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Research Article

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Evaluation of Local Processing of Tapioca and its Effect on the Starch and Cyanide Contents

Marcus AC, Kpee F

Department of Chemistry, Ignatius Ajuru University of Education, P.M.B. 5047, Rumuolumeni, Port Harcourt, Nigeria

Abstract Tapioca constitutes one of the staple foods prepared in different forms for consumption by many African dwellers, including Nigeria. It is a processed food from *Manihot esculenta* (cassava), rich in carbohydrate and contains linamarin that produces the toxic compound, hydrogen cyanide (HCN). Samples were collected from six selected areas: Bua-Yeghe, Sogho, Ebubu, Igbodo, Baa-Goi and Eneka in Rivers State to determine the starch and cyanide contents of the unprocessed cassava and tapioca, using the picrate and spectrophotometric methods respectively. The results of detectable values (%) for starch were: 4.65±0.74, 6.30±0.70, 3.80±0.43, 2.63±0.34, 5.81±0.57 and 6.29±0.60, while cyanide concentrations (mg/Kg) were: 3.20±0.43, 7.54±0.50, 5.57±0.55, 1.76±0.36, 11.31±0.92 and 10.31±1.06 respectively. The unprocessed *M. esculenta* gave 18.56±2.64 and 27.78±4.26 for starch and cyanide respectively. There was significant variation ($p<0.05$) between the processed and the unprocessed forms, implicating sources other than linamarin as the only probable source of cyanide. The results obtained from the unprocessed cassava which showed that cyanide concentrations were higher than the 10 mg HCN per kilogram as recommended by FAO/WHO was expected, but those from tapioca fell below the threshold limit, except those from Baa-Goi and Eneka, which are slightly above the international standard, signifying possible exposure of consumers of the food to toxicity. The study also noted differences (%) in starch and cyanide contents of the unprocessed cassava and tapioca, which ranged from 64.25-94.24% for cyanide and 62.15-88.41% for starch at the various sample sites as the extraction of starch and cyanide contents continued each day, following soaking, for five days. The study concluded that the processing method employed could be considered effective in reducing cyanide in tapioca, but may grossly affect the taste of tapioca and the dietary requirement for carbohydrate for such a meal.

Keywords evaluation, local processing, tapioca, starch, cyanide

Introduction

Tapioca is one of the processed foods of the bitter *Manihot esculenta* (cassava) in Nigeria and Africa in general. Locally, it is prepared and eaten as *abacha* with coconut, groundnut or any other suitable compliment by the lgbos; sometimes, it is mixed with the sliced processed form of oil bean seed (*ugba*) as *African salad* in some quarters. In other places such as Okrika, Kalabari, and even Bayelsa, a local delicacy of it is prepared with ripe plantain, blended with red oil, and eaten with dry or smoked fish as *apalapa* or as *pikiri-ijapu* mixed with well blended crayfish and red oil.

The local method of processing it for consumption is in most cases an attempt to reduce hydrogen cyanide, a toxic substance that is produced by a cyanogenic glucoside, known as linamarin in cassava. Of course, those who eat tapioca as a staple food can attest to the fact that this method is aimed at not only reducing hydrogen cyanide but also improves storability, convenience and palatability [1].

A number of studies have shown that a wide variety of traditional cassava processing methods which include maceration, soaking, boiling, roasting, fermentation, drying of cassava tubers, or combination of these processes partially remove cyanogenic glycosides during processing. However, these methods tend to leave some residual quantities which are responsible for the chronictoxicity associated with continued consumption of processed foods from cassava [2].

Consuming cassava roots and leaves raw therefore is of concern because the cyanogenic glucoside, linamarin and lotaustralin are decomposed by linamarase, a naturally-occurring enzyme in cassava, liberating hydrogen cyanide [3-5].

The raw cassava can contain about 130-200mg HCN/kg [6]. However, the hydrolysis of cyanogenic glucosides to hydrocyanic acid by the endogenous enzyme linamarase is possible when the plant tissues are damaged during harvesting or processing [7]. Fermentation process has been reported to enhance detoxification of cassava through the liberation of hydrocyanic acid. More so, this detoxification, process also leads to development of flavor, which also improves the quality of the final product [1, 8-9].

Hydrocyanic acid is an effective inhibitor of many metalloenzymes, with cytochrome oxidase, the terminal oxidase of aerobic organisms as the principal site of action. This in many cases results in cyanide poisoning and eventual death due to oxygen starvation at the cellular level [4].

Acute cyanide intoxication has shown symptoms such as vertigo, vomiting and collapse, about four hours after ingesting raw or poorly processed cassava. The mortality of the victim within a few hours is also an inevitable result in some cases. However, with an injection of thiosulfate, which makes sulfur available to detoxify victim's body by converting the poisonous cyanide into thiocyanate, treatment may be achieved [10].

Chronic or low-level cyanide exposure is associated with the development of goitre and with tropical ataxic neuropathy, a nerve-damaging disorder that renders a person unsteady and uncoordinated. Severe cyanide poisoning, particularly during famines, is associated with outbreaks of a debilitating, irreversible paralytic disorder called konzo and, in some cases, death. The incidence of konzo and tropical ataxic neuropathy can be as high as 3% in some areas according to Wagner (2010) [11]. In 2011, FAO reported that, brief soaking (four hours) of cassava is not sufficient, but soaking for 18–24 hours can remove up to half the level of cyanide and that drying may not be sufficient, either.

The question is how effective can any of the processing methods be without affecting the dietary requirement for starch (carbohydrate) in a tapioca meal? The paper therefore is an assessment of the local method of the processing of tapioca and the effect this method could have on the taste, starch and cyanide contents as well as comparing these with those of the original cassava tuber in order to draw plausible conclusion on the efficacy of the method.

Materials and Methods

Sample Collection and Preparation

Fresh tubers of *M. esculenta* were purchased upon being harvested from local farmers at selected sample locations in Rivers State namely: Bua-Yeghe, in Gokana Local Government Area; Sogho, in Khana Local Government Area; Ebubu, in Eleme Local Government Area; Igbodo, in Etche Local Government Area; Baa-Goi, in Tai Local Government Area, and Eneka, in Obio-Akpor Local Government Area. Farming activities are prevalent in these areas. Samples were transported to Ignatius Ajuru Research Laboratory of Department of Chemistry for preparation and analysis. The cassava tubers were cleaned and peeled using a stainless knife and later washed with clean water before analysis.

Preparation of Alkaline Picrate Solution

Twenty five grams of anhydrous sodium carbonate and 5*g* of anhydrous picric acid were added to a 1- *litre* volumetric flask. The mixture was dissolved in a minimal amount of warm distilled water and the solution was made up to the mark with cold distilled water. The alkaline picrate method as described by other authors [12-14] was used.

Construction of a Standard Curve for Cyanide Assay Using Alkaline Picrate Method

A sample of potassium cyanide to be used as standard was first dried in the oven to constant weight. A stock solution was prepared by dissolving 8*mg* of this salt in 100 *ml* of distilled water. This gives a concentration of 32 μg CN^-/ml. From this stock solution, a series of 10 *ml*-plastic stoppered test tubes containing from 3.2-64 μg of cyanide was set up. The volume of each was made up to 2 *ml* with distilled water and 4 *ml* of alkaline picrate added and mixed. The resulting solution was incubated in a water bath at 95 *ºC* for 5 minutes. Upon cooling to room temperature, the absorbance was taken at 490 *nm* of the deep orange color formed was read. The absorbance at 490 *nm* was plotted against cyanide concentration.

Determination of Hydrogen Cyanide in the Unprocessed *M. esculenta*

Five grams (5*g*) of each cassava variety was blended into a paste and dissolved in 50 *ml* of distilled water in a conical flask and allowed to stay overnight before filtering. Two (2)*ml* of the filtrate was poured into another conical flask and 4 *ml* alkaline picrate solution was added and the content incubated in a waterbath for 5 minutes for colour change (reddish-brown) and absorbance taken at 450 *nm*. The blank and standard test (which serves as control) was prepared using 1 *ml* distilled water and 4 *ml* alkaline picrate solution. The cyanide content was extrapolated using a cyanide standard curve.

Determination of Starch Content of the Unprocessed *M. esculenta*

The percentage of starch content was determined using spectrophotometric method. About 2.5 *g* of ground fresh cassava tubers was dissolved in 50 *ml* of cold water and allowed to stand for an hour. Twenty (20)*ml* of HCl and 150 *ml* of distilled water were added to the sample and refluxed for 2 hours in a round bottom flask.

This was later cooled and neutralized with 0.5 *N* NaOH. The content was used for glucose determination using anthrone reagent. Series of glucose solution was prepared containing 0 *ppm*, 2 *ppm*, up to 10 *ppm* to calibrate the glucose standard concentration. Five (5)*ml* anthrone reagent was added to each of the standard and test sample and boiled in a waterbath for 20 minutes for colour development. The test tubes were cooled and absorbance was taken at 620 *nm* against a blank containing only 1 *ml* of water and 5*ml* of anthrone reagent.

Preparation and Determination of Starch and Cyanide contents of Tapioca

For the determination of starch and cyanide in tapioca, the fresh cassava tubers having been peeled and washed with clean water were boiled for between 30-40 minutes. The boiled cassava was allowed to cool for about 10 minutes after which it was sliced into smaller strands. At this stage, it was believed to contain a high level of hydrocyanic acid (HCN), a toxic substance produced by linamarin in cassava, and so, had to be soaked overnight, presumably to extract some of the hydrocyanide acid before consumption. This was the rationale behind this research. The tapioca strands were soaked to monitor the levels of starch and cyanide in it each day for the five (5) days.

Five grams (5*g*) of each sample were weighed using weighing balance into a conical flask and 50*ml* of distilled water was added and corked. This was allowed to stay overnight and then filtered. The extract, which was taken in four hourly replicates per day, was used for cyanide and starch determination, following the procedures already described above.

Statistical Analysis

Means and standard deviations were calculated to represent the actual value of each parameter. Since samples were collected from six areas in Rivers State, a Single Factor Analysis of Variance (ANOVA) on the platform of Microsoft Excel, 2007, was conducted.

Results and Discussion

Results

The results obtained from the six locations within Gokana, Khana, Eleme, Etche, Tai and Obio-Akpo Local Government Areas of Rivers State, for starch and cyanide contents of unprocessed Manihot esculenta (cassava) and tapioca, a processed form of it, are presented in Tables 1 and 2 respectively.

Table 1: Results of Starch (%) and Cyanide (mg/kg) Contents of Unprocessed *Manihot esculenta* from six locations

Parameter	Location						$\bar{x}\pm$ SD
	Bua-Yeghe	**Sogho**	**Ebubu**	**Igbodo**	**Baa-Goi**	**Eneka**	
Starch	15.59	18.48	20.62	22.70	17.29	16.62	18.56 ± 2.64
Cyanide	20.65	29.55	24.86	30.57	32.18	28.84	27.78 ± 4.26

NB: $\bar{x}\pm$ SD are Mean and Standard Deviation

Table 2: Results of Starch (%) and Cyanide (mg/kg) Contents of Tapioca (Processed *Manihot esculenta*) from six locations

Parameter	Day/Location					$\bar{x}\pm$ SD
	1	**2**	**3**	**4**	**5**	
			Bua-Yeghe			
Starch	5.45	5.10	4.90	4.20	3.60	4.65 ± 0.74
Cyanide	3.80	3.45	3.10	2.95	2.70	3.20 ± 0.43
			Sogho			
Starch	7.20	6.90	6.40	5.70	5.30	6.30 ± 0.80
Cyanide	8.10	7.90	7.65	7.10	6.95	7.54 ± 0.50
			Ebubu			
Starch	4.35	4.00	3.90	3.60	3.20	3.80 ± 0.43
Cyanide	6.30	5.90	5.55	5.20	4.90	5.57 ± 0.55
			Igbodo			
Starch	3.10	2.80	2.60	2.45	2.20	2.63 ± 0.34
Cyanide	2.20	2.00	1.75	1.55	1.30	1.76 ± 0.36
			Baa-Goi			
Starch	6.55	6.10	5.90	5.40	4.10	5.81 ± 0.57
Cyanide	12.30	11.90	11.35	11.10	9.90	11.31 ± 0.92
			Eneka			
Starch	7.10	6.60	6.30	5.90	5.55	6.29 ± 0.62
Cyanide	11.62	10.95	10.50	9.40	9.10	10.31 ± 1.06

NB: $\bar{x}\pm$ SD are Mean and Standard Deviation

Discussion

The study was aimed at evaluating the local processing of tapioca by analyzing the starch and cyanide contents of tapioca, a processed food of *Manihot esculenta* (cassava) and the unprocessed form of it by collecting samples from selected areas in Rivers State. There seems to be inverse relationship in the pattern of extraction between the values obtained for starch and cyanide in the unprocessed *M. esculenta*, and this was replicated in tapioca in Bua-Yeghe and Igbodo. Sogho, Ebubu, Baa-Goi and Eneka showed a reverse trend (Tables 1 and 2). This can be explained on the basis of the factors other than linamarin is presumed to be the precursor of the toxic cyanide in cassava.

It has been reported that disposal from cassava processing waste into the soil contaminate soils, and because HCN dissolves in effluents and remains in solution, when it enters the soil, part of the cyanogenic glucoside remain unconverted by microorganism between the few enzymes present in cassava fiber which are not enough for complete conversion [15].

Apart from emissions from major uses of cyanide compounds and cyanide production [16], another area of principal concern is that related to hydrocracking and the Fluid Catalytic Cracking processes of the petroleum industry. In such activities that are not alien to Rivers State, where the present study was carried out, cyanide induced corrosion had been identified during turn-around maintenance of the catalytic cracking units of some refineries that could be traced to cyanide in cassava of contaminated environments.

It was further reported that, NH_3, HCN and other nitrogen compounds are inevitable by-products during the cracking of organic nitrogen compounds in Petroleum feedstock [17]. These findings are in agreement with the single factor analysis of variance which showed that there was no significant variation ($p<0.05$) in the values obtained for both parameters in all the samples collected from the different sampled areas which further implies that the production of cyanide in all the samples collected may have been influenced by factors other than linamarin.

The essence of analyzing the unprocessed cassava was to know how much of the starch and cyanide that may have been extracted from the cassava after being processed to tapioca; this would enable plausible conclusion to be drawn on the efficacy of the method.

Table 3 presents the percentage differences between values obtained from the unprocessed cassava and those from tapioca.

Table 3: Differences (%) in Starch and Cyanide Contents of the Unprocessed *Manihot esculenta* and Tapioca at the six Sampled Areas after Extraction

Sample Areas	Unprocessed *M. esculenta*		Tapioca		Differences (%) after Extraction	
	Starch	Cyanide	Starch	Cyanide	Starch	Cyanide
Bua-Yeghe	15.59	20.65	4.65	3.20	70.17	84.50
Sogho	18.48	29.55	6.30	7.54	65.90	74.48
Ebubu	20.62	24.86	3.80	5.57	81.57	77.59
Igbodo	22.70	30.57	2.63	1.76	88.41	94.24
Baa-Goi	17.29	32.18	5.81	11.31	66.40	64.85
Eneka	16.62	28.84	6.29	10.31	62.15	64.25

The reduction of cyanide ranged from 64.25-94.24%, while that of starch was from 62.15-88.41% as indicated in Table 3. Similar percentage differences of about 90% have also been reported in Burundi in a study on identification of highly effective procedures that reduce the cyanogens contained in cassava roots which require no sophisticated equipment [1].

The implication is that the processing method employed, was appreciably effective in reducing cyanide, but could however affect the taste of tapioca and perhaps the dietary requirement for carbohydrate in such a meal for palatability [9]. This calls for more effective method that will not only reduce the toxic compound to tolerable limits, but also seek to retain the food value in terms of its starch (carbohydrate) content.

The results of starch and cyanide contents of the unprocessed cassava are presented in table 1. The percentage of starch ranged from 15.59-32.18% with a mean value of 16.60±2.24. The highest percentage of these was obtained from Baa-Goi, while the least came from Bua-Yeghe. The concentrations of cyanide (mgHCN/Kg) followed a similar trend with a range of 20.65-32.18, having the highest (32.18) and the least (20.65) also at Bua-Yeghe and Baa-Goi respectively. The mean was 27.78±4.26mgHCN/Kg.

These results are comparable to a recent similar study conducted on fresh tubers of cassava in Abia State by Ezeigbo *et al*. [18] that reported 18.59±0.17 % for starch and36.65 ± 0.16 mgHCN/Kg for cyanide. The study conducted in Burundi [1] is however, significantly higher than the results of the present study with values as high as 275 ± 24 mgHCN/Kg for cyanide.

The two parameters starch and cyanide were measured in tapioca for five (5) days. Their distributions are shown in Table 2. The highest mean percentage (6.30±0.80 %) for starch was obtained from Sogho, while the least (2.63±0.34 %) came from Igbodo. The cyanide concentration on the other hand, was 11.31±0.92 mgHCN/Kg as highest at Baa-Goi, while the lowest concentration of 1.76±0.36 mgHCN/Kg was also from Igbodo. These results are significantly higher than those obtained from similar studies conducted for gari, also a processed food of cassava in Owerri by Kamalu & Oghome [19], who reported ranges of 1.75-7.70% for starch and 0.031-0.064 mgHCN/Kg for cyanide.

Apart from the results obtained from Baa-Goi and Eneka, which have mean concentrations 11.31±0.92 mgHCN/Kg and 10.31±1.06 mgHCN/Kg respectively, which are slightly above the 10 mg HCN equivalent per kilogram according to FAO/WHO amendment [20], all other results are within allowable limits.

It is interesting to note that the pattern of distribution is a downward trend from first day to the last in all cases (Tables 1 and 2). This was because all the measurements were taken after extraction in water, and so, as the number of days increased the values also decreased. This is in good agreement with the report that brief soaking (four hours) of cassava is not sufficient, but soaking for 18–24 hours can remove up to half the level of cyanide and that drying may not be sufficient either [10]. The study has therefore shown that this traditional or local method of processing cassava to foods such as tapioca is not out of place as shown in all cases (Table 3).

Hydrogen cyanide is responsible for the toxicity of cassava to humans when inadequately processed. Therefore, traditional processing methods should be aimed at reducing cyanide and improving storability, convenience and palatability.

It is believed that boiling in the present study which was also part of the processing to tapioca has helped in reducing the cyanide, judging from the analysis in Table 3. However, from the results of Baa-Goi and Eneka, where even the values in the unprocessed cassava are similar to others, the cyanide concentrations in tapioca were still high and slightly above the threshold limits except on the fifth day in both cases. This gives evidence to the report that boiling may have played an important role in reducing this toxicant, but may not be very effective in doing so, according to Machungu *et al*. [21].

The toxicity of individual cyanide compounds is dependent on the ease with which they release cyanide (CN^-) anion. For example, cyanide radicals have a low affinity for alkali metals and a high affinity for ferric iron (Fe^{3+}) and other metals; therefore, simple cyanide salts (for example, sodium cyanide or potassium cyanide) are toxic, whereas certain iron-containing cyanide compounds do not release CN^- readily and are nearly nontoxic.

Cyanide exerts its primary toxicological effects by binding to the metallic cofactor in metalloenzymes, thereby impairing enzyme and cell function. Cytochrome C oxidase (an enzyme in the mitochondrial respiratory chain) is the most significant target of cyanide exposure since its inhibition prevents tissues from using oxygen [22].

The different content values of the two parameters considered in the present study may be attributed to varying exposure of the food material to different environments from which the samples were collected. Although, this study may not authoritatively claim to concur with the method employed to reduce the cyanide contents of tapioca, the results seem to give credence to what is already being practiced by the traditionalists that see this food as a staple.

There is however, need to warn as has been noted by the study, that, as soaking continued to presumably reduce the cyanide concentration, the starch content was also being depleted; efforts therefore should be made to ensure that the percentage required for daily dietary consumption is retained.

Conclusion

The processing of *M. esculenta* to tapioca seemed to have given credence to the local method that was employed for this study as the cyanide concentration continued to decrease following prolonged soaking each day for five days. The concentrations of cyanide were below permissible levels except for those obtained from Baa-Goi and Eneka. The study, nevertheless, noted that even though by this method, cyanide was reduced below the permissible levels, the continuous consumption of food contaminated with cyanide even at concentrations lower than the threshold limits may lead to serious health problems. Moreover, the continuous extraction of the starch along with cyanide, may potentially affect the taste and the dietary carbohydrate requirement for a normal tapioca meal.

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