The Hepatic and Hematologic Toxic Effects of Pro-Vitamin A High Quality Cassava Flour Obtained by Different Processing Methods

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ABSTRACT

The hepatic and hematologic toxic effect of Pro-Vitamin A High Quality Cassava Flour (HQCF) by different processing methods was studied in male albino Wistar rats, fed for 28days on the Cassava diet containing 4.794mgCN kg^{-1} and 10% protein supplement, using spectrophotometry, enzyme assay and automated haematology analyzer. There was significant increase in the serum Glucose concentration, serum activity of Aspartate Aminotransferase and Alanine Aminotransferase of the test animals compared to the control, whereas the serum total protein concentrations showed a significant decrease between the test and control groups. The hematology studies showed no significant difference ($p > 0.05$)between the test and control groups. Toxicity study suggested that the in vivo metabolism of Pro-Vitamin A HQCF was capable of altering some biochemical parameters such as elevation of serum Glucose concentration and some enzymes such as Aspartate Aminotransferase and Alanine Aminotransferase, whereas a reduction in serum total protein concentration was observed. The findings indicated that the processing methods (most especially oven dry) have reduced the toxicity of HQCF to a minimum safe level.

Keywords: Toxicity, Concentration, Hematology, Metabolism

INTRODUCTION

Cassava (Manihot esculenta Crantz) is the common food of many people in the tropical region.[1-2]It belongs to the family of Euphorbiaceae and the root is rich in carbohydrates.[3] Different Cassava varieties vary in their starch content,[4] with older roots having less starch content than younger ones.[5] The root of Cassava is also rich in cyanogenic glucosides (Linamarin and Lotaustralin).[6,7] These cyanogenic compounds can undergo hydrolysis after ingestion to generate hydrogen cyanide (HCN) and other compounds containing cyanide. Ingestion of cyanide causes its higher levels in the liver than through inhalation. This knowledge is very useful in scientific investigations.[8]

After cyanide absorption, it is rapidly circulated throughout the body. A large proportion of cyanide in the body is protein-bound (60%). Cyanide reacts reversibly but with a high affinity with metals such as the Ferric ion (Fe3+) and Cobalt. Cyanide can also react with compounds that contain Sulfur. Tissues that contain cyanide include the heart, liver, spleen, brain, kidneys, blood, and lungs.[9] Cyanide poisoning inhibits enzymes that contain metals. The toxicity of cyanide appears in the inhibition of the enzyme, Cytochrome oxidase a_3, terminal enzyme of the respiratory chain which compromises oxidative phosphorylation leading to cytotoxic hypoxia[10]. Due to this toxicity, there is an important need in exploring the best Cassava processing method to reduce cyanide to the barest minimum.

The traditional methods of peeling and grating, dewatering and fermentation for 72 hours reduce the cyanogens in Cassava roots to a considerably safe level.[11,12] Consumption of poorly processed cassava food products can lead to devastating health disorder and even death in man.[13, 14]Studies have confirmed that peeling has shown to represent the first processing step to reduce cyanogenic contents and to lower the Cassava toxicity, as the cyanogenic glycosides (CNG) are distributed in large amounts in the roots cortex (skin layer).[15] Moreover, grating of the pulp as the second step of sample preparation, breaks compartmental barrier and creates a higher surface area enabling Linamarin to

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have contact with its hydrolytic enzyme (Linamarase), resulting in the hydrolysis and subsequent removal of the breakdown products.[16] Since some cyanogenic compounds are soluble in water, its amount is reduced by traditional detoxification methods such as dewatering.[17] Further reduction occurred due to the volatile cyanogenic compound (HCN), which evaporated into the air during different drying methods.

MATERIALS AND METHODS

Sample Collection

A variety of Pro-Vitamin A Cassava tubers (Manihot esculenta Crantz) identified as UMUCASS 36, were purchased from the Cassava programme farm of the National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria. The Cassava tubers were harvested after 8 months of planting.

Preparation of Pro-Vitamin A High-Quality Cassava Flour

A variety of Pro-Vitamin A Cassava tubers, named UMUCASS 36, was processed with the following procedures. The tubers were processed within 24 hours after harvest. The tubers were peeled, washed, grated, pressed, disintegrated and dried using four (4) different drying methods which include;

- Sun-dried method.
- Oven-dried method
- Solar dried method
- Tray dried method

Animal Study

Twenty-five (25) Albino rats of the Wistar strain with body weight of 120-200g were used for the experiment. The animals were purchased from the animal house of Veterinary College, Michael Okpara Federal University of Agriculture, Umudike, Abia State. All animals were kept at room temperature and were allowed to have free access to drinking water and their diets. The animals were also allowed to acclimatize to their environment and their diet for 7 days before the experiment commenced. The animals were fed throughout the experiments with Pro-Vitamin A High Quality Cassava Flour (HQCF) based diet as shown in the table below. The control diet was prepared as above with Pro-Vitamin A HQCF replaced with corn flour, which was given to the control animals.

All the animals were fed for 28 days, after which they were sacrificed by stunting and their blood was collected directly from the heart through cardiac puncture using a syringe and needle inside the red and purple blood sample bottles. The serum was separated from the whole blood by centrifugation for 10 minutes at 1,000 revolutions per minute. The urine samples were also collected. This was with the approval from the Animal Ethic Committees (AECs), College of Veterinary medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Determination of Total Cyanide Content in Pro-Vitamin A HQCF

Cyanogenic content of the samples was determined using simple picrate paper test as described by Trinder.[18]

Determination of Serum Glucose

The serum Glucose was determined using glucose oxidase/peroxidase (GOD/POD) method as described by Tietz.[19]

Determination of Serum Total Protein

The serum total protein was estimated using the Biuret method as described by Tietz.[20]

Assay of Aspartate Aminotransferase and Alanine Aminotransferase Activity

A Randox commercial Enzyme kit according to the method by Reitman and Schmidt was used.[21,22]

Determination of Hematology Parameters

The haematology parameters were obtained at once for each blood sample using an Automated Hematology Analyser (Mindray BC 2300, China).

Statistical Analysis

Analysis of variance test was carried out using the statistical package for social science (SPSS).[23] Results are presented as Mean Standard Error and significant means were separated using Duncan Multiple Test.[24]

RESULTS

The cyanogenic content of Pro Vitamin A Cassava cultivar showed significant reduction (88.7%) in the cyanogenic content of the dewatered sample with concentration of 11.088ppm compared to the fresh sample with concentration of 97.812ppm, resulting in reduction/removal of 86.724ppm of cyanogenic content. Further reduction of cyanogenic content was observed during the different drying methods. The Sun dried reduced cyanogenic content by 91.9% (89.892ppm), Oven dried by 97.4% (95.268ppm), Solar dried by 97.2% (95.040ppm), and Tray dried by 93.9% (91.872ppm), when compared to the fresh sample, as shown in table 3. The average of the cyanogenic content of the different drying methods was computed as 4.794mgCN^{-}kg^{-1} to obtain the actual cynogenic content of the sample (Pro Vitamin A HQCF).

The concentration of Glucose in the serum of rats fed with yellow Cassava diet, in table 5, shows a significant difference at $p < 0.05$ in the test groups compared to the control group. Group D (tray dried) was the highest with concentration of 80.601.435mg/dl, whereas the control group was the least with concentration of 61.40 $\pm$ 2.315mg/dl. Table 2 also shows the results of the concentration of total protein in the serum of rats fed with yellow Cassava diet. There were significant differences at $p < 0.05$ in the serum total protein concentration of group A and C and slight difference in group B and D compared to the control group. Group C (Solar dried) was the least with concentration of 6.378 $\pm$ 0.248g/dl, whereas the control group was the highest with concentration of 7.794 $\pm$ 0.101g/dl.

The results of the activity of Aspartate Aminotransferase in the serum of rats fed Pro-Vitamin A High Quality Cassava Flour (HQCF) diet shows a significant increase ($p < 0.05$) in the serum levels of Aspartate Aminotransferase activity of the animals fed cassava diet above those of the control group (Figure 1). Figure 2 shows the results of the activity of Alanine Aminotransferase in the serum of rats fed yellow Cassava diet. There was significant increase ($p < 0.05$) in the serum levels of Alanine Aminotransferase activity of the animals fed Pro-Vitamin A HQCF diet above those of the control group (Figure 2).

The haematological parameter results in the plasma of rats fed with yellow Cassava diet detect slight reduction in Red Blood Cell (RBC) count in the test groups when compared with the control group (although there is no statistically significant different at $p < 0.05$). Group A (Sun dried) has the least RBC count with 7.342 $\pm$ 0.104 x10^{12}/L, whereas the control group has the highest RBC count with 8.130 $\pm$ 0.153 x10^{12}/L. There was no significant difference at $p < 0.05$ in the White Blood Cell Count, Platelets Count, Packed Cell Volume, Haemoglobin, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration of the test groups compared to the Control group.

Table 1: Dietary Animal Feed Composition

Ingredients	Quantity (g/kg)	(%)
Pro -Vitamin A HQCF	750	75
Protein (Soya beans)	100	10
Groundnut oil	40	4
Vitamin Mixture	40	4
Banana Flavor	40	4
Minerals Mixture	30	3

Experimental Design

The twenty-five (25) rats were grouped into five (5) groups, namely: Group A, B, C, D and E. Each group is comprised of five (5) animals. The test animal groups (A, B, C and D) were fed throughout the investigation on Pro-Vitamin A HQCF based diet as shown in the table 1 below. While Group E (Control) were fed with corn flour-based diet.

Table 2: Groups and their Treatments

Groups	Treatment
A	Sun -Dried Pro - Vitamin A HQCF Based Diet
B	Oven -Dried Pro - Vitamin A HQCF Based Diet
C	Solar Dried Pro - Vitamin A HQCF Based Diet
D	Tray Dried Pro - Vitamin A HQCF Based Diet
E(Control)	Corn Flour based diet

Table 3: Cyanogenic Content of Fresh, Dewatered and Dried (Flour) Pro-Vitamin A High Quality Cassava (UMUCASS 36)

Samples	Total CN content (ppm)	CN content removed from fresh (ppm)	% of CN removed from fresh (%)
Fresh	97.812	-	-
Dewatered	11.088	86.724	88.7
Sun Dried	7.920	89.892	91.9
Oven Dried	2.544	95.268	97.4
Solar Dried	2.772	95.040	97.2
Tray Dried	5.940	91.872	93.9

Each sample(n) is collected in duplicate and the average is recorded.

Table 4: Cyanogenic content of Pro-Vitamin A HQCF.

Samples	Cyanogenic content (mgHCN equivalent per kg)
UMUCASS 36	4.794

Cyanogenic content of Pro-Vitamin A HQCF was obtained by taking the average of cyanide content of Sun dried, Oven dried, Solar dried and Tray dried samples. All samples are collected in duplicate and the average was recorded.

Table 5: Concentration of Total Protein and Glucose in the Serum of Rats Fed Pro-Vitamin A HQCF diet.

Groups	Serum total protein concentration (g/dl)	Serum glucose concentration (mg/dl)
A	$6.46 + 0.30^{b}$	$79.60 + 1.21^{a}$
B	$7.30 + 0.33^{a}$	$74.20 + 1.98^{a}$
C	$6.38 + 0.25^{b}$	$76.60 + 3.08^{a}$
D	$7.28 + 0.29^{a}$	$80.60 + 1.44^{a}$
E	$7.79 + 0.10^{a}$	$61.40 + 2.32^{b}$

Table 6: Haematological parameters of Rats Fed Pro-Vitamin A HQCF

Parameters (Control)	Group A	Group B	Group C	Group D	Group E
RBC count ($x10^{12}$/L)	7.34 ± 0.10^{b}	8.11 ± 0.18^{a}	7.96 ±0.15^{a}	7.67 ±0.16^{ab}	8.13± 0.15^{a}
WBC count ($x10^{9}$/L)	12.38 ± 1.03^{a}	14.10 ±1.58^{a}	11.60± 0.51^{a}	12.22± 0.92^{a}	11.78 ± 0.73^{a}
Platelets ($x10^{9}$/L)	462.60 ±49.06^{b}	639.40 ±70.76^{a}	511.60 ±17.44^{a}	453.20 ± 54.29^{b}	539.20± 49.71^{a}
PCV (%)	43.68± 0.32^{a}	47.10 ± 2.36^{a}	47.12±1.32^{a}	47.38±2.09^{a}	47.42±1.05^{a}
Hb conc. (g/dl)	13.02± 0.15^{a}	13.24± 0.45^{a}	13.02±0.16^{a}	13.52±0.26^{a}	13.50±0.28^{a}
MCV (fl)	59.62 ± 095^{a}	57.80± 2.22^{a}	58.38± 1.83^{a}	60.30± 1.24^{a}	59.62± 1.11^{a}
MCH (pg)	17.70 ± 0.37^{a}	16.18± 0.42^{b}	16.18± 0.53^{b}	16.78± 0.37^{ab}	16.90± 0.19^{ab}
MCHC (g/dl)	29.76 ± 0.22^{a}	28.16± 0.54^{b}	27.22± 0.70^{b}	27.66± 0.54^{b}	28.44± 0.33^{ab}

Means with different superscript along the columns are significantly different at $p < 0.05$. Values are recorded as mean ± SE.

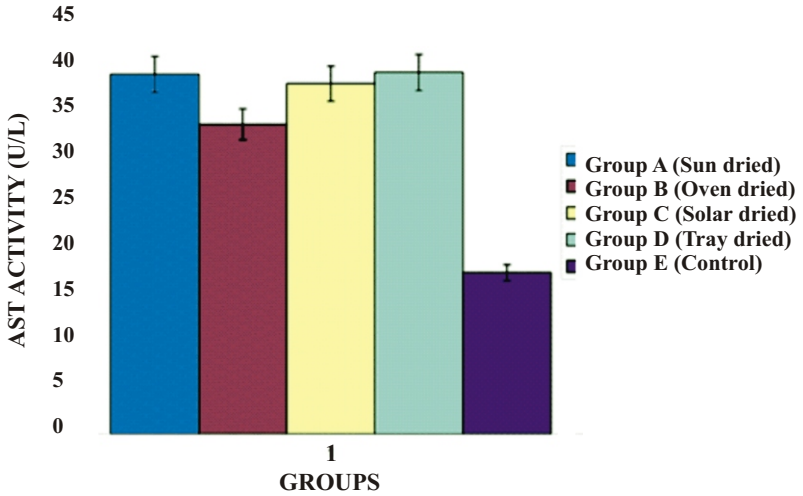

Figure 1: Activity of AST in the Serum of Rats Fed Pro-Vitamin A HQCF diet

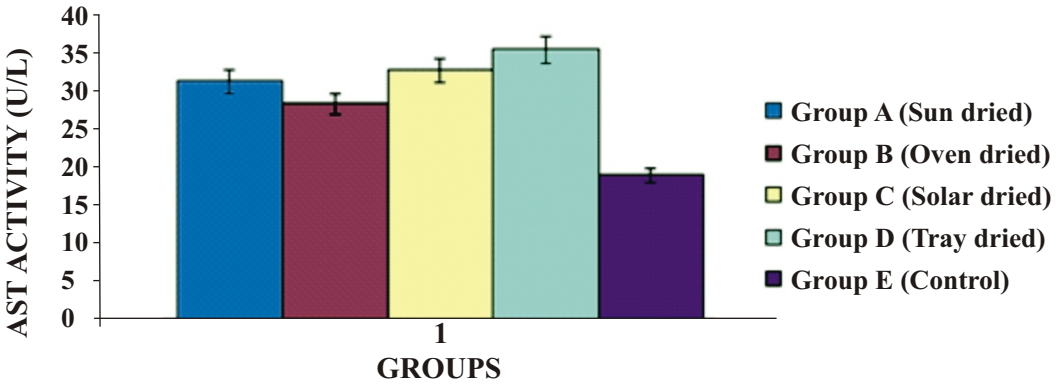

Figure 2: Activity of ALT in the Serum of Rats Fed Pro-Vitamin A HQCF diet.

DISCUSSION

Studies have confirmed that peeling represents the first processing step to reduce cyanogenic contents and to lower Cassava toxicity, as the cyanogenic glycosides (CNG) are distributed in large amounts in the root cortex (skin layer).[15] Moreover, grating of the pulp as the second step of sample preparation, breaks compartmental barrier and creates a higher surface area enabling Linamarin to have contact with its hydrolytic enzyme (Linamarase), resulting in the hydrolysis and subsequent removal of the breakdown products.[16] Since some cyanogenic compounds are soluble in water, its amount is reduced by traditional detoxification

methods such as dewatering.[17] Further reduction occurred due to the volatile cyanogenic compound, which evaporated into the air during different drying methods.

Data from this study showed that the serum Glucose concentration of rats fed Pro-Vitamin A HQCF increased significantly ($p < 0.05$) in the test Groups when compared to the Control group (Table 2). The increase suggests exposure of the test animals to dietary cyanide because studies have revealed that cyanide alters Glucose metabolism resulting in increased Glucose and lactic acid level and a decreased in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism. This is following work that reports Diabetes as a toxic effect produced by ingesting cassava, a cyanogenic plant, in various species.[25,26] There was a statistically significant decrease ($p < 0.05$) in the concentration of the serum total protein of the test animal groups below that of the control group (Table 5). The decrease indicates an attempt to use Sulphur-containing amino acids of their body to detoxify the cyanide ingested through diet. In the human body, cyanide is detoxified mainly by enzymatic conversion to the much less toxic thiocyanate. This detoxification requires Sulphur donors, which are provided from Sulphur-containing dietary amino acids, cysteine and methionine[27].

The activity of Alanine Aminotransferase and Aspartate Aminotransferase increase significantly in animal groups fed Pro-Vitamin A HQCF when compared to that of the Control group (Figure 1 and 2 respectively). This increase suggests that the samples are capable of causing hepatocellular injury. Alanine Aminotransferase and Aspartate Aminotransferase are important enzymes used in monitoring liver damage.[28] While Alanine Aminotransferase is cytosolic, Aspartate Aminotransferase is both cytosolic and mitochondrial. These enzymes leak out from injured hepatocytes into the blood during liver damage to the cell membrane of the hepatocytes. As a result, increase levels of enzymes are found in the serum and may be caused by a wide range of liver diseases.[13]

In the present study, the results obtained from haematology parameters, Red blood cell count, White blood cell count, Platelet, Packed cell volume, Haemoglobin, Mean Corpuscular Volume, Mean corpuscular Haemoglobin, and Mean corpuscular Haemoglobin concentration showed no statistically significant difference ($p < 0.05$) in all the test groups when compared to the control group (Table 6). This suggests that the cyanide content of Pro-Vitamin A HQCF was reduced to a tolerable level that the blood components were unhindered. All the data obtained for haematological study fell within the normal ranges according to Research Animal Resources, of 7.16-9.24 10^{12}/L for Red blood cell count, 5-8.9 10^{9}/L for White blood cell count, 37-48 % for Packed cell volume, 599-1144 10^{9}/L for Platelet count, 11-15g/dl for Haemoglobin, 67-77fl for Mean Corpuscular Volume, 11-17 pg for Mean corpuscular Haemoglobin, 27- 34g/dl for Mean corpuscular Haemoglobin concentration.[29]

CONCLUSION

In conclusion, the findings from this research work showed that the Pro-Vitamin A Cassava Cultivars was capable of altering some biochemical parameters such as elevation of serum glucose concentration and inactivity of some enzymes such as Aspartate Aminotransferase and Alanine Aminotransferase, whereas a reduction in serum protein was recorded. These effects were due to the presence of cyanide content in the roots of these Pro-Vitamin A Cassava Cultivars, in which the cyanide toxicity has been reduced to a safe barest minimum. Therefore, the oven dry method has shown the least toxic effect while the sun dry method has the most toxic effect.

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