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Effect of processing on amygdalin and cyanide contents of some Nigerian foods

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ABSTRACT

Amygdalin is a phyto-toxin which occurs in many plant species, of which a number of species are usually consumed by humans. Hydrolysis of amygdalin to produce hydrogen cyanide occurs during processing of plant foods. Consumption of food containing amygdalin and or cyanide can cause serious health problems to animals including humans. Amygdalin and cyanide were extracted from raw cassava roots, cassava products, processed fruit products, nuts, sorghum, cocoyam, bitter leaf, Africa star apple seeds and Cirina forda and determined using spectrophotometric method. The level of amygdalin and cyanide in some raw and processed Nigerian foods was investigated in this study. Our results showed that amygdalin content of cassava roots ranged from 8.8mg/g to 48.3mg/g. Amygdalin was completely hydrolyzed to cyanide during processing of cassava roots to garri and lafun. At each stage of garri and lafun production, amygdalin was not detected but cyanide was detected. The cyanide content of processed cassava was relatively low (5.2-19.1 ppm for garri and 3.5-13.2 ppm for lafun). Amygdalin content of processed fruit products was very low. The results showed that amygdalin and cyanide contents of most of the Nigerian foods analyzed in this study were low and are unlikely to cause health problems to consumers if adequately processed before consumption.

Key words: Amygdalin, Cyanide, Hydrolysis, Cassava, phyto-toxin

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INTRODUCTION

Amygdalin is one of the most common cyanogenic glycosides. Cyanogenic glycosides are plant natural toxicant that has the ability to produce toxic hydrogen cyanide. Concentrations of cyanogenic glycosides vary widely in plants as a result of genetic and environmental factors, location, season and soil types [1, 2]. Cyanide concentrations vary from one plant variety to another, for example cassava may contain 15 – 400 mg equivalent cyanide/kg fresh weight. Occasionally, some varieties of cassava tubers may contain 1300 – 200 mg cyanide/kg fresh weight, and cassava leaves contain 1000 – 2000 mg equivalent cyanide/kg on a dry matter basis [3].

Enzymatic hydrolysis of cyanogenic glycosides in edible plants occurs during the preparation of foods. During food processing, enzyme β-glucosidase from the plant is responsible for the hydrolysis of cyanogenic glycosides to produce toxic hydrogen cyanide[4,5]. After food ingestion, glucosidases of the intestinal microflora and, to a lesser degree, those of the liver are responsible for the production of hydrogen cyanide from ingested cyanogenic plant foods [6,7].

Cyanide can be lethal to humans and the acute dose is in the region of 1 mg/kg body weight. Because the body can rapidly detoxify cyanide, an adult human can withstand 50 – 60 ppm for an hour without serious consequences. However, exposure to concentrations of 250 – 500ppm for 30 minutes is usually fatal. Clinical symptoms of acute cyanide poisoning include; rapid respiration, drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhea, mental confusion, stupor, cyanosis, twitching and convulsions [8]. Aside from death, acute cyanide toxicity at small doses between 0.5 and 3.5 $mgkg^{-1}$ body weights can cause headache, tightness in throat, and muscle weakness [2].

In order to prevent cyanide toxicity, processing procedures such as peeling, grating, crushing, grinding, soaking, fermenting and drying have been used for centuries to reduce potential toxicity of cyanogenic plants before consumption. Amygdalin is the cyanogenic glycoside responsible for the toxicity of the seeds of many species of rosaceae, such as bitter almonds, peaches, cassava and apricots. Although the use of almond in marzipan production is common, their preparation procedures should eliminate most of the cyanide [9]. Cassava, an important source of carbohydrate for people in Africa and South America, is detoxified by chopping and grinding in running water prior to preparation. However, cases of acute cyanide poisoning have been associated with inadequate preparation of cyanogenic plants such as apricot pits, bitter almonds, cassava and apple seeds [10]. Goitre and cretinism due to iodine deficiency have been reported to be exacerbated by consumption of insufficiently processed cassava [8].Hence, the aim of this study was to determine the effect of processing on the amygdalin and cyanide content of some Nigerian foods.

EXPERIMENTAL SECTION

Food Samples

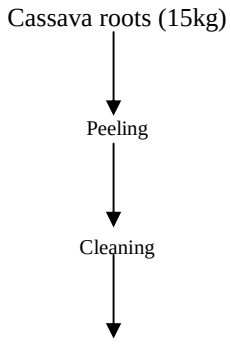
Freshly harvested cassava roots used in this study were obtained from Ladoke Akintola University of Technology farm, Ogbomoso, Nigeria. Cocoyam, eggplant (garden egg), bitterkola, kolanut, Cirinaforda (*monimoni*), African star apple (*agablumo*), watermelon, bitter leaves, sorghum, golden delicious apple, mango and pawpaw were purchased from local market in Ogbomoso, Oyo state, Nigeria. Moringa seeds, almond fruits were obtained from local farmers in Ogbomoso Southwest, Oyo state Nigeria. The fruits were cleaned and their seeds were removed using a knife. The seeds were dried in an oven (60^oC)for 1 hr and stored overnight in airtight container until extraction.

Chemicals and Reagents

Amygdalin (98%purity) was purchased from Sigma Aldrich, UK, ethanol (98%)from BDH Chemicals Ltd Poole, England, diethyl ether (98%)from Surechem Products Ltd Needham market, Suffolk England. Polypropylene plastic, Whatman No1 filter paper were all purchased from Rite laboratory, Ogbomoso, Oyo State, Nigeria.All other reagents were of analytical grade.

Production of *Garri*

Cassava roots were peeled with a knife and washed in several changes of clean water to remove dirty particles. They were then coarsely grated with the help of a grater machine. The grated cassava was collected in jute sacks and left to ferment for four (4) days.The fermented cassava mash was pressed by the means of jacking machine to remove water. The pressed cassava mash was sieved to remove the fibres by means of a sieve and heated with constant stirring in wide, shallow, non-stickly metal pans till it beaome light crisp *garri*. The *garri* was cooled and stored in an air tight container prior to analysis. The flowchart for the production of *garri* is presented in Fig. 1.



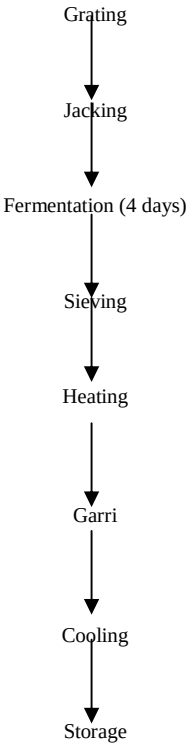


Figure 1: Flowchart for *garri* production

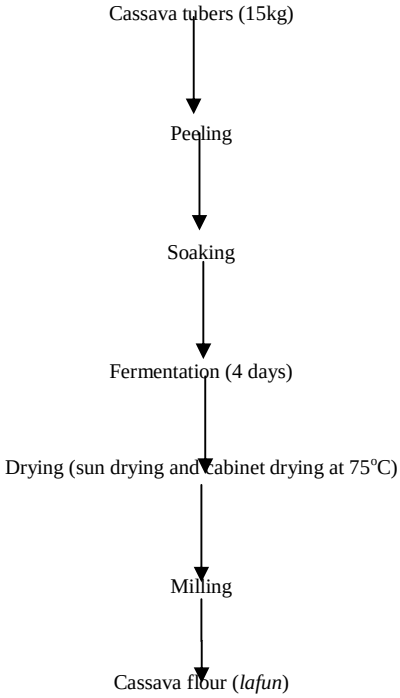


Figure 2: Flowchart for the production of cassava flour (*lafun*)

Production of Cassava Flour (*lafun*)
Freshly harvested cassava roots were soaked in water inside a container for four days to facilitate thorough fermentation and softening of cassava roots. The fermented cassava roots were sun dried for five days, milled and stored in an airtight container prior to analysis. The flowchart for the production of cassava flour is presented in Fig. 2.

Production of Sorghum Ogi
Sorghum ogi was prepared using the wet-milling process described by [11].Two hundred grams of the cleaned sorghum samples were soaked in a plastic bucket containing 300 ml of water and steeped for 72 h at room temperature (28 ± 2°C). The steeped water was discarded by decantation and the steeped grains were wet-milled using a Kenwood chef grinder. The milled slurry was then sieved through a fine mesh sieve to remove the over tails which were discarded. The over tails were further washed off with 700 ml of distilled water. The troughs were allowed to stand and further fermented for 48h by allowing it to stand and sediment at room temperature [12]. The souring water was decanted from the sediments and the o*gi* slurry obtained was collected into a muslin cloth and hand squeezed to remove excess water leaving behind the cake of *ogi* samples.

Production of Watermelon Juice
Fresh ripped watermelon fruit was cleaned and peeled. The watermelon fruit was divided into 2 parts. The seeds were removed from one part and left in the other part. The two parts were blended separately in a Kenwood blender for 2 mins to obtain watermelon juice.

Determination of Amygdalin Content of Food Samples
The method of amygdalin extraction described by [13] was used to extract amygdalin from cassava products (*garri* and *lafun*)at each stage of their production process and from other foods. The food samples (5 g) or cassava roots were ground in a blender and 4g of it was weighed into a round bottom flask (500ml). Ethanol (100ml) was added and the mixture was boiled under reflux for 20 minutes. The extract was filtered using Whatman No1 filter paper and the mixture was transferred into polypropylene tubes. Ethanol was completely evaporated from the filtrate with a rotary evaporator. Diethyl ether (10ml) was added to the dried samples and the mixture was shook vigorously for two mins at room temperature (32 ± 2^{o}C) to precipitate amygdalin. The diethyl ether was allowed to evaporate overnight in a fume hood and the precipitated amygdalin was dissolved in water (5 ml), filtered and determined using a UV spectrophotometer set at 256 nm. The amount of amygdalin in the sample was determined with reference to amygdalin standard curve.

Amygdalin Calibration Curve
Amygdalin standard was dissolved in water to obtain a stock solution of 100µg/ml. The absorption of amygdalin standard concentration of 1, 50, 100, 200, 300, 500 and 1000µg/ml was determined at 256nm using a spectrophotometer. A calibration curve was constructed by plotting the concentration of the seven amygdalin standard solution against their absorbance. The calibration curve was used to calculate the concentration of amygdalin in the extracted samples.

Determination of cyanide in food
Food sample (5 g) was weighed into a beaker (50 ml) and 5ml of freshly prepared Mg $(OH)_2$,Cl^- free was added to the food sample. The mixture was titrated with 0.1N $AgNO_3$, using K_2CrO_4 as indicator [14].

Statistical Analysis
All data obtained were subjected to analysis of variance (ANOVA), and means were separated using Duncan's multiple range tests with significance level at $p < 0.05$. SPSS software version 15 was used for all statistical analysis.

RESULTS AND DISCUSSION

Amygdalin Standard Curve
Amygdalin detection was achieved by UV detection in an isocratic elution with an excellent linearity (correlation R^2=0.9984) between the concentration and the absorbance of amygdalin (Fig. 3).

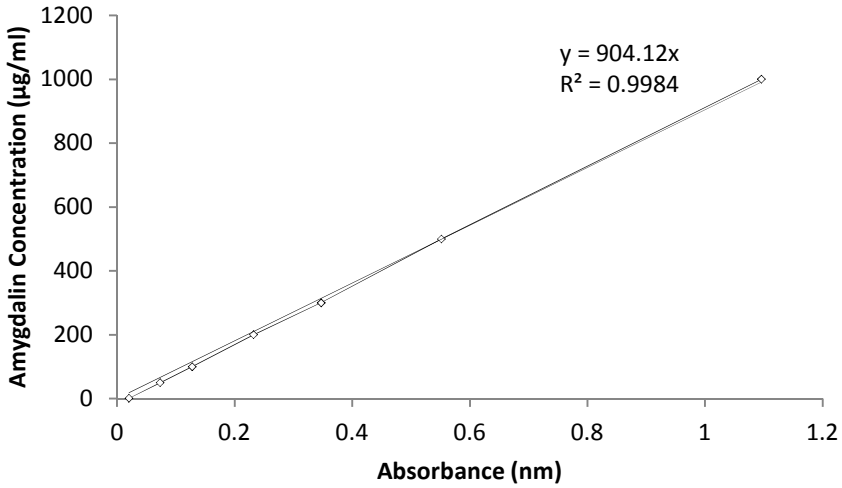


Figure 3: Amygdalin calibration curve

Amygdalin Content of Different Varieties of Cassava Roots

Amygdalin contents of cassava from different varieties of cassava are presented in Table1. Amygdalin content of cassava roots ranged from 8.84mg to 48.33mg. Among the six varieties of cassava analyzed in this study, *Okoyawo* TME7 cassava variety had the highest amygdalin content (48.33mg/g) followed by White cassava (17.36mg/g), Olekanga (16.57mg/g), Black cassava (13.79mg/g), Arubielu cassava (11.19 mg/g) and Yellow cassava had the lowest (8.84 mg/g) amygdalin content. The variation in the amygdalin content of the cassava roots may be due to cultivation practices and variation in the species. Application of fertilizer to field before planting has been reported to decrease cyanogenic glycosides levels in cassava roots [15].

Table 1: Amygdalin Content of Cassava Roots

Cassava variety	Amygdalin content (mg/g)
Yellow colour	8.84 ± 0.15^{a}
Arubielu colour	11.19 ± 0.89^{b}
Black colour	13.79 ± 0.22^{b}
Olekanga colour	16.57 ± 0.56^{c}
White colour	17.36 ± 0.35^{c}
Okoyawo colour	48.33 ± 0.79^{d}

Each value is expressed as mean ± standard deviation (n = 3 extractions). Means with the same superscripts along the same column are not significantly different ($p < 0.05$).

Table 2: Cyanide content of *garri* from okoyawo variety and each processing stage

Processing stage/Product	Equivalent cyanide content (mg/kg)
Tuber after peeling	19.14 ± 0.16^{d}
Grating	15.73 ± 1.33^{c}
Second day of fermentation	14.80 ± 0.11^{b}
Third day of fermentation	14.53 ± 0.01^{b}
Fourth day of fermentation	14.08 ± 0.87^{b}
Jacking	13.50 ± 0.12^{b}
Sieving	6.76 ± 1.11^{a}
Garri frying	5.78 ± 0.05^{a}
Garri	5.19 ± 0.02^{a}

Each value is expressed as mean ± standard deviation (n = 3 extractions). Means with thesame superscripts along the same column are not significantly different ($p < 0.05$).

Cyanide Content of *Garri*

Gaari was produced from the cassava variety with the highest amygdalin content(Okoyawo) among the six cassava roots that were analyzed in this study. Surprisingly, amygdalin was not detected at each stage of *garri* production, instead, cyanide (Table 2) was detected after peeling, grating, fermentation, jacking, sieving and *garri-frying*. This result showed that amygdalin of cassava was hydrolyzed to cyanide shortly after peeling. The cyanide content of the

garri ranged from 19.17mg/kg to 5.00mg/kg. It was observed that the cyanide content of the cassava mash decreases as fermentation period increases. Other unit operations (jacking, sieving, garri-frying) in *garri* processing also reduced the cyanide content of the final product significantly (Table 2). This is possible because cyanide is soluble in water, thus jacking of cassava mash will lead to removal of dissolved cyanide from the mash and since cyanide is volatile (boiling point is 26^{o}C) it is evaporated at the high *garri-frying* temperature.

Cyanide Content of Cassava Flour (L*afun*)

Cassava flour (*lafun*)was produced from the cassava variety with the highest amygdalin (Okoyawo) content. The results obtained were similar to that of *gaari* above (i.e. amygdalin was not detected during the processing of cassava root). The cyanide content of processed cassava roots reduced significantly after each processing stage for*lafun* production (Table 3).The reduction in the cyanide content could be as a result of the solubility of cyanide in the soaking water. Soaking and fermentation of cassava roots has been reported to be one of the principle mechanisms of cyanide removal from *lafun*[16]. During soaking of cassava roots linamarase and or amygdalin are separate from cassava tissue [17] and hydrolyzed to cyanide which are discarded with the soaking water. The cyanide content of the cassava flour (3.51 mg/kg) obtained in this study was similar to the cyanide content of cassava flours (2.43 – 3.4 mg/kg) reported by [18].

Table 3: Cyanide content of *Lafun* from okoyawo variety and each processing stage

Processing stage/Product	Equivalent cyanide content mg/kg)
Cassava (*Okoyawo* variety) after peeling	13.16 ± 0.76^{c}
First day of soaking /fermentation	11.86 ± 0.26^{b}
Second day of soaking /fermentation	10.22 ± 0.06^{b}
Third day of soaking /fermentation	9.50 ± 0.96^{b}
Third day of sun drying	5.87 ± 0.04^{a}
Fifth day of sun drying	5.02 ± 1.06^{a}
Cassava flour(*Lafun*)	3.51 ± 0.09^{a}

Each value is expressed as mean ± standard deviation (n = 3 extractions). Means with thesame superscripts along the same column are not significantly different (p < 0.05).

Amygdalin Content of Fruit Seeds

Amygdalin contents of the fruit seeds varies significantly from seed to seed as shown in Table 4. Among the fruit seeds analyzed, sorghum has the highest amygdalin content (122.31 mg/g), followed by African star apple (*agbalumo*) seeds (69.73 mg/g), golden delicious apple seeds (12.16 mg/g), mango seeds (4.41 mg/g), moringa seeds (4.29 mg/g), watermelon seeds (3.97 mg/g) egg plant seeds (3.17 mg/g), almond seeds (3.00 mg/g) with pawpaw seeds having the lowest amount of amygdalin (0.90 mg/g). Amygdalin content of golden delicious apple seeds (12.16 mg/g) obtained in this study is higher than the amygdalin content of the seeds (3.91 mg/g) reported by [19]. The difference in the amygdalin content of the apple seeds could be as a result of differences in method of analysis, ripening stage of the apple as well as source of the apple and cultivation practices[19, 15].

Table 4: Amygdalin content of fruit seeds samples

Fruit seeds	Amygdalin content (mg/g)
Sorghum grains	122.31 ± 0.11
African star apple	69.73 ± 1.05
Watermelon	65.09 ± 0.87
Eggplant	3.17 ± 0.12
Apple	12.16 ± 0.99
Almonds	3.00 ± 1.22
Mango	4.41 ± 0.03
Pawpaw	0.90 ± 0.91
Cucumber	0.79 ± 0.32

Each value is expressed as mean ± standard deviation (n = 3 extractions).

Amygdalin Contents of Raw and Processed Foods

Amygdalin content of raw and processed food products are presented in Table 5.*Cirina forda* has the high amygdalin content (61.44 mg/g) among the raw food samples analysed in this study. Thus, *Cirina forda*has the potential of generating 3.68 mg/g of cyanide. This value is close to the toxic level of cyanide for adult (0.5 – 3.5 mg/kg) reported by [2]. Amygdalin content of unsieved (88.83 mg/g) and sieved (44.71 mg/g) sorghum ogi were lower that of raw sorghum grains (122.31 mg/g). Amygdalin content (56.45 mg/g) of watermelon juice (pulp and seeds) was lower than that of raw watermelon seeds (65.09 mg/g). Indicating that processing of raw foods resulted

in significant reduction in the amygdalin content of the processed food products. Amygdalin was not detected in watermelon juice produced from pulp only. It is recommended that watermelon juice should be produce from watermelon pulp only in order to prevent health problems associated with the consumption of amygdalin (see introduction section for details). Amygdalin was not detected from juice produced from golden delicious appleflesh, this is because the flesh and the skin of apples are reported to be non-cyanogenic[19]. However, amygdalin was detected in apple juice (2.79 mg/g) produced from whole apple (flesh + skin + seeds) fruits. This value differs from the amygdalin content of apple juice (0.43 mg/g) reported by [19].

Table 5: Amygdalin content of foods

Food Sample	Amygdalin Content (mg/g)
Cirina forda	61.44 ± 0.21
Sorghum ogi (unsieved)	88.83 ± 1.04
Sorghum ogi (sieved)	44.71 ± 0.33
Watermelon juice (pulp + seeds)	56.45 ± 0.22
Watermelon juice (pulp only)	nd
Apple juice (flesh + skin + seeds)	2.79 ± 0.52
Apple juice (flesh + skin only)	nd
Cucumber (flesh + skin + seeds)	1.19 ± 0.45
Sorghum stem sheath (*poporo*)	1.08 ± 0.89
Fresh bitter leaf	206.21 ± 1.06
Scrubbed bitter leaf	38.09 ± 0.98

Each value is expressed as mean ± standard deviation (n = 3 extractions). nd – not detected.

Amygdalin Content of Different Varieties of Cocoyam Tubers

As shown in Table 6, amygdalin content of cocoyam is cultivar dependent. Purple flesh cocoyam variety had the highest amygdalin content (10.84 mg/g) followed by white flesh cocoyam (6.29 mg/g) while cream flesh cocoyam (commonly called Ghanaian cocoyam) had the lowest (5.88mg/g) amygdalin content. Differences in theamygdalin content of the cocoyam varieties may be due to differences in their genetic composition and source of the cocoyam tubers. All parts of raw cocoyam plant have been reported to contain a toxic compound which must be removed before consumption [20]. Purple flesh cocoyam has the potential of generating 0.65 mg/g equivalent cyanide. This value is very low and could not result in cyanide toxicity in humans.

Table 6: Amygdalin content of different varieties of cocoyam

Cocoyam Varieties	Amygdalin Content (mg/g)
Purple colour	10.84 ± 0.01^{b}
White colour	6.29 ± 0.45^{a}
Cream colour	5.88 ± 0.69^{a}

Each value is expressed as mean ± standard deviation (n = 3 extractions).

Amygdalin Content of Nuts

Two different varieties of kolanut and one variety of bitterkola were analyzed. *Cola nidita* (gbanja) had the highest (55.05 mg/g) amygdalin content while *Cola acuminate* (abata) had the lowest (44.53 mg/g) amygdalin content (Table 7). Amygdalin content of bitter kola was low (3.28 mg/g).*Cola nitida* has the potential of generating 3.30 ppm equivalent cyanide while *Cola acuminate* can generate 2.67 mg/g cyanide. Although these values were relatively low, continuous consumption of raw kolanut may lead to chronic cyanide toxicity.

Table 7: Amygdalin content of nut samples

Nuts	Amygdalin content (mg/g)
Kolanut (*Cola nitida*)	55.05 ± 0.15c
Kolanut (*Cola acuminata*)	44.53 ± 0.19b
Bitter kola	3.28 ± 0.04a

Each value is expressed as mean ± standard deviation (n = 3 extractions). Means with the same superscript along the same column are not significantly different (p < 0.05).

CONCLUSION

Amygdalin and cyanide content of cassava roots are cultivar dependent. Processing methods such as fermentation, soaking, dewatering, drying and roasting/frying reduced the cyanide content of cassava products significantly. Since the acceptable level of cyanide in cassava products is 10 ppm according to WHO and the level of cyanide in cassava

roots soaked for three days is less than 10 ppm. It can be concluded that *lafun* produced from cassava roots soaked or fermented for three days would not cause health problems to consumers. The production of fruit juice whole fruits (i.e. flesh + seeds) is discouraged due to the presence of amygdalin in fruit seeds. Most Nigerian foods would not cause health problems if adequately processed.

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