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Evaluation of effects of processing on levels of selected pollutants in gari

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ABSTRACT

Human health is an outcome of the trio of the environment, individual susceptibility and time. Contamination of Cassava (*Manihot esculenta*) roots by the environment or during processing presents health hazards. This study evaluates the effects of processing on the level of selected pollutants in Gari diet and the effect on selected organs of albino rats. Cassava tubers from Kokori and Ogbomoso were varied in the method of processing to produce Gari. Gari and fish samples sundried in the vicinities of flared gas were used to produce four nutritionally similar Gari/fish diet regimen. Twenty-four, 28-day old male albino rats were divided into four groups of six each to fit into four diet regimen and acclimatized for a 7-day period on commercial rat feed. The four groups were afterward placed on different diet regimens for 13 weeks during which the body weight and food intakes were measured weekly. Result shows that the least percentage weight gain (27.9%) occurs in Diet IV-Kokori/SPDC followed by Diet II-Kokori (37.4%), Diet III-Kokori/Ogbomoso (44.7%) and Diet I-Ogbomoso (55.3%) in ascending order. Albino rats on Diets II and IV had a negative food utility index and high concentrations of nickel, lead, cadmium and chromium were found in the feed formulation. The levels of toxicants differ with the processing method and protein efficiency ratio varied with the processing method. Examination of selected organs shows clinical anomalies. The study reveals that pollutants load in gari-diet depend on the environment of cultivation and the method of processing.

Keywords: Pollutants; gari-diet, processing, environment

INTRODUCTION

Food chain contamination is becoming a burning issue because of their potential accumulation in biosystems through contaminated water, soil and air. The main sources of heavy metals to crops are their growth media (1). Several human activities release lethal concentrations of toxic components that may impair the ability of individual organisms to function properly. The status of human health is determined by a complex interaction of the trio of the environment, individual susceptibility and time. Santoz and Gomez (2) noted that of the trio, only the environment is within human direct control. Individual susceptibility is an inherent factor that affects the response of an individual to an environmental exposure and time refers to the period of exposure before a factor manifest. The bioaccumulation of pollutants in food materials leads to various health consequences. Wyatt *et al.*, (3) noted that various industrial activities involving crude oil are connected to pollution of the environment with adverse effect on human health. Oil spillage causes oxygen imbalance, displacement of nutrients and

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introduction of toxic substances (4). Plants grown in a polluted environment can accumulate trace elements at high concentrations, causing a serious risk to human health (5). A relationship has been shown between atmospheric element deposition and elevated element concentrations in plants and top soils, especially in cities and in the vicinity of emitting factories (6, 7). The effect of the pollutants in human could be acute or chronic. Howard *et al.* (8) identified changes in the brain, liver, kidney and intestines due to pollutants. Tremblay *et al.* (9) found adaptive thermogenesis and weight loss in obese individuals as a result of pollutants.

One of the well known benefits of food processing is the removal or reduction in the quantity of unwanted materials in the food. Effective food processing addresses food toxicology, nutritional quality, and technology as well as public health significance of foods. It can significantly reduce the levels of pollutants and toxins in food materials and make food fit for human consumption.

Cassava (*Manihot esculenta*) is widely consumed in the tropical countries of the world and constitutes a staple food in some West African countries especially Nigeria. The roots are the main part consumed although the leaves are also edible. Cassava is processed and consumed in various forms in different agro-ecological zones of Nigeria where many poor households consume a cassava-based diet at least once a day. Gari is a granulous gritty, starchy staple food with high energy content derived from cassava (10). It is the most common form in which cassava is sold in Nigeria and many other African countries (11, 12). Though cassava contains natural toxicants, several pollutants may be present in it due to ineffective industrial wastes disposal. Oke (13) observed that the consumption of polluted cassava can lead to health hazards. Bourdoux (14) detected that symptoms of cassava toxicity may show within four hours of ingestion. The efficiency of fermentation and cooking in reducing levels of pollutants in Cassava products is known (12, 15, 16).

This study evaluates the effect of Gari processing on the level of selected environmental pollutants in the food product and the effect of the pollutants on organ weight and histology of experimental animals.

MATERIALS AND METHODS

Sample preparation

Cassava tubers collected from two sampling sites were varied in the method of processing. The cassava tubers were peeled, grated and processed into Gari using the traditional processing method and SPDC method. Fish samples for commercial feed were sundried in the vicinities of flared gas to simulate the pollution process. Four nutritionally similar Gari diets were developed from Cassava tubers from Kokori and Ogbomoso. Diet I (Ogbomoso) was prepared with cassava tubers from Ogbomoso and processed at Ogbomoso with production period of four days. Diet II (Kokori) was prepared with cassava tubers from Kokori and processed at Kokori with production period of one day. Diet III (Kokori/Ogbomoso) was prepared with cassava tubers from Kokori and processed at Ogbomoso with production period of four days. Diet IV (Kokori/SPDC) was prepared with cassava tubers from Kokori and processed using SPDC method with production period of three days.

Four diet regimens were developed in the ratio indicated in the table below;

Table I. Diet Composition

Diet/Composition	Diet I	Diet II	Diet III	Diet IV
Ogbomoso Processed Cassava (kg)	2.56	-	-	-
Kokori Processed Cassava (kg)	-	2.56	-	-
Kokori/Ogbomoso Processed Cassava (kg)	-	-	2.56	-
SPDC, Ogulagha Processed Cassava (kg)	-	-	-	2.56
Fish (kg)	0.8	0.8	0.8	0.8
Groundnut Oil (kg)	0.56	0.56	0.56	0.56
Vitamin Premix (g)	52	52	52	52
Mineral Premix (g)	28	28	28	28

Each formulated diet was thoroughly mixed in a Vortex Mixer to ensure homogeneity and thereafter packed, sealed, labeled and kept in a deep freezer.

Feeding Regime

Twenty-four, 28-day old male albino rats were used for the experimental phase of the study and six rats were used as external control. The experimental rats were divided into four groups of six each to fit into four diet regimen (Diet I, Diet II, Diet III, and Diet IV) and were individually housed in galvanized steel mesh cages. The laboratory conditions were maintained at relative humidity of 50±10%, temperature of 22°C±3°C and 12 hour light/dark cycle. The rats were acclimatized for a 7-day period during which the rats were placed on normal rat feed. The experimental stage of the study lasted 13 weeks during which the different rats were placed on the four different diet regimens and the fifth group was continued on the normal rat feed. The body weight and food intakes were measured weekly.

Analytical Procedure

At the end of the 13 weeks experimental feeding regime, the rats were decapitated by group after chloroform treatment in a dessicator for laboratory assessment. 2.0ml of blood was collected from each rat into centrifuge tubes placed in ice for one hour and centrifuged to obtain serum for analysis using assay kits obtained from *Quinica Clinica Apliada* S.A.

Histopathology

The heart, kidney, liver and intestine of all the rats were removed and fixed in 10% buffered formalin (pH 7.4). These tissues and organs were thereafter treated in graded alcohol, embedded in paraffin, sectioned to 5™"m thickness, and stained with haematoxylin and eosin for light microscopic enumeration. Autopsy findings were carried out on all the rats at the end of the experiment.

Metal analysis

A Varian Techron AA-5 Atomic Absorption Spectrometer was used. The absorption signal was recorded on a Yokogawa 3046 strip-chart recorders and on Hewlett-Packard Model 5050-B printer, single element cathode lamps were employed. The instrument settings for each element complied with manufacturer's instruction and the gas mixture was adjusted in each case to give maximum signal response.

High purity certified reagents were used for analysis. Concentrated nitric acid was obtained from British Drug Houses (BDH), chemical stock solutions (1000mg/L) of the various elements were used to make working standard. Doubly distilled water was used to dilute samples and standards.

Sample preparation

The various samples obtained from the food and animal parts were reduced to small pieces, oven dried at 80°C to constant weight. Each sample was pulverized in a coffee mixer mill 800 to less than 200 mesh (<74μm) using alumina ceramic cylinders.

Sample digestion for Atomic Absorption Spectography determination

A 2.5g ground oven-dried sample was placed in a 50cm³ long neck digestion flask. 10cm³ of concentrated acid mixture was added with occasional string to obtain a homogenous mixture and digested slowly at 70°C using a hot plate. 5.0 cm³ portion of the acid mixture was later added and the temperature was increased to 100°C and maintained until approximately 5.0 cm³ solution remained.

The solution was allowed to cool and few drops of hydrogen peroxide were added and heated gently. The heating was continued until the digest was clear. The final digested solution was transferred into 50cm³ calibrated flasks and diluted to volume with de-ionized water. A blank digest was carried out in the same way.

Analysis

Aliquots of the digest were analyzed for the trace elements and heavy metals by flame Atomic Absorption Spectrophotometry (GBC Avanta version 1.31). Copper was analyzed

by using NBS certified reference materials that were digested together with the samples and analyzed quantitatively using the same procedure.

Determination of serum parameters

Serum total protein was analyzed using the Biuret method, Inorganic Phosphate was analyzed using Fiske-Subarrow method and Modified Jaffe method (17) was used in Creatine analysis. Other parameters measured were cholesterol, alkaline phosphatase, glutamate oxaloacetate transaminase and glutamate pyruvate trans-aminase. The cyanide content of the pulp and gari was determined by silver nitrate titration procedure.

RESULTS AND DISCUSSION

The mean weekly food intake of rats according to the diet group is presented in Table 2. Food intake varies significantly across the Diet group. The albino rats on Diet IV had the highest food intake (215.91±15.5g) followed by Diet III (213.06±9.3g), Diet II (203.35±11.0g) and then Diet I (200.89±13.8g) in descending order. There was no significant difference (P>0.05) in the levels of food intake of the experimental animal. The mean body weight and percentage gain in body weight according to Diet group is presented in Table 3. The least percentage weight gain (27.9%) occurs in Diet IV-Kokori/SPDC followed by Diet II-Kokori (37.4%), Diet III-Kokori/Ogbomoso (44.7%) and Diet I-Ogbomoso (55.3%) in ascending order. Diet I thus promote highest percentage gain in body weight. Comparison of feed intake with relative percentage weight shows that weight gain is not proportional to the feed intake in rats fed on Diets II and IV. Albino rats on Diets II and IV had a negative food utility index calculated as ratio of the average weekly body weight gain to food consumed. Rats on Diets I and III had a proportional weight gain of 55.3% and 44.7% respectively relative to the food consumed. The result shows improved food utility index with reduction in the pollutant level as found by Tremblay *et al.* (9).

The proximate composition of the experimental diets (g/100g) is presented in Table 4. The values obtained for crude protein were not statistically different from each other. The closeness of the proximate values is an indication that all the diets contained identical basic food ingredients (P>0.05).

Table 2. Weekly food intake (g) of rats fed on experimental diets

Diet Group	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Total
DIET I	207.4 ±32.9	200.1 ±30.2	200.0 ±26.0	190.6 ±15.5	200.0 ±16.3	197.3 ±16.6	199.4 ±11.8	191.6 ±13.2	205.3 ±10.9	208.5 ±7.3	206.1 ±10.7	205.6 ±11.25	200.7 ±2.4	200.89 ±13.8
DIET II	204.2 ±21.6	216.3 ±2.0	197.1 ±8.4	184.2 ±27.0	202.3 ±30.2	196.2 ±29.0	191.1 ±29.6	206.8 ±8.4	205.6 ±6.2	208.3 ±2.3	213.2 ±1.8	209.5 ±0.7	208.8 ±1.9	203.35 ±11.0
DIET III	213.9 ±21.6	223.6 ±2.0	207.1 ±23.6	203.5 ±30.6	211.8 ±17.9	219.3 ±2.0	218.5 ±14.1	217.1 ±13.1	207.0 ±7.5	207.8 ±7.2	213.4 ±6.1	218.8 ±5.9	208.0 ±2.2	213.06 ±9.83
DIET IV	211.8 ±31.9	216.7 ±47.4	196.0 ±33.8	190.9 ±23.8	216.8 ±18.1	225.6 ±15.3	234.4 ±9.3	239.2 ±6.6	222.1 ±1.9	224.2 ±7.6	212.8 ±2.2	208.8 ±6.8	207.5 ±9.3	215.91 ±15.5

Table 3: Body weight (wt) Gains of rats

Diet Group	Weeks 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Wt gain (g)	Gain %
DIET I	260.0 ±5.2	268.4 ±3.2	280.1 ±11.2	289.6 ±7.5	299.7 ±9.0	307.5 ±5.8	318.6 ±16.6	330.4 ±9.2	341.0 ±6.9	353.8 ±11.3	236.5 ±9.4	386.1 ±6.4	399.9 ±7.0	408.9 ±8.8	143.9	55.3
DIET II	250.0 ±5.0	261.2 ±6.2	269.0 ±9.4	274.2 ±8.2	283.8 ±6.0	291.0 ±2.0	299.3 ±3.3	308.1 ±6.9	317.2 ±11.2	326.5 ±3.3	335.9 ±2.9	340.3 ±1.2	344.3 ±10.1	343.7 ±7.5	93.7	37.4
DIET III	255.5 ±2.1	261.4 ±5.0	268.8 ±7.0	277.6 ±3.2	288.5 ±4.8	297.2 ±12.2	306.8 ±9.6	313.8 ±3.2	322.4 ±10.0	336.2 ±6.6	343.5±3 .5	350.4 ±4.9	350.8 ±12.1	369.9 ±8.2	114.4	44.7
DIET IV	252.3 ±7.1	260.0 ±6.3	267.0 ±7.0	175.4 ±6.5	280.8 ±3.0	290.1 ±2.1	289.0 ±8.2	295.8 ±6.4	304.3 ±10.1	311.3 ±7.0	319.1±3 .8	316.2 ±4.2	321.6 ±11.2	322.7 ±9.4	70.4	27.9

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Table 4. Proximate composition of the experimental diets (g/100g)

Components	DIET I	DIET II	DIET III	DIET IV
Moisture	3.0	3.3	3.4	3.7
Crude Protein	12.3	12.5	12.6	12.5
Ether Extract	9.1	9.6	9.5	9.6
Ash	2.8	2.7	2.8	2.7
Crude Fiber	3.8	3.8	3.8	3.7

The metal ion composition of the feed composite and the experimental diets is presented in Table 5 and 6 respectively.

Table 5. Mineral Composition of Cassava and Smoked Fish

Diet	Concentration (mg/Kg) Metal									
	Zn	Cd	Cu	Pb	Ni	Fe	Cr	Mn	As	Hg
Raw Cassava	236.3 ±4.7	35.5 ±0.3	61.3 ±1.5	150.3 ±3.0	533.3 ±13.3	1394.0 ±27.9	4.8 ±0.0	250.1 ±5.9	9.3 ±0.4	3.3 ±0.1
Smoked Fish	109.1 ±2.2	54.4 ±1.1	34.5 ±1.1	307.1 ±6.1	428.8 ±5.7	3264.0 ±13.6	2.7 ±0.0	306.3 ±4.6	8.6 ±1.0	5.3 ±0.2

Table 6: Mineral Composition (mg/Kg) of Formulated Diet

Diet	Zn	Cd	Cu	Pb	Ni	Fe	Cr	Mn	As	Hg
I	253.2 ±6.3	32.1 ±0.7	81.4 ±2.1	150.0 ±2.8	262.7 ±5.2	1642.7 ±21.3	4.3 ±0.2	101.5 ±6.3	ND	ND
II	296.8 ±7.7	51.7 ±1.8	141.5 ±3.8	562.2 ±8.3	1240.1 ±15.3	2670.1 ±22.8	6.0 ±0.2	127.2 ±3.6	Tr	Tr
III	548.7 ±11.3	42.0 ±3.4	189.4 ±6.2	591.3 ±6.8	800.9 ±4.3	2600.6 ±19.7	21.0 ±1.27	98.5 ±9.9	Tr	Tr
IV	716.6 ±15.9	48.1 ±5.6	148.2 ±2.1	533.4 ±4.3	1360.9 ±17.3	1351.2 ±11.3	16.9 ±1.1	115.2 ±3.5	Tr	Tr

ND- Not Determined Tr- Trace amount

There was a high concentration of nickel, lead, cadmium and chromium in the raw materials used for feed formulation (cassava/fish). The high levels of these metals can be attributed to the rearing environment of the fish (polluted water bodies in the Niger Delta region of Nigeria) and accumulation during sundrying (smoking) in the presence of flared gas. Iron was the predominant nutritive metal followed by zinc, manganese and copper. Mercury (Hg) and Arsenic (As) were not detected in Diet I while only trace amounts was found in Diets II, III and IV; this is attributable to the varying source of cassava tubers and difference in processing method.

A comparison between Diet I and III that were processed in Ogbomoso shows that Diet I has less metal concentration because the cassava tubers were from the south-west region. Diets II, III and IV were high in lead, nickel, copper and iron. Diet III has significantly ($P<0.05$) lower levels of cadmium, nickel and manganese probably as a result of the thorough and prolonged processing method even though it was cultivated in Kokori. Diet IV prepared in the SPDC method contained significantly high levels of the toxicants though the processing period was over three days. Though significant reduction ($P<0.05$) in the concentration of some toxicants is observed and this varies with the processing method, high levels of toxicants are inherent in the samples from Kokori. Although the high level of the iron could be health promoting but the inimical effects of the other non-beneficial metal has a more profound effect on health and well being.

The protein concentration and protein efficiency ratio (PER) of each diet regimen is as presented in Table 7. Diet I has the highest protein efficiency ratio while Diet II with the highest protein content (16.3) had the least PER of 0.9. Diet IV has a protein content of 13.8 and PER of 1.1. This result shows that the degree and duration of processing of cassava products significantly affect either the bio-availability or the bio-utilization of the protein content. Kinney and Tucker (18) described that toxicants forms complexes such as dimmers or trimers with protein molecules and makes them resistant to digestive enzymes thus reducing the

bio-availability of protein. The presence of toxicants therefore reduces protein digestibility and consequently the protein quality. Statistical analysis shows no significant difference between the values of protein content and PER of Diets I and III.

Table 7. Food Intake, Weight Gain, Protein Content and Protein Efficiency Ratio

Diets	Food Intake (10 days)	Weight Gain (10 days)	Protein Content	PER
I	134.0	27.5	15.0	1.91
II	129.0	14.9	16.3	0.9
III	121.0	23.7	14.1	1.7
IV	108.0	15.1	13.8	1.1

Depressed growth was observed in rats on Diets II and IV, consequently selected organs in all the experimental rats were weighed. The percentage body weight for the livers is presented in Table 8. The least percentage gain in weight occurred in rats placed on Diet IV. Jequier and Tappy (19) attributed distension of the lipid bilayer leading to oedema to toxicants load. Similar values were observed for the kidney and spleen in both diets II and IV. The values observed for brains are not statistically different.

Table 8. Weights of organs as percentage of Body Weight

Diet Group	Animal wt	Liver weight		Kidney weight		Spleen weight		Heart weight		Brain weight	
		g	%BW	g	%BW	g	%BW	g	%BW	g	%BW
Diet I	260.0	4.97±0.6	1.91±0.0	1.10±0.2	0.42±0.0	0.29±0.0	0.11±0.0	0.63±0.3	0.24±0.0	1.51±0.1	0.58±0.0
Diet II	250.0	5.55±0.3	2.22±0.0	1.23±0.4	0.49±0.0	0.38±0.0	0.15±0.0	0.66±0.1	0.26±0.0	1.64±0.1	0.66±0.0
Diet III	255.5	5.49±0.9	2.15±0.0	1.17±0.2	0.45±0.0	0.44±0.0	0.17±0.0	0.50±0.0	0.23±0.0	1.71±0.1	0.67±0.0
Diet IV	252.0	6.05±0.5	4.40±0.0	1.29±0.2	0.51±0.0	1.02±0.1	0.40±0.1	0.63±0.0	0.25±0.0	1.78±0.0	0.71±0.0

Legend: g – gram; %BW- percentage body weight

CONCLUSION

The study shows that pollutants load in cassava depend on the environment of cultivation. Processing is effective in reducing the levels of these pollutants but effectiveness varies with period and method of processing. The adoption of appropriate timing and method of processing can minimize the pollutant intakes and thereby increase the nutrient availability from cassava products, the environment influences the toxicants load in cassava based diets.

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