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Effects of Processing Techniques on the Level of Cyanogenic Glycosides and Hydrogen Cyanides in Cassava Flour

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ABSTRACT

This work investigated the toxicity level of Cyanogenic glycosides, hydrogen cyanide in palm oil, fermented and unfermented cassava flour. The cassava flour processed by different methods was made to undergo qualitative and quantitative using Zhou S., method of analysis. Results show that the three cassava flour samples (dry fermented garri, oil garri and water submerged fermented garri) contain 0.986mg/kg, 0.864mg/kg and 0.986mg/kg Cyanogenic glycoside, hydrogen Cyanide (0.0049 mg/kg, 0.0043mg/kg and 0.0049mg/kg) respectively. Both compounds concentration are reduced in oil garri. This is attributed to the glycosides and non glycosides undergoing hydrolysis, which is in line with Cooke who said that microorganisms are not necessarily involved in the breakdown of Cyanogenic glycosides. This study clearly shows that addition of palm oil to garri helps in delaying the decomposition of Cyanogenic glycosides while it also reduces the formulation of hydrogen cyanides. The three methods of preparation yields are below the tolerable level of acute reference dose (ARFD) of 0.09mg/kg bw (body weight) and provisional maximum tolerable daily intake (PMTDI) of 20mg/kg bw/d as recommend by World Health Organization. Fermentation by submerging cassava in water for days before frying, contain higher amount of Cyanogenic glycoside which releases higher concentration of hydrogen cyanide due to hydrolysis. Cassava flour made of palm oil (yellow garri) is highly recommended for those without diabetic issue since it contains some amount of vitamin A, E and K from palm oil and lesser amount of HCN which made it less toxic.

1. INTRODUCTION

Cassava root production has been increasing steadily since the 1960s but between 1997 and 2007, its production has increased by over 40% (from 161 to 224 million tons) and its use in animal feed increased by 76 million tons [1].

The composition of cassava and its nutritional properties depends on the specific tissue (root or leaf) being consumed. These aspects in turn, depend on several factors such as geographic location, variety, age of the plant and environmental conditions. Cassava roots and leaves are deficient in

the Sulphur containing amino acids, methionine, cysteine and some nutrients are not optimally distributed within the rest of the plant's physiology [2].

Cassava also contains its own share of anti-nutrients, which have either positive or negative effects on the health; depending upon the amount of the component being ingested. They basically interfere with the digestibility and uptake of some nutrients. Nevertheless, depending on the amount consumed, these substances can also bring benefits to humans. [3].

Cyanide is present in all the tissues of cassava but the highest level is in the leaves. Most of the cyanide in the tuber is found in the peel compared to the cortex. The enzyme responsible for the hydrolysis of Linamarin and lostaustralin is the β-glycosidase, linamarase. In cassava, Linamarin and lostaustralin are found in the cell vacuole while linamarase is localized in the cytoplasm hence the hydrogen cyanide is released when crushed [4]. Cyanide is the most toxic factor restricting the consumption of cassava root and leaves. Several health disorders and diseases have been reported in cassava eating Populations, owing to the presence of improperly processed cyanide [2].

Cassava eating populations are naturally exposed to high amounts of cyanide, nitrates and nitrites- chemical compounds which are known to contribute to the risk of developing stomach cancer. Cassava eating individuals tend to have a high amount of thiocyanate in the stomach due to cyanide detoxification by the body, which may catalyze the formation of carcinogenic nitrosamines. The consumption of lower cyanide amounts is not lethal but long term intake could cause severe health problems such as tropical neuropathy [5]. The nitrate content in cassava leaves ranges from 43 to 310 mg/100g dry matter [3].

Fermentation of cassava brings a new line of food products altogether. Fermentation, either naturally or with selected microbial inoculums, has been extensively used to enhance the nutrient potentials of cassava and its by-products both for human and livestock consumption. Two popular fermentation techniques, namely, the liquid substrate or submerged fermentation are used. Fermentation process has also played a significant role in the nutritional enhancement of the agro-industrial by-products generated through the harvesting and processing of cassava roots.

Apart from the food industry, cassava starch is used for textiles and the paper industry, and in the manufacture of plywood, veneer adhesives, glucose and dextrin syrups. Through fermentation, it can be used for alcohol production and as a waste material, it can be processed to biogas, pharmaceutical and cosmetic ethanol, potable ethanol such as bear and whiskey [6].

The potential toxicity of a Cyanogenic plant depends primarily on the potential that its Consumption will produce a concentration of cyanide that is toxic to humans. In humans, the signs of acute cyanide intoxication include; rapid respiration, vomiting, drop in blood pressure, mental confusion, dizziness, headache, stomach pains, diarrhea, cyanosis with twitching and convulsions followed by terminal coma. Death due to cyanide poisoning can occur when the cyanide level exceeds the limit an individual is able to detoxify.

Cassava being a staple food for many communities, ensuring the safety of its product is very important. Understanding the variations in toxicity levels between fermented and unfermented cassava flour is important for assessing the safety of these commonly consumed products and implementing measures to mitigate health risks associated with cyanide exposure.

This Research Work focuses on quantifying Cyanogenic Glycosides levels in dry fermented, water fermented and oiled cassava flour; investigates the impact of fermentation on cyanide

release during food processing, assessing the bioavailability of cyanide from these products during digestion and identifying potential health implications associated with the varying levels of cyanide exposure.

2. MATERIALS AND METHODS

The materials include: Distilled water, Whatman no.1 filter paper, Para-dimethylaminobenzaldehyde (4-4dimethylaminobenzaldehyde: DMBA), Sodium hydroxide, Hydrochloric acid, Hot plate, Water bath and Spectrophotometer.

2.1 Batch Experiments

Oiled cassava flour (yellow garri): The cassava tubers were harvested, peeled, washed, ground, mixed with oil, pressed with presser, and then fried the next day. This gave rise to our yellow garri sample (YGS).

Dry fermented cassava flour or white garri sample (WGS) was made by harvesting cassava tubers, peeled and washed, ground and left in Sacs for five days before pressing it in a presser and fried the sixth day.

Water submerged fermented cassava flour or Fufu garri sample (FGS) was made by harvesting cassava tubers, peeled, soaked in water for 5 days, ground, pressed and fried the sixth day.

2.2 Determination of Cyanogenic Glycoside and Hydrogen cyanide

Determination of cyanogenic glycoside and hydrogen cyanide was carried out according to [7]. 5g of each cassava variety was blended into a paste and 50 ml of conc. HCL is added in a conical flask and the mixture is heated in a water bath or hot plate until it boils for 10 -30minutes and the solution is allowed to cool over night. The cooled solution is transferred to a volumetric flask and diluted to 10 ml before filtering. Few drops of sodium hydroxide is added to the filtrate to maintain the pH and it is kept for determination of hydrogen cyanide using a spectrophotometer.

2.3 Preparation of cyanide Standard Curve

The stock solution of HCN is dissolved in distilled water to produce different concentration of HCN ranging from 5µg to 50µg in 500ml conical flask and 20ml of 1mol/l HCL added. Each of the standard solution is added to a separate corvettes and the absorbance is measured at a wavelength of 565 nm. The standard curve for HCN using 4-4 Dimethylaminobenzaldehyde (colorimetric indicator for cyanide ion), is carried out by the addition of the different series of standard solution HCN into the corvette followed by the addition of the 5ml DMBA Solution. This allows for the reaction between HCN and DMBA to occur in the corvette developing a colour which is measured in the spectrometer. A graph of absorbance versus concentration gives the standard curve

2.4 Determination of hydrogen cyanide

In the experiment, 2 ml of the filtrate was poured into another conical flask and 4 ml4-4Dimethylaminobenzaldehydeis added for colour change (pale yellow to bright red which is due to the formation of diazonium picrate) and absorbance taken at 450 nm using a spectrum label 23A Spectrophotometer. The blank and standard test (which serves as control) was prepared using 2ml distilled water and 4ml DMBA solution. The cyanide content was extrapolated using a cyanide standard curve. The concentration of HCN is calculated using Beer Lambert law which sate that the absorbance of a solution is directly proportional to the concentration of the absorbing species and the path length of the light through the sample i.e. ($A=\in IC$; where A is the absorbance measured, $\in = molar\ absorptivity$, I = path length and C the concentration of the absorbing species.

2.5 Cyanogenic glycoside determination

The Cyanogenic glycoside concentration is

calculated from the HCN concentration. The amount of HCN released is directly related the amount of Cyanogenic glycoside present. Therefore Cyanogenic glycosides (mg/g) =HCN concentration (mg/l) X molecular weight of Cyanogenic glycoside released (laminarin). The cassava flours contain laminarin with molecular weight of 201.16g/mol. The HCN for WGS from the analysis is .0049mg/l; the concentration of the laminarin is therefore 0.0049mg\l x201.16g/mol = 985.684mg/g (0.986mg/kg) as shown in table 2 below.

3. RESULTS DISCUSSION

3.1 Finding of research

It was clearly observed from Table 2, figure 2 below that the three cassava flour samples contains Cyanogenic glycosides in good quantity, which on hydrolysis produce the toxic hydrogen cyanide which is reduced in the presence of palm oil. This is as a result of supplementary oil delaying the decomposition and therefore prevents absorption of the Cyanogenic glycosides. Results from this research work have shown that fermented garri, either dry or wet fermentation has the highest value for Cyanogenic glycosides with trace release of hydrogen cyanide which is toxic to man in acute dose. This is as a result of the glycosides form (laminarin) and non-glycosides bound form (cyanohydrins) undergoing hydrolysis under normal condition and on decomposition gives acetone and hydro cyanide acid. This is in line with [8] that found that microorganisms are not necessarily involved in the breakdown of Cyanogenic glycosides.

White garri (dry fermented) which was kept in the sack to undergo dry fermentation has same amount of Cyanogenic glycosides and hydrogen cyanide as liquid fermentation. This is as a result of heat generation and mould growth during fermentation which could have led to hydrogen cyanide losses. In a nutshell, the major toxic substance in the three cassava flour samples is Cyanogenic glycosides and hydrogen cyanide.

Table 1: Concentration of hydrogen cyanide (mg/kg) present in cassava flour

Hydrogen cyanide (mg/kg)	1st run (mg/kg)	2nd run (mg/kg)	3rd run (mg/kg)	mean (mg/kg
GS	0.0048	0.0048	0.0049	0.0049
YGS	0.0043	0.0043	0.0043	0.0043
FGS	0.0049	0.0049	0.0050	0.0049

WGS= Dry Fermented Garri
YGS= Palm Oiled Garri
FGS= Water Submerged Fermented Garri

Table 2: Calculated cyanogenic glycoside concentration (mg/kg) from HCN concentration in the three samples of cassava flour

Samples (mg/kg)	HCN (mg/kg)	Cyanogenic Glycoside (mg/kg)
WGS	0.0049	0.986
YGS	0.0043	0.864
FGS	0.0049	0.986

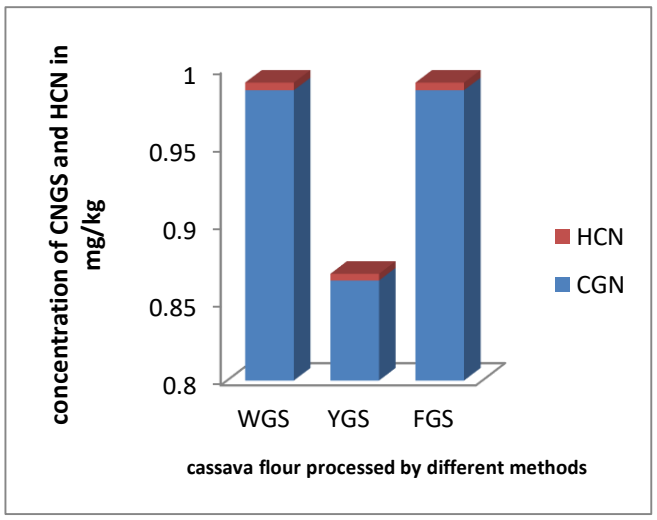

Figure 1: Concentration (mg/kg) of Cyanogenic glycosides (CNGS) and hydrogen cyanide (HCN) present in cassava flour processed by different methods

4. CONCLUSION

This study shows that the different methods of processing cassava flour determine the toxicity levels of Cyanogenic glycosides in the three garri samples. The study revealed that addition of palm oil to garri helps in delaying the decomposition of Cyanogenic glycosides while it also reduces the formulation of hydrogen cyanides. From all indications, liquid fermentation of cassava for days before frying though sweet and contain lesser sugar contains a higher Cyanogenic glycoside and hydrogen cyanide compared to oiling method. The three method of preparation are very good in preparation of garri but oil method is the best followed by dry fermentation and the liquid fermentation the least. The method of preparation yields are below the tolerable level of acute reference dose (ARFD) of 0.09mg/kg body weight per day(b w/d) and provisional maximum tolerable daily intake (PMTDI) of 20mg/kg bw/d cyanide as recommended by world health organization [9].

RECOMMENDATIONS

From this study, yellow garri is highly recommended followed by the white dry fermented garri and Fufu garri the least though very sweet .Palm Oil garri is highly recommended for consumptions since it contains lesser amount of HCN which is toxic and some good amount of vitamin A, E and K from palm oil that may be added to the oiled garri. The producer as well as the consumers should be encouraged to use the palm oil method in preparation of garri since accumulation of HCN in the body can lead to various diseases that can lead to death.

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