Rapid Detection And Quantification Of Cyanide In Cassava (Manihot Esculenta Crantz) Via UV/Vis Extinction Spectroscopy

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Abstract: Cassava (*Manihot esculenta crantz*) is a cyanogenic edible plant tuber which is usually considered as a staple food in many developing countries. Cassava is often characterized by the presence of a cyanogenic glycoside linamarin, whose enzymatic hydrolysis produces hydrogen cyanide (HCN) as a byproduct. HCN is poisonous to humans when ingested in more than acceptable amounts; hence, rapid detection of HCN is needed to prevent the effects of this toxic compound in people who rely on cassava meals. In this study, cassava samples obtained from two different regions of Zambia were investigated to probe the levels of cyanide in processed and raw (i.e., soaked and unsoaked) cassava samples. The results show that the concentration of cyanide in cassava samples varied with geographical location, as well as the age of cassava plant and processing methods. The highest concentration of 12.033 mg/mL of cyanide was recorded in unsoaked cassava samples collected from Luampa District, whilst the lowest cyanide level of 3.1136 mg/mL was detected in soaked cassava harvested from Mansa, suggesting that soaking of cassava reduced the amount of cyanide concentration as observed in this study. In virtually all cases, the concentration of cyanide detected in original cassava tubers from Luampa were higher than the WHO recommended level (10 mg of HCN/kg), irrespective of the implemented processing strategy (soaked *vs* unsoaked).

Keywords: Extinction spectroscopy, Hydrogen cyanide, Quantitative analysis, Cassava, WHO.

Introduction

Cassava is a starch tuberous root of a tropical tree that is used as staple food in tropical countries. There are many cultivars of cassava which are identified according to the structural features of the plant. Features such as tuber shape, early maturity, yield and the cyanogenic glycoside content are some characteristics used to distinguish different varieties of cassava [11]. Cassava can also be classified by virtue of the taste exhibited by tubers that is mainly: sweet cassava and bitter cassava depending on levels of cyanide in cassava. Sweet cassava roots can be made less toxic by peeling the cassava skin and cooking the inner white part. On the other hand, one way of reducing cyanide content in bitter cassava involves grating and prolonged soaking (for about 24 h), followed by drying. Irrespective of processing steps, high cyanide concentrations (>1000 mg/kg) have been frequently reported in post-processed cassava in various regions of Africa [7]. In Africa, improperly processed cassava is a major problem which is usually associated with several cyanide-related health disorders, particularly in the malnourished people [1]. Chronic cyanide toxicity is associated with goiter growth, and tropical ataxic neuropathy, a nerve-damaging disorder that renders an affected person unsteady and uncoordinated. Severe cyanide poisoning, especially during famines is often linked to the irreversible paralytic disorder called Konzo (spastic paraparesis) which may lead to cognitive impairment and even death [9].

In Zambia, cassava is mainly consumed when prepared as nshima (hardened porridge of cassava flour) or dried and roasted and to some extent raw fresh roots are eaten as snacks [3]. The cassava root contains carbohydrates (64% to 72 %) of which is made up of starch, mainly in the form of amylose and amylopectin [6]. The lipid and protein content of cassava are about 0.5% and 2%, respectively. Cassava is also a source of vitamin C, thiamine, riboflavin and niacin vitamins. Cassava leaves, which are also edible if cooked properly, can contain up to 25% proteins [8].

Cassava samples investigated in the present study were grown and harvested in Luampa and Mansa districts of Western and Luapula provinces of Zambia respectively. Luampa and Mansa Districts were selected as sampling sites because the main economic activities in the two districts are fishing and agriculture which also includes large-scale farming of cassava for household consumption and commercial value according to Zambia Central Statistics Office [13]. It is perhaps worth noting that both Luampa and Mansa Districts produce a variety of cassava species [e.g., Kasonta, Kalimabeni and Kanakashi [10]; however, cassava samples tested in the present work were randomly collected from the two districts with no recourse to nomenclature.

Here, for the first time, we quantified cyanide compound levels in cassava samples collected from Luampa and Mansa Districts using UV/vis extinction spectroscopy and statistical data processing. Wet chemistry analysis based on picrate test, which is often used for cyanide quantification in food samples, was used to extract the analyte prior to spectral analysis. UV/vis extinction spectroscopy measures combined absorption and scattering of light upon interaction of radiation with the tested sample. The intensity of the output spectral signal derived from molecular (e.g., cyanide) excitation was employed to quantify cyanide content in cassava samples, and the results validated using the picrate color test. UV/vis extinction spectroscopy took advantage of simple sample handling steps, portability and rapid analysis of cyanide in cassava, with potential for on-site/on-field assessment of cyanide and food quality in farms. More importantly, spectral data exhibited desirable analytical performance as shown by high coefficient of determination and sensitivity of the spectral data.

Materials and methods

Research design and study area

A single experimental method for determining cyanide content in cassava roots was developed in this study, with a view to developing low-cost UV/vis extinction spectroscopy as an analytical tool for rapid cyanide quantification in cassava. This is because gold standard techniques, such as high-performance liquid chromatography (HPLC) and mass spectrometry-based methods, are expensive equipment and not readily available locally. The sampling sites were chosen mainly based on three factors: firstly, the importance of cassava crop and as one of the staple foods in the area; second, geographical location; and third, accessibility of the study areas. Luampa is a district located in Western province of Zambia, with an elevation of 1142 meters and located within the coordinates 15º 17' 26.6" S and 24º 44' 39.8'' E. On the other hand, Mansa district is located in Luapula province of Zambia, and the district lies within the coordinates 59.1360 S/11º11' and 39.5160/ 28º53', with an area of 7681 km^2.

Samples from Luampa district were randomly selected from four different farm fields within the coordinates 14º56'11.226''S / 24º25'7.422''E (farm A), 14º56'18.906''S / 24º24'37.842''E (farm B), 14º56'14.844' S / 24º24'57.246'' E (farm C) and 14º56'21.342'' S / 24º24'29.082'' E (farm D), respectively. Samples collected from Mansa farm fields within the coordinates 11º12'21.846'' S / 28º55'18.708'' E (farm E) were used as control samples for comparing the levels of cyanide in cassava samples from different places (i.e. Luampa *vs* Mansa). Cassava roots were isolated from the sampling sites and immediately stored on ice before being transported by road to the laboratory for sample processing and analysis. Since the researcher was required only to analyse cassava roots of cassava, the samples collected from the farm sites were enough for this proof-of-concept study and this was done in areas where cassava plants are grown as shown in Figure 1.

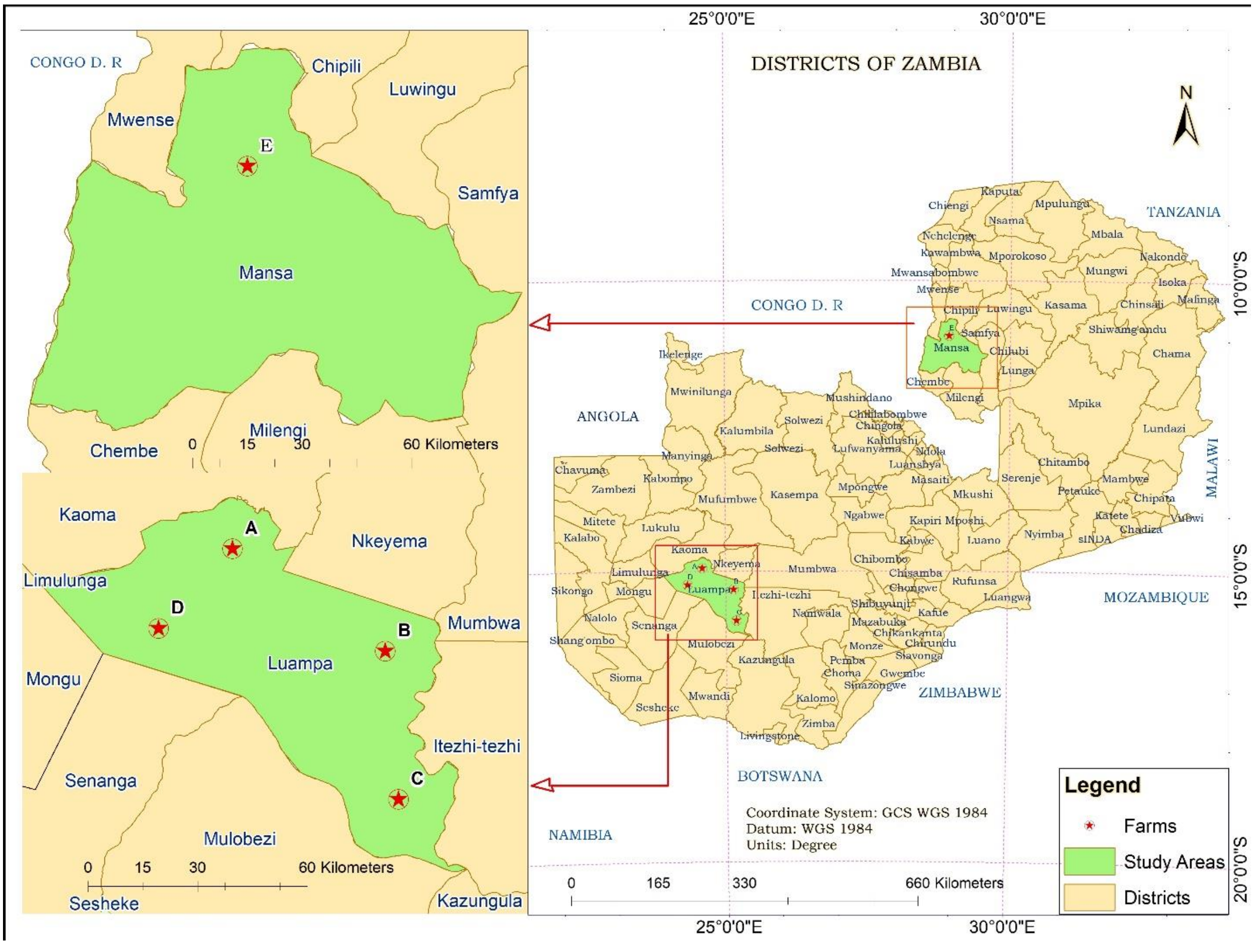

Figure 1. A map of Luampa and Mansa Districts showing sampling areas (Adapted from Google Maps, 2022). The study sites where cassava samples were collected are shaded in green trace.

Sample preparation and procedures

Four different cassava varieties were considered, sample roots were properly labelled as they were taken from the plant and transported to the laboratory on ice using a cooler box to keep the samples fresh before subsequent sample processing steps. The soil was removed from cassava samples by washing using copious tap water and properly labelled. Thereafter, cassava roots were peeled and cut into smaller pieces using a clean knife, and then placed in separately cleaned and labelled petri dishes of 140 mm by 15 mm dimensions. Small cassava pieces were subsequently kept in an oven operating at a temperature of 95 °C for 24 h. Thereafter, dried cassava samples were pounded in a clean mortar and pestle and sieved using a Retch stainless-steel test sieve of 150 mm diameter pore size, whose height was 40 mm (Fisher Scientific Inc., USA) with ISO 3310/1 standard grade. Then, 2.5 g of each powdered sample was measured using an electronic analytical balance (OHAUS-Parsippany, USA) and transferred into separate labelled glass vials until further analysis.

Preparation of alkaline picrate solution
Equal volumes of 2.5% (*w/v*) picric acid supplied by Copperbelt University laboratories (Zambia) while 5% (*w/v*) of Na_2CO_3 and 2% (*v/v)* of KOH solutions supplied by Zambia Bureau of Standards (Zambia) were prepared in 100 mL and mixed to have alkaline picrate solution for sample analysis.

Preparation of standard potassium cyanide stock solution for calibration curve analysis
A mass of 16.2 g of potassium cyanide was measured and transferred into 250 mL volumetric flask containing distilled water and the concentration normalized to 64.8 mg/mL. Next 1 mL of the stock solution was dissolved in 250 mL of distilled water to make up a 0.1034 mg/mL standard solution. From this standard solution, a series of working standard cyanide concentrations: 0, 0.01, 0.015, 0.025, 0.04 and 0.05 mg/mL were prepared in triplicates and used to generate a calibration curve which was in turn used to determine cyanide levels in unknown cassava samples.

The picrate test was performed using Whatman number one filter papers with 6 x 1 cm dimensions. The filter papers were initially dipped into an alkaline picrate solution for 15 min, followed by acidification with 20% (*v/v*) HCl solution. Acidified picrate-cyanide impregnated papers were then heated to 80 °C for 5 min. Finally, the cyanide-picrate system was incubated at room temperature for 24 h. The red-colored complex (cyanide-picrate) formed on picrate paper was eluted with 50% (*v/v*) ethanol solution for 30 min and the absorbance of the eluates were measured using a UV-vis spectrophotometer.

Preparation of cassava samples for cyanide analysis
The filter paper strips for sample measurements were prepared under identical conditions as for the calibration steps for standard cyanide solutions described above. Briefly, each sample (2.5 g) was loaded into a clean and dry glass vial and acidified with 10 mL of 20% (*v/v*) HCl acid solution. The vials were sealed with six picrate impregnated strips suspended above the acidified samples, and thereafter strips heated at 80 °C in an oven for 5 min. The heated strips were then left at room temperature for 24 h. The red-colored picrate paper strips were then rinsed in 5 mL of 50% (*v/v*) ethanol solution before absorbance readings were taken using a UV-vis spectrophotometer.

Results and data analysis
Figure 2 shows the standard curve was computed via regression of the absorbance measurements of CN^- calibrants detected at λ_{max} of 510 nm using UV/vis extinction spectroscopy.

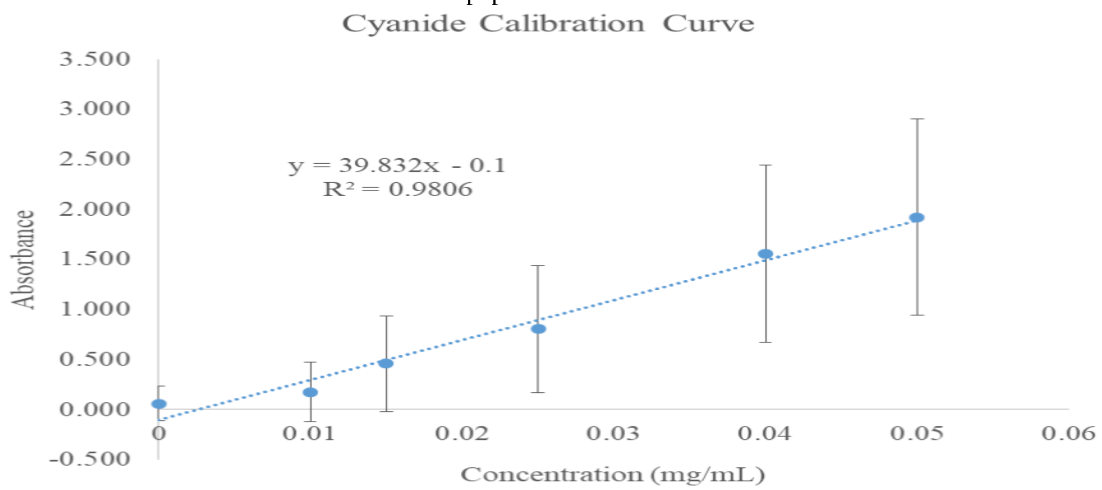

Figure 2. Calibration curve for cyanide determination using the alkaline picrate color method. Each data point on the calibration line represent an average of triplicates per concentration point of the standard sample. Error bars represent one standard deviation of the average measurement (shown as circles on the graph).

A linear relationship was obtained between cyanide concentrations in the range of 0 – 0.05 mg/mL standard solutions and extinction spectra. A high coefficient of determination (R^2 = 0.9806) for the line of best fit (Figure 2) indicate a strong linearity for absorbance *versus* concentrations of CN^- standards. The values obtained for CN^- quantitative analysis were reproducible, and concentration as low as 0.01 mg/mL of CN^- were detected. Figures of merit, including the limit of detection (LOD) and limit of quantification (LOQ), are often used to assess the performance of analytical techniques in quantitative analysis.

In this study, LOD and LOQ were calculated based on the standard error of the *y*-intercept of the calibration curve, in order to demonstrate the analytical performance of the picrate method prior to CN^- detection in cassava. With this strategy, the LOD and LOQ were found to be 0.0038 mg/mL and 0.0116 mg/mL, respectively. Having established the standard calibration curve (Figure 2), the CN^- content of cassava samples were calculated through interpolation of absorbance for test cassava samples, and sample concentrations summarised in Table 1.

Table 1. Details of the concentrations of cyanide in 2.5 g, as well as the age of investigated cassava samples

Sample identity	Districts	Sample condition	Mass of sample (grams)	Age (years)	Absorbance	Unknown Concentration (mg/mL)
1	Mansa	Unsoaked	2.5088	2	0.271	9.319
2	Mansa	Soaked	2.5079	1	0.082	4.57
3	Luampa	Soaked	2.5004	2	0.154	6.379
4	Luampa	Unsoaked	2.5132	2	0.024	3.113
5	Luampa	Soaked	2.5077	1	0.169	6.756
6	Luampa	Unsoaked	2.528	1	0.379	12.033

The unknown concentration in cassava samples were calculated using the standard curve presented in Figure 2. The results presented in the Table show the cyanide concentrations of cassava flour samples in unsoaked and soaked of one and two years old, with the highest concentration being 12.033 mg/mL and the lowest reading was 3.113 mg/mL in 2.5 g analytical sample. Samples 1 and 2 were collected from Mansa and were deployed as control samples. Although Mansa and Luampa Districts are ~1149 km apart, the levels of cyanide in cassava sampled from these two Zambian districts were comparable; hence, cassava samples from Mansa were regarded as positive control samples in the present study. It can also be seen in Table 1 that the concentration of cyanide varied significantly within-sample and between-samples, with higher cyanide levels detected in unsoaked cassava than in soaked samples, regardless of the age of cassava plant. In general, the concentration of cyanide varied inversely with the age of cassava plants; that is to say, higher concentrations of cyanide were detected in younger cassava plants than in older plants.

The difference in concentration (CN equivalent) of cyanide collected from different provinces that are about 1149 km apart can be attributed to the fact that, the chemical composition of cassava crop varies according to variety, location, age, and environmental conditions [4 - 5]. The cyanogenic potential of cassava crops changes with agro-ecological zone. Apart from ecological differences, the variations in the levels of cyanide may also be attributed to different soil chemistries of Luampa and Mansa regions [2]. This is mainly as a result of the variation in key components of soil like potassium, calcium and magnesium ions that adversely affect uptake of cyanide by cassava plants. The results in Table 1 were summarized as bar graphs illustrated in Figure 3 for clearer visualization of the variations in cyanide concentrations with respect to sampling area and the age of cassava plants.

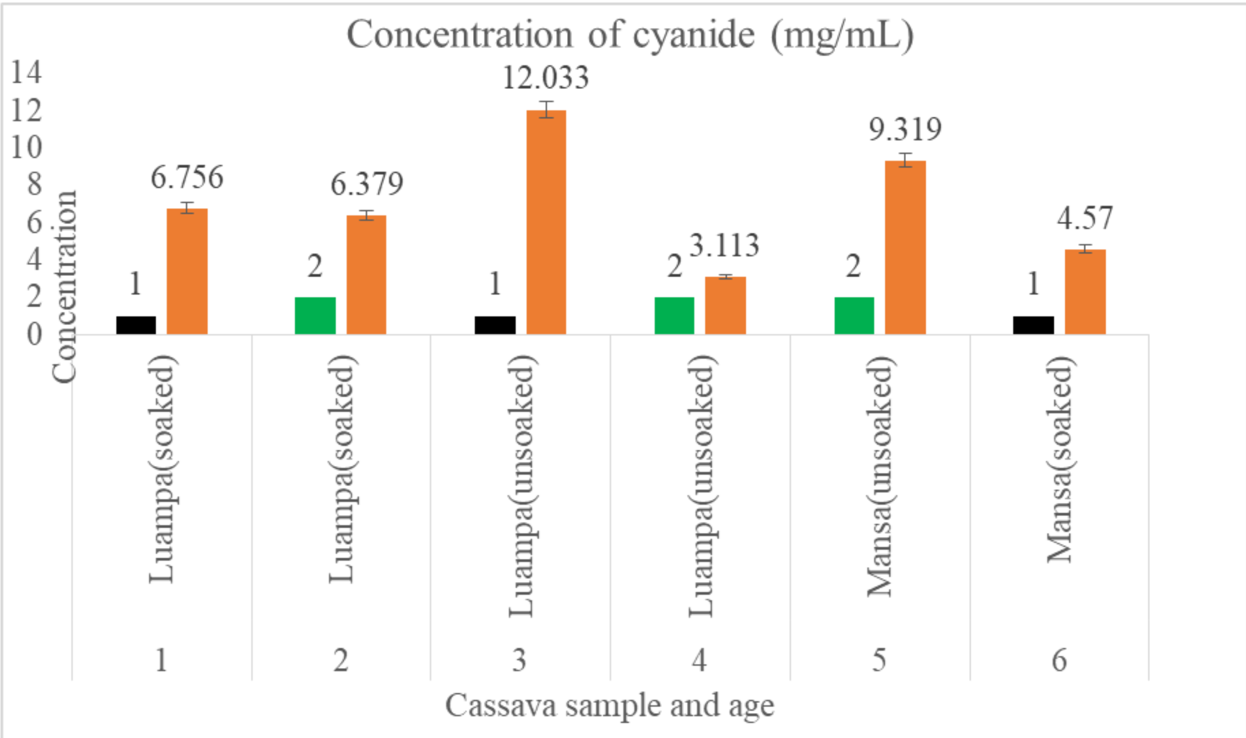

Figure 3. Bar graphs for cyanide concentration dynamics in soaked and unsoaked cassava according to plant age. Bars in black and green traces indicate plant ages for soaked (1-year old crops) and unsoaked (2 year-old crops) respectively whilst orange trace represent the concentrations of cassava samples with respective quantities on top of the bars graphs. The numbers on top of the black and green bars indicate the age of the plants: 1 = 1-year old and 2 = 2 years old crops. Error bars represent one standard deviation of the mean.

Soaked cassava samples contained cyanide levels that were above the world health organisation (WHO) guidelines for safe concentration which is set at 10 mg of CN/kg body weight [12], except one sample which gave a concentration of 6.193 mg/kg equivalent of CN which was below the safe level recommended by WHO. The probable reason for such high concentrations of cyanide in most of the soaked cassava could be due to the limited duration of the soaking period, and this requires careful consideration in future work. As the levels of cyanide concentration differ from one variety to another so the number of days for soaking may differ. By contrast, all unsoaked samples exhibited cyanide amounts that were higher than the safety limit, with the highest amount having 23.799 mg/kg equivalent CN. It is also worth noting that the cyanide content in cassava roots decreased with the increase in age of the plant as observed in this study.

Conclusions

This study has demonstrated that there were varying levels of cyanide in cassava samples collected from two geographically different Zambian districts (*viz.*, Luampa and Mansa). Cassava samples from Luampa District displayed higher cyanide levels than those harvested from Mansa District, which was utilized as positive control in this study. The differences in cyanide concentration levels between unsoaked and soaked cassava was clear, with unsoaked cassava exhibiting higher cyanide levels compared to soaked cassava despite the amount of cyanide in both samples being above the WHO recommended limit.

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