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Determination of cyanide content in cassava tubers (*Manihot esculenta*) and apple seed (*Pyrus malus*)

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Abstract

The research was conducted in order to determine the concentration of cyanide in cassava turbers and apple seeds consumed in Zaria and to compare the level of cyanide in cassava tubers using different drying methods. This was determined using alkaline titration method as described by Kamalu and Oghoma. The results obtained shows that oven dried cassava has the highest concentration (2.61±0.31ppm) followed by air dried cassava (2.54±0.05ppm), fresh apple seed (1.94±0.09pmm) and undried Cassava (1.53±0.16ppm). When wet apple seeds are compared with wet cassava, the apple seeds have higher concentration (1.94±0.09ppm and 1.53±0.16ppm respectively. According to Food Safety Focus (2008), the acute lethal dose of hydrogen cyanide for humans is reported to be 0.5 to 3.5 mg per kilogram of body weight. Children are particularly at risk because of their smaller body size. These results indicate that consumption of 6 – 7 apple seeds (0.7g) is enough to poison a child of 7 -10 kg weight. We recommend that all products obtained from cassava should be analyse for cyanide concentration to ensure safety of the masses.

Keywords: Cassava Tubers, Apple seeds, Cyanide and Toxicity

Introduction

Cassava is the third largest source of carbohydrates food in the tropics, after rice and maize. Cassava is the staple food of more than 500 million people in the tropics, many of whom are very poor (Katz and Weaver,2003). According to New World Encyclopedia (2008), it is one of the world's most perishable tuber crops with a high post-harvest loss. Anomically cassava root is not a tuberous root but a true root which cannot be used for vegetative propagation. The three distinct tissues of the mature cassava tuber include; the periderm, cortex and pulp which is the edible portion of the fresh root.Apple(*Malus*) is a genus of about 30–55 species of small deciduous trees or shrubs in the family Rosaceae, including the domesticated orchard apple – also known as the eating apple, cooking apple, or culinary apple. It is dealt with under Apple. The other species are generally known as crabapples, crab apples, or wild apples

According to Wheatley and Chuzel (1993), the cyanide concentration in cassava varies in different part of the plant. Cassava is of a lower nutritional value than cereals, legumes, and even some other root and tuber crops such as yam (Charles *et al.*, 2005).

Cassava roots contain significant amount of iron, phosphorus, calcium and vitamin C, but is a poor source of protein. The root contains carbohydrate 64-72% of which is made up of starch, mainly in the form of Amylose and Amyl pectin. About 17% sucrose is found in sweet varieties and small amounts of fructose and dextrose have been reported (Hendershort, 1972), the lipid content of cassava is 0.5%, protein content is about 1-2% and the amino profile of the cassava root is very low,cassava is reasonably rich in calcium and vitamin C, but the thiamine and riboflavin and niacin contents are not high (Hair, 1998; RMKDC, 2004; New World Encyclopedia, 2008).

So therefore, the two distinct types of cassava are the sweet cassava (Manihot *dulcis*) and the bitter cassava (Manihot *esculenta*). The bitter cassava is associated with high level of cyanogenic glucoside.

Cyanide

Cyanide is a chemical compound that contains the group C≡N. this group known as the cyano group consist of a carbon atom triple bonded to a nitrogen atom.

According to Gosselin et al, (1984), cyanide can be produced by certain organisms (e.g bacteria, fungi, and algae), and equally in plant. According to Greenwood and Eamlaw (1992) free cyanide is defined as the sum of the cyanide present as CN^- . As a result, low level of cyanide can appear in naturally occurring surfaces or ground water, which normally would not be expected to contain it (US fish and wildlife service, 1991).

According to Duffey (1981), anthropogenic sources of cyanide include synthetic catalytic process involving reaction of ammonia and natural gas with or without air. Hydrogen cyanide may be obtained as a by-product in the production of acrylnitrite.

Other cyanide like sodium and potassium are principally prepared by direct reaction of hydrogen cyanide released to air as a result of chemical manufacturing and processing industries, volatilization of cyanide waste deposit in landfill and waste pond. Some sources of cyanide arise from cigarette smoke, are generated when some certain synthetic material such as plastics are burned.

Much of the cyanide in soil, water and air comes from industrial processing, gold mining, and waste waters from starch industries. The major sources of cyanide in water are discharged from metal mining processes. Other sources include exhaust released from certain chemical industries, municipal waste burning and use of pesticides containing cyanide. Underground water can be contaminated by cyanide present in landfills. In other body, cyanide can be combined with plant foods including almonds, millet sprouts, lima beans, soy spinach, bamboo shoot and cassava root. Cyanide occurs as part of naturally occurring sugars or other complex organic compound.

In humans, cyanide is a well known poison with potential acute and chronic metabolic effect (Kakes, 1990). Cyanide exacts its toxic effect by binding ferric ion with cytochrome, an enzyme that account for about 90% of total oxygen uptake in most cells through electronic transport chain. Human get exposed to cyanide mainly by breathing, drinking water, eating foods and touching soils.

Some symptoms of acute poisoning include, mental confusion, muscular paralysis and respiratory distress (Kakes, 1990). Large amount of cyanide may be responsible for visible manifestations as goiter and cretinism (Bradbury, 2004).

Statement of the Problem

Cassava is grown in Zaria as a subsistence crop, its utilization as food varies from region to region, it is a source of food, serenity not only because it can be grown on less productive land, but because it is a source of income for the urban and rural population.

People in many parts of Nigeria rely largely on starchy foods for their carbohydrate intake, cassava being a major source of carbohydrate is becoming a major source of food. The crop's agronomical advantages such as high productivity ease and flexibility of cultivation, tolerance to drought and its ability to grow well on relatively poor soil has made it rapidly and extensively adaptable.

The status of cassava as a food security crop to most subsistence farmers is however threatened by pest, diseases and weed, and potential toxicity in cassava is due to the presence of cyanogenic glycoside potential for production of poisonous hydrogen cyanide.

Apple is widely consumed for its attractive look, taste and other medicinal purposes. The seeds are usually not consumed but often consumed accidentally or ignorantly and its contains (cyanide) are harmful. Apple seeds contain amygdalin, which is converted into cyanide when the seeds are chewed or crushed. Cyanide is highly poisonous and can be deadly in high doses.

The research seeks to determine the concentration of cyanide in cassava and apple seed consumed in zaria and its environs and to compare the concentration of cyanide in cassava under different drying processes (moist, sun-dry, air-dry and oven dry cassava).

Significance of the Study

Information obtained in this study on the determination of cyanide content in cassava and apple seeds will be crucial for awareness campaign to its users. Such information will be very important for extensive services towards training of farmers and consumers by agricultural officers and other regulatory body on effective method of processing cassava.

Cyanide Toxicity

Cyanide toxicity is generally considered to be a rare form of poisoning. However, cyanide exposure occurs relatively frequently in patients with smoke inhalation from residential or industrial fires (Holstege and Kirk, 2015). In addition, intensive treatment with sodium nitroprusside or long term consumption of foods containing cyanide is a possible source of cyanide poisoning (Abraham, Buhrke and Lampen, 2016; Akyildiz, Kurtoglu, Kondolot and Tunc, 2010). Historically, cyanide has been used as a chemical warfare agent, and it could potentially be an agent for a terrorist attack (Armstrong 2002).

Depending on its form, cyanide may cause toxicity through inhalation, ingestion, dermal absorption or parental administration. Clinical administration may vary widely, depending on the dose and route of exposure, and may range from minor upper airway irritation to cardiovascular collapse and death within minutes. In severe cases, aggressive therapy consisting of supportive care and antidote administration can be lifesaving.

Effect of Cyanide on Human Body

The poison blocks the cellular enzyme (cytochrome oxidase), which is responsible for the assimilation of oxygen by the cell. Therefore, when poisoning with cyanide accesses the cells, metabolic processes inside the cell stops, and the body quickly dies. The effect is equivalent to the fact that the poisoned simply suffocated due to lack of air.

According to Njue (2011) consumption of unprocessed, raw and whole cooked cassava lead to food poisoning and fatal cases in the Northern Province of Nigeria.

Biochemical Basis for Cyanide Poisoning

The toxic effect of cyanide is ascribed predominantly to the production of anoxia following inhibition of cytochrome oxidase, a terminal mitochondrial respiratory chain enzyme. This enzyme contains two heme A and two copper ions. Cyanide has a special affinity for heme ion and the reaction of cyanide with the multimeric iron enzyme complex is facilitated by first penetration of cyanide to protein cervices, with initial binding of cyanide to the protein followed by binding of cyanide to heme ion. Thereby, a cyanide-heme cytochrome oxidase complex is formed which renders the enzymes incapable of utilizing the oxygen. The resultant oxygen saturation of the blood imparts a cherry red color, which aids the diagnosis in most instances of cyanide poisoning. Inhibition of cytochrome oxidase results in interruption of electron transport chain and the oxidative phosphorylation. Resultant anaerobic metabolism with severely decreased Adenosine triphosphate (ATP) generation and concomitant increase in tactic acid production eventually leads tissue hypoxia and metabolic acidosis. The inhibitory properties of cyanide may be ascribed to its ability to complex with metals.

Besides, iron containing cytochrome oxidase, there are other metallo-enzymes containing molybdenum, zinc or copper which are equally sensitive to cyanide. Other mechanism for cyanide inhibition may be attributed to its affinity to Schiff base intermediates, e.g ribulose diphosphate carboxylase and 2-keto-4-hydroxyl glutarate aldolase involving formation of cyanohydrins intermediate. Therefore, cyanide toxicity may not be attributed solely to a single biochemical lesion but a complex phenomenon.

Cyanide Level in Cassava Cultivar

The different cultivars of cassava can be distinguished by such features as size, color and shape of the leaves, stem and petiole, blanching habit, plant height, tuber and amount of the root tuber produced per plant. The nutritive content of the tuber, the resistance to certain diseases and weed, the climatic and nutritional requirement, such as fertilizers for maximum yield of the plant, sweet or bitter depending on the level of cyanide content (Nweke *et al*.., 1999; RMRDC, 2004). The pulp of most cultivars varies from white to yellow (Booth *et al*.., 1976).

Cassava due to the presence of cyanogenic glycoside is potentially toxic with the exception of the seeds. The cyanogenic glycosides LINAMARIN (95%) and LOTAUSTRALIN (5%) are present in all part of natural cassava plant(Conn, 2000). Lotaustralin (ethyl linamarin) is located in the plant cell vacuoles, and enzyme linamarase is located in the cell wall (Jorgensen, 2005). The cyanogenic glycosides and corresponding hydrolytic enzymes (beta glycosidase) are brought together when the cell structures of the plant is disrupted by predators, leading to subsequent breakdown to sugar and a cyanohydrins, the unstable cyanohydrins under natural condition rapidly decomposes hydrogen cyanide(HCN) and ketones.

Alkaline Titration Method of Cyanide Determination

This method was previously used by Onyesom *et al.* (2008) in determining the levels of cyanide in cassava fermented with lemon grass (cymbopogoncitratus). The cyanide content of each sample was determined using the alkaline titration method (AOAC, 1990). 5% potassium iodide was used as indicator and the end point of the titration was reached when the solution changed from a clear solution to a faint turbid solution. The amount of cyanide in the sample was determined from the relation: $1cm^3$ 0.02M $AgNO_3$ =1.08mg HCN.

This is one of the most popular cyanide measured methods used in gold extraction industries. It measures free cyanide when dominant ion is CN^- more appropriately termed as "titratable cyanide" rather than free cyanide (Heath *et al..,* 1999). The method involves the addition of silver ions to the solution to complex with the "free cyanide". When all the free cyanide is consumed as silver cyanide complex, the excess silver ions indicate the end point of the titration. The end point is indicated by either color or potential. The easiest possibility is to use an indicator such as potassium iodide or p-dimethyl amino-benzal-rhodanine that changes color upon appearance of free silver ions. The colour observed is due to formation of diammine silver (II) complex (Courtney, 2008).

The three methods from the two techniques, colorimetric (picrate paper and picric in solution methods) and titration are the most commonly used in determination of cyanide.

Materials and Methods

Sample preparation

Cassava samples were randomly brought from different sellers in popular markets in Zaria. The apples were sliced open and the seeds removed and rinse with water and pounded to smaller size. The pounded seeds were put in tightly sealed envelopes and kept in field cellophane bags prior to analysis. The cassava sample was peeled, sliced and pounded. A portion was oven dried at 100˚c, the other was air dried at room temperature and the third portion kept in a cellophane bag prior to analysis. All reagent used were of analytical grade and were prepared as follows;

Preparation of 5% potassium iodide

The solution (5%) of KI was prepared by weighing 10g of KI and dissolving it in a 200ml volumetric flask using distilled water to the mark.

Preparation of 0.02M $AgNO_3$

Silver nitrate (0.02M) was prepared by weighing 0.85g of $AgNO_3$ into a 250 ml volumetric flask and dissolved using distilled water to the mark.

Preparation of 25% NaOH

This was prepared by weighing 83.3g of NaOH and dissolving it in a 250 ml volumetric flask to the mark.

Analysis of Total HCN

Total HCN (ppm) in the sample was analyzed using the alkaline titration method as obtained from AOAC (1990). 5g of grounded sample was soaked in a mixture of distilled water (50 ml) and orthorphosphoric acid. The samples were each thoroughly mixed and stored then left at room temperature overnight. This was done to set free all bounded hydrocyanic acid. The resulting sample (mixture) was then transferred into distillation flask and a drop of paraffin (antifoaming agent) was added to the cassava samples. The flask was then fitted to other distillation apparatus and distilled. About 50ml of the distillate was collected in the receiving flask containing 4ml of distilled water and 0.1g of Sodium Hydroxide pellets. The distillate was then transferred into 50ml volumetric flask and make up to mark with distilled water, 1.6 ml of 5 % Potassium Iodide was added and titrated against 0.02M $AgNO_3$.

Results

The results obtained from the analysis of cyanide using alkaline titration are shown in tables below. Table 4.1 shows the mass of each sample used for the titration analysis which was carried out in triplicate. The table also reveals the titrant value obtained for each of the sample triplicate analysis.

Table 4.1: Titrant values for the titration analysis.

Sample	Trial	Mass of sample (g)	Titre value (ml)
	1st	5.00	0.96
Air Dried Cassava	2nd	5.00	0.92
	3rd	5.00	0.94
Oven-Dried Cassava	1st	5.00	0.90
	2nd	5.00	1.10
	3rd	5.00	0.90
	1st	5.00	0.60
Undry Cassava	2nd	5.00	0.60
	3rd	5.00	0.50
	1st	5.00	0.69
Apple Seed	2nd	5.00	0.63
	3rd	5.00	0.67

Table 4.2: shows the total hydrogen cyanide contained in each sample analyzed. The result was obtained from the titre value in table 4.1 and multiply by 13.5(a constant) and divided by the mass of the sample used.

Table 4.2: Cyanide content (ppm) in analyzed samples

Sample	Trial 1	**2**	**3**
Air dried	2.59	2.48	2.54
Oven dried	2.43	2.97	2.43
Un-dry cassava	1.62	1.62	1.35
Fresh apple seed	2.03	1.83	1.97

Table 4.3: Concentration of cyanide in cassava and apple seeds

Sample	Cyanide content (ppm)
Air Dried Cassava	2.54±0.05
Oven Dried Cassava	2.61±0.31
Un-Dry Cassava	1.53±0.16
Fresh Apple Seeds	1.94±0.09

Table 4.3 reveals the average total hydrogen cyanide in the different dry cassava, fresh cassava and apple seeds. Also, the table showed the standard deviation which is expressed in plus or minus (±).

Discussion

Table 4.1 shows the titrant values of each triplicate sample analyzed by alkaline titration. For each sample of cassava, 5g was weighed and analyze three times. For air-dried cassava sample, the titrant volumes used were 0.96ml, 0.92ml and 0.94ml; the average used titrant volume for air-dried cassava was 0.94ml. For oven-dried cassava sample, the titrant volumes used were 0.90ml, 1.10ml, and 0.90ml; the average used titrant volume for oven-dried cassava sample was 0.96ml. For the un-dried cassava sample, the titrant volumes used were 0.60ml, 0.60ml, and 0.50ml; the average used titrant volume for un-dry cassava sample was 0.56ml. From the three processed cassava samples, the oven-dried sample used higher titrant volume of 0.96ml which is similar to those reported by Famurewa and Emuekele (2014). The un-dry cassava sample used the lowest volume of titrant which was 0.56ml similar to those reported by Nwokoro *et al*.(2010). This result shows that in alkaline titration, samples that are dried will use moretitrant solution than fresh or un-dried sample. For fresh apple seed, the volumes of titrant used were 0.64ml, 0.58ml, and 0.62ml. The average volume of titrant used by apple seed was 0.61ml. The result for the fresh apple seed and the fresh cassava for volume of titrant solution used were similar which shows that samples with high moisture will require lesser amount of titrant solution for titration.

Table 4.2 shows the cyanide content in each triplicate sample. The cyanide content in the air-dried cassava samples were 2.59ppm, 2.48ppm and 2.50ppm. The cyanide content in each triplicate oven-dried sample were 2.43ppm, 2.97ppm, and 2.43ppm. The cyanide content in each triplicate un-dry cassava sample were 1.62ppm, 1.62ppm and 1.35ppm. The cyanide content in the fresh cassava sample were 1.87ppm, 1.70ppm, and 1.82ppm. The lowest cyanide content in table 4.2 is 1.35 in the un-dry cassava sample, this is because the moisture present in the sample is high and it used the lowest volume of titrant solution. The highest cyanide content in table 4.2 is 2.97ppm in the oven-dried cassava sample. This is because the moisture present in oven-dried cassava sample is low and it used the highest volume of titrant solution. In table 4.2, when the cyanide content in both fresh cassava sample and fresh apple seed sample are compared, the cyanide content in the apple seed is higher than that in the cassava sample, this shows that apple seeds contain more cyanide than cassava.

Table 4.3 shows the average concentration of cyanide present in all samples analyzed. The average concentration of cyanide were 2.54ppm in air-dried cassava sample, 2.61ppm in oven-dried cassava sample, 1.53ppm in un-dry cassava sample and 1.80ppm in fresh apple seed sample. The highest value of 2.61ppm and lowest value of 1.53ppm for cyanide concentration present in cassava agrees with the report of Fawurewa and Emuekele (2014) but slightly disagree with Nwokoro *et al* (2010)report. The result also reveals that when cassava and apple seed are been consumed, the cyanide consumption will be higher in apple seeds. Also, processing cassava in terms on increasing the moisture content like boiling before consuming it will further reduce the cyanide concentration significantly as seen in fresh cassava (1.53ppm) and in oven dry cassava (2.61ppm). The WHO has set a safe level of cyanogens in cassava as 10ppm and all samples analyzed in the study are acceptable value of cyanide consumption 9FAO/WHO, 1991). From the result of this study, it shows that the higher the moisture content of cassava the greater the loss in the cyanide content drying which was seen in both the dried and un-dry cassava samples. Also, cutting of cassava tubers into smaller sizes can create easy access for contact between the enzymes and cyanogenic glycosides resulting in higher hydrolysis (Tivana and Bvochora, 2005). This research help to show that though cassava contains cyanide in most species, the edible specie found in Zaria falls under the World Health Organizations regulated/allowable standard of cyanide in cassava. Although the cyanide levels of the test samples employed in this study are within the acceptable level recommended by FAO, but epidemiological studies have shown that exposure to small doses given over a long period of time can result in increased blood cyanide levels with the following symptoms: dizziness, headache, nausea and vomiting, rapid breathing, restlessness, weakness and even in severe cases paralysis, nerve weakness and even in severe cases paralysis, nerve lesions, hypothyroidism and miscarriage (Soto-Blanko *et al.,* 2002; Rachinger *et al.,* 2002).

Cyanide is a chemical compound that contains the group carbon triple bonded to nitrogen. Cyanide has several sources which includes all species of cassava and apple seeds. Cyanide in humans is a well-known poison with potential acute and chronic metabolic effects. Cassava samples were randomly bought from different sellers in popular Zaria market while apple were bought from fruit market in Zaria. The two samples were prepared prior to the determination of their cyanide concentration by alkaline titration. The results showed that all the samples studied contained different concentration of cyanide ranging from 2.61ppm in oven dried cassava sample to 1.53ppm in un-dry cassava sample as well as 1.94ppm in apple seeds.

Food Safety Focus (19th Issue, February 2008) stated that in humans, the clinical signs of acute cyanide intoxication include rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pain, vomiting, diarrhoea, mental confusion, twitching and convulsions. Death due to cyanide poisoning can occur when the cyanide level exceeds the limit an individual is able to detoxify. The acute lethal dose of hydrogen cyanide for humans is reported to be 0.5 to 3.5 mg per kilogram of body weight. Children are particularly at risk because of their smaller body size. Chronic cyanide intoxication may lead to the development of certain conditions including disturbance of thyroid function and neurological disorders. It tends to affect those individuals who have regular long-term consumption of cassava with poor nutrition status. This indicates that consumption of 6 – 7 seeds of apple seeds is enough to poison a child of 5 – 10 kg weight.

Conclusion

Although, all the cassava samples and apple seeds sample studied contained cyanide, the cyanide content present in all the studied samples is within the acceptable safe level or concentration for human consumption, therefore, consumers of cassava and apple in the right quantity are not at risk of cyanide poisoning or other serious health implications. This study concludes by this assertion that processing food containing cyanide like cassava before consuming them will significantly reduce the cyanide present in the food as the processing will create easy access for contact between enzymes and cyanogenic glycosides resulting in higher hydrolysis thereby loss in cyanide will occur. And consumption of apple seed avoided.

Recommendation

The following recommendations were drawn from this study, they include;

(1) As this study has reveal that high quantity of cyanide in human is poisonous, it is therefore recommended that food especially those containing cyanide like cassava should be further process before eating to significantly reduce cyanide consumption.
(2) This study recommends that other sources through which cyanide can enter human system be study and preventive strategy be given
(3) It also recommends that consumption of unprocessed cassava and apple seed be reduced or avoided completely due to its cyanide content.

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