RESIDUAL CYANIDE CONTENT OF PROCESSED CASSAVA-BASED PRODUCTS IN GUYANA

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ABSTRACT

Cassava products are emerging as sustainable, economical solutions to food security in developing countries. The tuber is known to contain anti-nutritional compounds, notably cyanogenic glycosides, but may be eaten without ill effect once properly prepared. It is processed to make value-added products like cassava bread, farine, tapioca and cassava flour. The CARICOM Regional Standard – "Specification for Cassava bread" (CRS 19) recommends that the maximum level of cyanide in food for human consumption should be 10 mg/kg dry weight. In this study, cassava flour, cassava bread, farine and tapioca were analyzed for total cyanide content utilizing the picrate paper method. The cyanide levels in the farine and tapioca samples, one cassava flour sample and the garlic-flavored cassava bread sample fell within the acceptable range. Cyanide levels in the remaining products were above 10ppm. These findings suggest that cyanide levels are dependent on processing techniques, processing times and the presence of additives.

KEYWORDS

cassava bread, farine, tapioca, picrate paper method, & cyanogenic glycoside

1. INTRODUCTION

Cassava, *Manihot esculenta* is a perennial shrub, which produces enlarged tuberous roots [1]. Cassava is one of at least 2500 species of plants that produce cyanogenic glycosides. These compounds break down to form hydrogen cyanide which can result in both acute and chronic toxicity in humans. Varieties of cassava can be classified into two broad groups, bitter or sweet, based on the level of cyanide in the plant tissues. Sweet varieties of cassava typically contain less than 50 mg/kg hydrogen cyanide on a fresh weight basis while the bitter varieties contain more than 50 mg/kg [2]. In Guyana, cassava has always been the main staple of the Indigenous people, but its use has transcended ethnicity; cassava and cassava-based products are consumed by all sections of society. There are more than 30 varieties of cassava, both sweet and bitter, cultivated in Guyana [3]. Cassava is grown by smallholder farmers in all ten (10) administrative regions of Guyana on different soil types [4]. Some local cassava products include cassava bread, cassava pone, cassava ball, farine, quinches, cassava flour, cassava puff, tapioca, cassareep and a wide variety of fermented beverages. Of these products, cassava bread and cassava flour are the most utilized and commercially available. The indigenous people of Guyana traditionally prepare cassava bread by first peeling, washing, and grating the tuber. The grated pulp is then placed into a long, woven sieve called a matapee and the juice is squeezed out of the grated cassava. The low moisture, grated cassava is placed on a flat metal sheet over a fire to cook. It is flipped to cook the upper side and then placed out in the sun to dry. Small scale manufacturers usually have an additional stage where the low moisture, grated cassava is sifted before being baked. Grating ruptures the plant cells releasing the enzyme linamarase which converts cyanogenic glycoside to hydrogen cyanide and sugar. The baking and sun-drying processes reduce the level of volatile hydrogen cyanide that remains in the product.

Traditionally, cassava flour can be produced from washed, peeled roots that are grated, chipped or sliced, then sun dried on trays and finally milled into flour [5]. Alternatively, the roots may be peeled, washed, oven dried, milled and sieved [6]. The grating and heating processes reduce the cyanide content in the same way as occurs during the production of cassava bread.

The Food and Agricultural Organization of the World Health Organization (FAO/WHO) in 1991 recommended that the maximum level of cyanide present in food for human consumption is 10 mg/kg DW or 10ppm [7]. The ability of food processors and manufacturers to meet this standard depends both on the cyanide content of the cassava cultivar being utilized and the processing techniques being employed. In 2012, Chand studied the effects of processing on the cyanide content of cassava products in Fiji and compared the levels to those accepted by Codex Alimentarius (10 mg/kg) [8]. The findings of this study showed that grated products had the lowest total cyanide levels, less than 10 mg/kg and the fried products had the highest total cyanide levels. This was attributed to the low solubility of cyanogenic glycosides in oil.

Cyanogenesis is the ability of living organisms to liberate hydrogen cyanide from stored cyanogenic glycosides, cyanogenic lipids or cyanohydrins by tissue damage and hydrolysis and/or decomposition [9]. This characteristic is utilized during the processing of the cassava tuber which includes soaking, boiling, baking, drying, frying, steaming and fermenting. In many preparations a combination of these processes is used. However, Montagnac and others in 2009 noted that regardless of the processing technique, or combination of processing techniques used, there is still residual cyanide [10]. They also found that the effectiveness of the techniques at reducing cyanide levels depended on the processing time and whether single or multiple processing methods were used. Crushing, pounding and grating were found to be very effective techniques. This was highlighted by the observation that when the tubers were used to prepare flour following crushing and sun-drying; cyanide removal was 99%. Soaking and sun drying; and soaking and fermenting gave lower cyanide removal percentages.

In 2010, the Guyana National Bureau of Standards (GNBS) adopted the CARICOM Regional Standard – "Specification for Cassava bread" - CRS 19 [11]. The standard was applied to cassava bread and other cassava products obtained from the processing of bitter cassava and intended for human consumption. The standard includes procedures for sampling of cassava bread, the determination of moisture content and ash yield, and the detection of cyanogenic glycosides by the Picrate Paper method. This research focuses on the determination of residual cyanide content of processed cassava products available on local markets with a view to assessing product quality as it relates to residual cyanide.

As the global population continues to increase, and food security is threatened by changing global climates, there has been a move to focus on alternative staples and reduce reliance on wheat imports in tropical countries. Cassava is considered a good alternative for several reasons; it has high starch content (high caloric value), it grows in poor soils and it is drought resistant[12]. In Guyana there have been several government-led initiatives, in partnership with the FAO, aimed at increasing cassava production. These include distribution of cultivars that are more resistant to diseases, funding for small farmers [13], establishment of cassava flour factories [14] and adoption of a regional standard for cassava bread and cassava products manufactured from bitter cassava [15]. However, because of its potential toxicity, processed cassava products must meet the minimum safety standards for consumption. Non-conformity has both public health and economic implications.

The hazardous effects of cyanide consumption to human health are well documented [16, 17]. This research is timely given that the local economy is now opening to many foreign markets. Consequently, value-added cassava-based products will have to adhere to both local and international standards before they can be exported to some foreign markets. One very important standard that must be met is the cyanide level. Additionally, in a bid to reduce revenue that is expended on the importation of wheat by developing countries, alternative sources of flour e.g., cassava, are being used to prepare flour composites for use in baked commodities [18].

This research aimed at investigating the residual cyanide contents of value-added cassava-based products – cassava bread, cassava flour, farine and tapioca and seeks to address the following questions

- Is there a difference in the residual cyanide content of the various cassava-based products?
- Is there a difference in the residual cyanide content of various brands of cassava-based products?
- Does the residual cyanide content of the cassava-based products change with time?
- What is the recommended daily intake for the cassava-based products?

2. METHOD

Comparative analysis of residual cyanide in cassava-based products - cassava bread, cassava flour, farine and tapioca - were carried out using spectrophotometry and picrate kit (Protocol B2) analyses adapted from Bradbury, Egan & Bradbury, 1999 [19].

2.1. Sample Collection

Samples for this research were obtained in triplicate from supermarkets in Georgetown, Guyana. For each product, all locally available brands were purchased. Samples were named based on the cassava product, for example, cassava bread 1 was named CB1, cassava flour 1, CF1 and farine 1, F1. Tapioca samples were labelled Ta.

Cassava bread – Four brands of cassava bread were bought from supermarkets in Georgetown.

Three packets of each brand were bought and stored at -18 ^{0}C in the freezer section of a refrigerator until time for analysis.

Cassava flour - Two brands of cassava flour were purchased and frozen until time for analysis. Cassava flour is available for retail purchase in packets of 450 grams mass.

Farine – Two brands of farine were bought from supermarkets in Georgetown. Three packets of each brand were purchased and stored in the freezer until time for analysis. Farine is available for retail purchase in packets of 450 grams mass.

Tapioca – Three packets of the only brand of tapioca available in supermarkets were purchased and stored in the freezer until time for analysis.

Samples were collected in this same manner three months later.

2.2. Equipment and Reagents

Picrate Kit

Precise electronic balance, flat bottom plastic bottles with screw caps, graduated 1mL pipettes, buffer/enzyme papers, yellow indicator papers, colour chart, pink standard paper

Spectrophotometer

UV/VIS Spectrophotometer; Agilent Technologies; Cary 60, Product No. G6860A, Serial No. MY17260006

Blender

250 Microns Black and Decker Cyclone 10-speed blender Distilled water.

2.3. Methods of Analyses

Picrate Kit

100 mg of finely ground sample and 1 mL of water were placed into airtight containers containing enzyme paper discs and yellow indicator paper just above the level of the liquid. The sample was then left to stand for 16–24 hrs. This experiment was done in triplicates for each sample. A positive and negative control were run with each sample. In the negative control the experiment was set up as described but without the cassava-based sample. While for the positive control, a pink standard paper disk is added in the place of the cassava sample. At the end of the stated period the bottles were opened and the colour of the indicator paper was matched against the shades of colour on the colour chart. The negative and positive control were also checked to ensure that they are zero and 50 ppm respectively.

Spectrophotometry

The plastic backing on the indicator paper in each sample was carefully removed and placed in separate test tubes to which 5.0 mL of water was added. They were left to stand for 30 minutes with occasional stirring. The absorbance of the solutions was then measured at 510 nm using a glass cuvette and the value of the negative control was subtracted from each sample reading.
The total cyanide content was then calculated by the equation:

Total Cyanide Content (ppm) = 396 x Absorbance

2.4. Analysis of Data

Analysis of this data was exploratory since the expected outcome is not known as it relates to the food items being studied in Guyana. The independent variable(s) were the type of cassava based product and the brand of cassava-based product. The dependent variable was the total cyanide content for each food product. These values are expressed as means ± SD. Sample size varied from three to fifteen dependent on the product. All data was analyzed using SPSS version 28 software. One-way ANOVA was used to test the null hypotheses for the products and brands. All tests were carried out at a 95% level of significance ($p \geq 0.05$). The hypotheses used were:

H_{0a} – Total residual cyanide content was the same in all cassava-based products.
H_{1a} – Total residual cyanide content was different for different cassava-based products.

H_{0b} – Total residual cyanide content was the same in all brands of a cassava-based product. H_{1b} – Total residual cyanide content was different for different brands of a cassava-based product.

3. RESULTS

3.1. Residual Cyanide Concentrations in Cassava-based Products

Residual cyanide concentration in the cassava-based products was found to be highest in cassava flour and lowest in tapioca; p = 0.000 (Table 1). The very high concentrations observed for cassava flour was due mainly to the contribution of a specific brand of cassava flour referred to as CF1, this brand of cassava flour had 45 times more residual cyanide than the other brand studied (Table 3).

Table 1. Mean residual cyanide concentration in cassava-based products

Cassava product	**Number of samples**	**Mean cyanide concentration (ppm)**
Cassava bread	26	15.5 ± 8.9^{a}
Cassava flour	12	78.1 ± 47.9^{b}
Farine	10	5.0 ± 1.2^{c}
Tapioca	3	0.8 ± 1.2^{d}

^{a,b,c,d}denotes significantly different concentrations at the 95% confidence level

3.1.1. Residual Cyanide Concentrations in Cassava Bread Samples

The average residual cyanide concentration in cassava bread products was 15.5 ppm but there were variations between brands ranging from 6.0 ppm to 24.6 ppm. CB2 was the only brand that had significantly lower cyanide content than the other brands (Table 2).

Table 2. Residual cyanide concentration in five (5) local cassava bread samples

Cassava bread	**Mean cyanide concentration (ppm)**
Cassava bread 1	16.1 ± 8.6^{a}
Cassava bread 2	6.0 ± 3.1^{b}
Cassava bread 3	21.1 ± 7.7^{a}
Cassava bread 4	14.3 ± 8.9^{a}
Cassava bread 5	24.6 ± 1.4^{a}

^{a,b}denotes significantly different concentrations at the 95% confidence level

3.1.2. Residual Cyanide Concentrations in Cassava Flour and Farine Samples

The two brands of cassava flour samples which were analyzed yielded widely varying results. Cassava flour sample 1 (CF1) contained 45 times more residual cyanide than cassava flour sample 2 (CF2) (Table 3). This brand was analyzed initially in month 1, then 3 months later in month 4, using different samples and both times similar concentrations were obtained. The two samples of farine had different cyanide concentrations, farine sample 2 (F2) had approximately 50% more residual cyanide than farine sample 1 (F1).

Table 3. Residual cyanide concentration in two (2) local cassava flour samples

Cassava flour	**Mean cyanide concentration (ppm)**
Cassava flour 1	103.5 ± 16.5[a]
Cassava flour 2	2.3± 0.5[b]
Farine 1	4.1 ± 0.55[c]
Farine 2	6.3± 0.49[d]

[a,b]denotes significantly different concentrations between CF samples at the 95% confidence level

[c,d]denotes significantly different concentrations between farine samples at the 95% confidence level

3.2. Residual Cyanide Concentrations in Cassava-based Products Over Time

There was an observed loss of residual cyanide in the cassava-based products as time elapsed. For cassava bread samples, the samples analysed in month 4 generally had lower cyanide concentrations than the samples analysed in month 1 but these differences were only significant for CB3 and CB4. These two brands showed reductions of 50% and 72% respectively from month 1 to month 4 (Table 4). The cyanide concentration of the cassava flour sample 1 decreased by 15% over the 3-month period (Table 5). Additionally, it was observed that cassava flour samples produced by the same manufacturer had similar cyanide contents when samples were purchased 3 months apart. The average cyanide concentration for cassava flour 1 samples purchased in month 1 and month 4 are 107.3 ppm and 90.9 ppm respectively ($p = 0.759$). Samples of farine showed marginal decreases in cyanide concentrations over the 3-month period but the sample population sizes were too small for the variations to be deemed statistically significant (Table 5 and Figure 1).

Table 4. Residual cyanide concentration in cassava bread samples over time

Cassava bread	**Mean cyanide concentration in Month 1 (ppm)**	**Mean cyanide concentration in Month 2 (ppm)**
Cassava bread 1	10.7± 0.8[a]	13.5 ± 2.0[a]
Cassava bread 2	6.2 ± 0.1[b]	5.8 ± 0.3[b]
Cassava bread 3	28.1± 0.7[c1]	14.1 ± 1.5[c2]
Cassava bread 4	22.4± 0.6[d1]	6.2 ± 0.4[d2]

[a,b,c,] denotes significantly different concentrations among brands at the 95% confidence level [1,2,] denotes significantly different concentrations over time at the 95% confidence level

Table 5. Residual cyanide concentration in cassava flour and farine samples over time

Cassava flour	**Mean cyanide concentration in Month 1 (ppm)**	**Mean cyanide concentration in Month 2 (ppm)**
Cassava flour 1	107.3 ± 2.7[a]	90.9 ± 6.2[b]
Farine 1	4.16 ± 1.4[c]	4.04 ± 0.8[c]
Farine 2	6.53 ± 0.8[d]	5.75 ± 0.2[b]

[a,b] denotes significantly different concentrations in CF samples over time at the 95% confidence level
[c,d] denotes significantly different concentrations in farine samples over time at the 95% confidence level

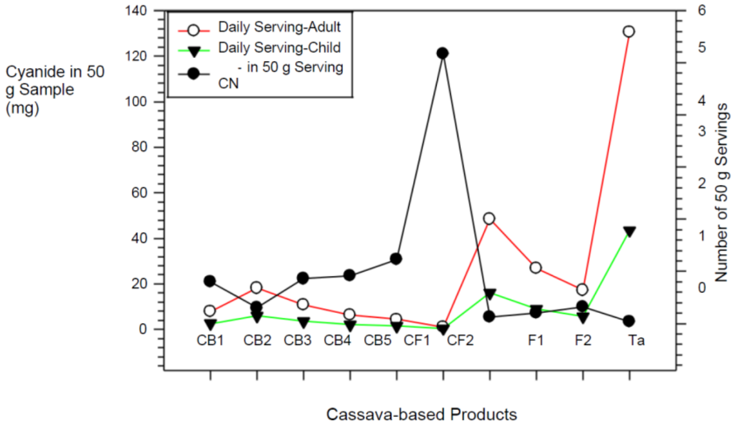

Figure 1. Cyanide content in 50 g serving of cassava product and maximum number of 50gservings that an average adult or child may consume daily before reaching toxic levels

3.3. Acute Reference Dose of Cassava-based Products

The Acute Reference Dose of cyanide is stated as 0.09 mg of cyanide per kg of body mass [20]. The average cyanide concentrations for the various cassava-based products were used to determine the cyanide content in a 50 g serving of each cassava-based product. This value was then used to determine the maximum number of servings of each product that may be consumed daily by an adult of average mass 60 kg and a child of average mass 20 kg, respectively. The results of these computations are displayed in Table 6. An adult of mass 60 kg may consume as much as 4 to 18 servings of cassava bread, dependent on the brand, without being at risk for cyanide toxicity. These amounts represent 200 g to 900 g of cassava bread. Similarly, an adult of mass 60 kg may consume 860g to 1340 g of farine or 6525 g of tapioca per day without the risk of cyanide toxicity. Due to the large variation in the cyanide concentrations of the cassava flour samples, daily allowable doses range from 55 g to 2425 g and depend greatly on the brand of cassava flour consumed.

4. Discussion

The residual cyanide concentration expectedly varied among products since the cyanide volatilizes during processing of the cassava in methods which include grating or grinding and heating or drying. These methods rupture the cell walls and bring the cyanogenic glycosides into contact with the enzyme linamarase. Linamarase hydrolyzes linamarin (major cyanogenic glycoside in cassava) into glucose and acetone cyanohydrin which vaporizes readily [21]. Under neutral conditions the acetone cyanohydrin decomposes to acetone and hydrogen cyanide (Figure 2).

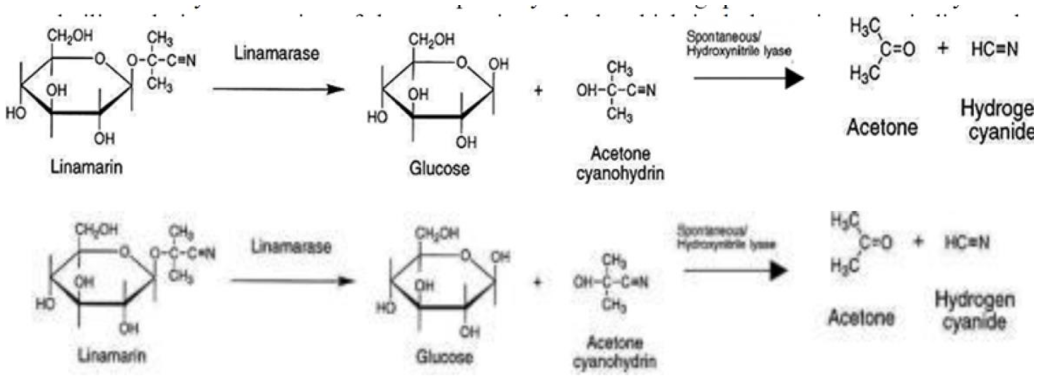

Figure 2. Hydrolysis of linamarin to produce hydrogen cyanide [22]

The finely divided products such as cassava flour, farine and tapioca are therefore expected to have the lowest concentrations of residual cyanide. The findings of this study corroborates this with one notable exception; CF1 had very high levels of cyanide (45 times the cyanide content of CF2 and more than 3.5 times the cyanide content of any of the other products studied). This brand of cassava flour may be manufactured from the bitter variety of cassava. It is equally likely that the processing regime employed differs from that of the other cassava flour product analysed.

Four of the five cassava bread samples which were examined had similar cyanide concentrations. The fifth sample (CB2) had lower levels of cyanide than the others; this sample was garlic flavored and compounds in the garlic are known to interact with the cyanogenic glycoside through side reactions. Results of an investigation into the effects of acillin, the main compound in garlic, on cyanide toxicity in rats revealed that rats fed a garlic diet showed a marked reduction in the lethality of cyanide intoxication. Further, the rate of protection increased with dosage. The authors suggested that sulphur compounds in garlic may have a protective effect against cyanide intoxication [23]. The action of sulphur donors in combating cyanide toxicity involves the conversion of cyanide to thiocyanate [24]. The binding of cyanide to the sulphur prevents the binding of cyanide to ferric ions of the cytochrome oxidase three in the mitochondria causing the cessation of aerobic cell metabolism [25].

Some results suggest a decrease in cyanide levels with age of the product. This was observed for samples of farine. Farine is usually utilized as an additive to food, many indigenous people of Guyana sprinkle it atop their meals or add it to porridges as a thickener. Over a 3-month period, the cyanide content in two brands of farine decreased by 7.4% on average. Reduction in cyanide content over time may be attributed to post-process volatilization of the HCN compound. The cyanide content of most of the cassava-based products analysed in this study allowed for at least moderate consumption of the product without reaching acute toxicity doses. The CB5 sample is flagged for its relatively high residual cyanide content and consequent low daily allowable consumption. This brand of cassava bread has a daily allowable consumption of less than 5 servings in adults and less than 2 servings in children (less than 250g and less than 100g respectively). Cassava bread is a staple in many indigenous communities of Guyana and thus it is likely to be consumed in much larger quantities. Cyanide toxicity presents as headaches, dizziness, confusion and mydriasis in the early stages. In the later stages, seizures and coma result which can eventually lead to death [25]. Methods of preparation of cassava bread should therefore allow ample opportunity for hydrolysis of the cyanogenic glycosides and consequent volatilization of the cyanohydrin produced. The cassava flour that displayed exceptionally high cyanide content, CF1 presents a serious health concern. However, since cassava flour is usually mixed with wheat flour to make baked goods such as bread, effective residual cyanide concentrations in the final product is likely to be significantly reduced.

5. CONCLUSIONS

This research investigated cyanide levels in locally available cassava-based products with a view to exploring potentials for export. Most of the products analysed contained cyanide concentrations above the 10-ppm threshold recommended by the World Health Organization. The mean cyanide concentration in cassava bread, cassava flour, farine and tapioca was 15.5 ppm, 78.1 ppm, 5.0 ppm and 0.8 ppm respectively. Samples of cassava bread and cassava flour accounted for 75% of the samples analysed. Local producers therefore need to adjust processing techniques for the reduction of residual cyanide concentrations. Recommended suggestions include longer soaking periods, longer baking and drying periods, and the addition of sulphur containing additives such as garlic. Further, improvements in packaging and labelling are needed so labels can more accurately reflect net contents, serving size, number of servings and expiration dates.

Recommendations for future work include studying the effect of processing parameters such as soaking time, baking time, baking temperature, and drying time on residual cyanide content. Studies on shelf-life and storage techniques are also recommended.

ACKNOWLEDGEMENTS

The authors would like to thank the Faculty of Natural Sciences at the University of Guyana for funding this research.

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