0.77 ± 0.06 |
| FSY-1108 | 1.08 ± 0.06 | 0.82 ± 0.13 |
| FSY-1109 | 0.96 ± 0.03 | 0.59 ± 0.15 |
| FSY-1121 | 1.11 ± 0.03 | 0.84 ± 0.08 |
| FSY-1125 | 1.27 ± 0.03 | 0.90 ± 0.10 |
| FSY-1126 | 0.91 ± 0.05 | 0.77 ± 0.12 |
| overall mean | 0.96 ± 0.05 | 0.73 ± 0.10 |

Figure 5 shows the UV absorption spectra of caffeic and chlorogenic acids and an ethanolic extract of morning-glory seeds. The ethanolic extract had an absorption peak at 326–328 nm, identical in wavelength to the chromophoric peak of chlorogenic acid. The maximum absorbance of caffeic acid (a hydrolysis product of chlorogenic acid; see structure above) appears at a lower wavelength. To calculate the total chlorogenic acid content, all three isomers were assumed to have the same molar extinction coefficient as chlorogenic acid.

In order to establish the applicability of the ultraviolet procedure for measuring chlorogenic acid in morning-glory and possibly other toxic weed seeds, ground seeds were spiked with several levels of authentic chlorogenic acid before extraction. Table II shows that the recovery of the four spiked samples was about 95% or better. Recovery of chlorogenic acid was independent of the amount added in the range shown. These observations suggest that the ultraviolet method can be used to estimate the chlorogenic acid content of morning-glory seeds.

The data derived from the ultraviolet analysis for chlorogenic acid were compared to the corresponding data from the more complex colorimetric procedure. Table III shows that the values obtained by colorimetry were generally lower than those obtained by the UV method. The lower values might be due to instability of the red color produced in the Hoepfner reaction and/or to the interference of other compounds in the formation of the chromophore or its absorbance.

The higher values with the ultraviolet method could also result from the presence of additional chromophores that absorb in the same region as chlorogenic acid. Caffeic acid is one possibility. However, the thin-layer chromatographic studies (Figure 2) show that caffeic acid is not present in the extracts.

Table III also shows that all of the morning-glory seeds tested contained relatively large amounts (0.55–1.3%) of chlorogenic acid based on fresh weight of the seeds. These values compare to about 1.5% in sunflower seeds (Pomenta and Burns, 1971), 0.5% for dry tea shoots (Ullah and Jain, 1980), 0.01–0.03% in apple juice (Van Buren et al., 1973), and intermediate values for a number of other foods such as fruits (Sioud and Luh, 1966; Corse et al., 1965; Swain, 1962; Hulme, 1953), potatoes (Vertregt, 1968), coffee (Clifford and Wight, 1976; Trugo and Macrae, 1984), and other foods such as coffee, potatoes, etc. (Sondheimer, 1964; Walter and Purcell, 1979).

In conclusion, large variations, ranging from 0 to 0.035%, were observed in ergot alkaloid content of morning-glory seeds. The cause of this variation is not known. All of the seeds tested also contain relatively high amounts of chlorogenic acid. The ultraviolet method used appears more accurate and reliable for determining this acid in morning-glory seeds than a colorimetric procedure. Further studies are needed to establish possible reasons for the higher values of chlorogenic acid obtained with the ultraviolet method than by colorimetry. The nutritional and toxicological significance of these findings and the possible relationship of seed composition to food safety are the subject of current studies. Finally, it would be of interest to establish whether conditions that inactivate the mutagenicity of polyphenolic compounds such as quercetin (Friedman and Smith, 1984) would also reduce or eliminate the clastogenic activity of chlorogenic acid. In a related study (Friedman and Dao, 1989), we examine the effect of baking on the ergot alkaloid and chlorogenic acid content of morning-glory seed flour.

ACKNOWLEDGMENT

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**Registry No.** Chlorogenic acid, 327-97-9.

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

G. Padmaja

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

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