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Contents of nitrosamine and its precursors in some roasted Nigerian food grains, tubers and animals and their potential ingestion in the diet

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ABSTRACT

Nitrate, nitrite and N – nitrosamine levels were determined in three varieties of food materials. Nitrate in tubers varied from 60.00 mg/kg in cocoyam (Colocasia esculenta) to 108.00 mg/kg in white yam (Discorea rotunda), while nitrite was from 23.67 mg/kg in cocoyam (Colocasia esculenta) to 80.00 mg/kg in white yam (Discorea rotunda), and nitrosamine was from 0.05 mg/kg in cocoyam (Colocasia esculenta) to 0.12 mg/kg in white yam (Discorea rotunda) varieties. Their levels in grains varied between 38.00 mg/kg in white maize (Zea may) and 82.00 mg/kg in coconut (Cocos nucifera) for nitrate and 10.00 mg/kg and 52.00 mg/kg for nitrite while nitrosamine was from 0.02 mg/kg in yellow maize (Zea may) to 0.05 mg/kg in cashew nut (Anacardium occidentale). Nitrate in meats varied between 47.67 mg/kg in cow meat to 63.33 mg/kg in fish (Tilapia), while nitrosamine was from 0.09 mg/kg in pork to 0.14 mg/kg in fish (Tilapia). On roasting, nitrite and nitrosamine increase in the food samples. Daily intake estimated that the nitrate, nitrite and nitrosamine contents of the food tubers, grains and meats seem to be higher than current acceptable daily intake.

Key Words: Nitrate, nitrite, n-Nitrosamine, roasted foods materials grains, tubers, meat, Nigeria.

INTRODUCTION

The relationship of nitrate and nitrite to infant methaemoglobinaemia and to the possibility of formation of carcinogenic nitrosamine has been well reviewed and appreciated (1); (2). Nitrite is reported to arise from microbiologic reduction of nitrate in foods or drinking water which such foods are stored at room temperature (3) ;(4). Hence if nitrate ions were not reduced to nitrite, little or no concern should be felt about its level in foods and drinking water (5).

N – Nitrosamines have been considered as an environmental problem. Since the early 1960. This is as a result of many literature reports on the hazards of nitrates and nitrites in food as precursor

of caranogenic nitrosamine (6); (7). During the past three decades, there have been considerable interests in the analysis of food for volatile N- Nitrosamine because of the known carcinogenicity and mutagenicity of these compounds. Nitrosamines which are one of the groups of nitroso compounds are formed when primary, secondary or tertiary amines react with nitrogen oxides or nitrite obtained from nitrite salts or nitrous acids.

The conversion of nitrites or nitrates to N-Nitrosamines is dependent on particular precursors, nature of catalyst such as ozone or metal ions and the $_{P}$H of the medium used (8).

The agronomic practice of large application of nitrogenous fertilizer to obtain heavier yields and improper disposal of human and animal waste may lead to accumulation of nitrite in food plants (9). In this regard, high amounts of nitrate, nitrite and nitrosamine could possibly be present in roasted foods and could pose health risks especially to infants.

In view of the health implications of these factors and the need to safe guide the public health, this study seeks to determine and evaluate the nitrosamine and its precursor's content of some commonly consumed roasted food grains, tubers and animals from Nigeria.

MATERIALS AND METHODS

Treatment of samples

Three varieties of tubers (white yam, Groundnut, Cocoyam), four varieties of grains (Yellow maize, White maize, Cashew nut, Coconut) and four of animals (Fish, Bush meat, Cow meat, Pork) were collected from the Oba Market, Akure in Ondo State, Nigeria. Twenty each of the roasted tubers, grains, animals and raw food materials were macerated with 80ml of double distilled water until fine slurry was formed. The slurry was then centrifuged. A spatula full of mercuric chloride was added to the supernatant as a deproteinizer. The mixture was allowed to stand for 15mins and then it was filtered through whaltman No. 32 filter paper to obtain a clear sample extract.

Determination of Nitrite

An aliquot (10 – 40ml) of sample filtrate was transferred to a 50ml volumetric flask. Then 2.5ml sulfanilamide reagent (0.5g sulfanilamide in 150ml 15 %(v/v) acetic acid) was added and mixed. After 5mins; 2.5ml of NED reagent (0.2g N - (1 –naphythyl) ethylenediamine – 2 – HCL in 150ml 15% acetic acid) was added, dilute to volume, mixed and held 15 min for colour development. Absorbance was read at 540mm against a blank of 45ml water, 2.4ml sulfanilamide reagent, and 2.5ml NED reagent (10). The standard curve was prepared by adding 10, 20, 30 and 30ml of sodium nitrite working solution (1 mg/l sodium nitrite) to 50ml volumetric flasks, followed by addition of NED and other reagent as described for samples. The standard curve was a straight line to 1mg/L sodium nitrite in final solution (11)

Determination of nitrate

Another 10ml aliquot of the solution obtained after ion-exchange clean up was mixed with 5ml NH_4CL butter; PH9.6, prepared as follows: 20ml of HCL were diluted to 500ml with water, mixed with 50ml NH_4OH, then brought to 1:1 with water. The buffer PH was adjusted to PH9.6 with HCL or NH_4OH as needed. The diluted, PH adjusted sample solution (10 sample aliquot +

5ml buffer) was then passed through a cadmium (Cd) column to reduce all nitrate to nitrite. Nitrite concentration was then determined. This value was a measure of total nitrite (Nitrite + Nitrate) sample nitrate concentration = total nitrite – free nitrite. The value for sample nitrate was multiplied by 1.23 to obtain results expressed as sodium nitrate (12).

Anion – exchange clean up

The roasted foods were passed through an ion-exchange column to reduce turbidity or coloured extracts that interfere with the final colorimetric estimation step (Sen and Lee, 1979). About 100g of the resin (Dowex 1 – X_1, 50 – 100 mesh, chloride form, strongly basic anion exchange resin, J.T Baker chemical Co. Phillipsburg, N.J) was allowed to soak in water over night. A 25ml glass burette was used to prepare the column. The dimensions of the resin bed were about 3 cm high x 1 cm diameters. The resin column was first washed until the PH of the washings was 7 – 8 care was taken to keep the resin bed filled with liquid at all stages. A fresh column was used for each analysis.

A 2 – 25ml aliquot of filtrate (depending upon the expected nitrate concentration in the sample) was adjusted to PH 7 – 8 by the addition of 1M NaOH, and the solution was passed through the resin column at a flow rate of 2 – 4ml/min. (13). The column was then washed with 50ml of water and the washing discarded. Finally, the nitrate and nitrite from the column were eluted with 20ml of sodium chloride solution. The eluate was collected and brought to 25.0ml in a volumetric flask. The solution was mixed well and used for the colorimetric estimation of free nitrite and nitrate.

Preparation of cadmium column

Metallic zinc sticks (3 -5) were placed in each of two 800ml beakers containing 50ml CdSO4 solution. Zn sticks were removed every 2 – 3 hours and spongy metallic Cd was scrapped off by rubbing the sticks against each other. The Cd must be removed with aqueous solutions at all times. After 6 – 8hours, the solution was discounted scrapings were then blended for 2 – 3 secs in a high speed blender. The blended materials were passed through 8 – 40 mesh sieves, and the particles on the 40 mesh sieves were retained. Blending and sieving of large particles were repeated to increase the yield of 40 mesh particles. The particles were washed in a beaker of O.IM HCL, stirring occasionally with a glass rod and left overnight in acid. Particles were then stirred to de-gas, decanted, and washed again with two 500ml portions of water.

A 50ml calibrated buret was used for the Cd column. The buret was plugged with glass wool and filled with water. The spongy Cd particles were added to a depth of 8 – 10cm, draining occasional, but taking care not to let the liquid level fall below the top of the Cd bed.

The Cd column was washed with 25ml NH_4OH buffer just before use, and drained to the top of the Cd bed. The sample filtrate was passed through the Cd column at a rate of 2 – 5ml/min, and effluent was collected in a 50ml volumetric flask. After the sample has passed, the column was washed with 15ml water. The wash water was determined using a standard solution (1mg $NaNO_3$/ml; Sen and Donaldson, 1978). Column efficiency was > 90%.

Determination of nitrosamine

Ammonium sulphamate was added to 10g of the roasted food samples to stabilizer any N-nitrosamine and also as a free nitrite scavenger. An agneous sodium chloride solution was then added to liberate the nitrosamine from the nitrosamine-water emulsion. The aqueous mixture was quantitatively transferred to a separating funnel where it was extracted with pentane to remove any non polar components. The aqueous phase was extracted with ethylacetate and the organic phase was washed with water and then dried with (Na_2SO_4). The solvent was concentrated in vacuo using a rotary evaporator. The residue was dissolved in glacial acetic acid and an aliquot of denitrosation reagent (3% v/v) HBr in glacial acetic acid) was added. Sulphanilamide was mixed with the test aliquot and the N – naphythyl reagent was added. The absorbance of the test sample was measured at 540nm using spectrophotometer 20 (14).

RESULTS AND DISCUSSION

Table 1 shows the nitrate, nitrate and nitrosamine levels in the raw and roasted food grains, tubers and meats. Nitrate levels in raw tuber (white yam, groundnut, and cocoyam) varied from 60.00mg/kg in cocoyam to 108.00mg/kg in white yam varieties. These are much lower values than the 500mg/kg nitrate limit recommended by WHO/FAO (15). The nitrate values in the raw tubers and grains were much higher (Table I) and this may be the result of residual nitrogen from organic fertilizer applied during planting (16).

Nitrate contents of grains (yellow maize, white maize, cashew nuts, coconuts) varied from 38.00mg/kg in white maize to 82.00mg/kg bush meat, cow meat, and pork) ranges from 47.67 mg/kg of cow meat to 89.00mg/kg of pork

Nitrite levels in the tubers ranges from 23.67mg/kg in cocoyam to 80.00mg/kg yam, while in grains the range is between 10.00mg/kg and 52.00mg/kg. These are high levels higher than the limits of the recommended normal acceptable daily intake (ADI), level (0.1mg/kg body weight). The nitrite levels for meats range between 25.00 mg/kg and 63.33mg/kg.

The nitrosamine levels in the tubers (yam, groundnut and cocoyam) varied from 0.05mg/kg in cocoyam to 0.12mg/kg in yam varieties. Nitrosamine levels in grains (yellow maize, white maize, coconut, cashew nut) varied from 0.02mg/kg in yellow maize to 0.05mg/kg in cashew nut, while nitrosamine levels in meats (fish, bush meat, cow meat, pork) varied form 0.09 mg/kg to 0.14 mg/kg in fish.

These are much higher values than the American Food and Drug Administration (FDA) action level. Higher levels may result from microbial reduction of nitrate stored under in appropriate conditions (17)

While roasting process increased the nitrite contents in all the samples (Table I), the nitrate levels increased in the tuber, grains and meat samples. Also, the nitrosamine levels increased in all the samplers as shown in table I. Similar changes in levels of nitrate and nitrite in food after cooking were observed for vegetables by Ezeagu and Fafunso (18).

The apparent difference in the nitrate, nitrite and nitrosamine contents between the raw and roasted food sample might be due to reconstitution and chemical interactions between the various component effect by heating or boiling during processing (19). An increased nitrate, nitrite and nitrosamine level in roasted tubers (yam, groundnut and cocoyam), roasted grains (maize, cashew nut, and coconut), and roasted meat (fish, bush meat, cow meat, pork) was observed result consistency of samples, quality of heat applied or systematic error. On the other hand some intrinsic factors such as nitrogenous compounds may have decomposed into nitrates and nitrosamines.

The daily intakes of various food grains, tubers and meats are 18.5g, 39.3g, 7.2g, 0.6g, 3.11g, 2.21g, 1.25g, 0.45g, 0.81g, and 0.2g respectively for yam, groundnut, cocoyam, maize, cashew nut, coconut, fish, bush meat, cow meat and pork (Field survey data, July – Oct 2010.)

Based on the mean nitrate/nitrite/nitrosamine contents of each tuber types the potential daily intake of nitrate from the roasted food tubers ranges from 0.104mg/kg in groundnut to 11.203mg/kg in yam (Table 2) for average consumer (70kg body weight) (Bowen, 1966) therefore, the total daily intake of nitrate would be 16.320mg (0.23mg/kg body weight) which is quite below the limit of WHO/FAO (WHO, 1974). The potential daily intake of nitrate from the roasted food grains from 0.062 in coconut to 0.138mg/kg in maize (table 3) and for daily intake of nitrate from the roasted food meats ranges from 0.20 in pork to 0.81mg/kg in cow meat (table 4). For average consumer (70kg bodyweight) (Bowen, 1996) therefore the total daily intake of nitrate would be 23.426mg 90.33mg/kg0 body weight which is quite below limit of WHO/FAO 9WHO, 19740 ADI of 3.7mg (5mg $NaNO_3$)/kg body weight allocated to nitrate, through a later study has recommended 18.5mg (45mg $NaNO_3$)/kg body weight (20). Conversely, the daily total nitrite intake from the tubers of 0.070mg, 0.047mg of grains and 3.998mg for meats appears to be significant relative to the recommended ADI of 0.2mg $NaNo_3$/kg body weight.

The daily total N – Nitrosamine intake from the tubers of 0.033mg, 0.014mg of grains and 0.579mg of meats appears to be significant relative to the recommended value of FDA.

Table 1: Level of nitrate, nitrite and nitrosamine in raw and roasted tubers, grains and animals (mg/kg)

		Nitrate			Nitrite			Nitrosamine		
		Raw	Roasted	% changes	Raw	Roasted	% changes	Raw	Roasted	% change
Tuber:	white yam	108.00	175.00	(+)62.01	80.00	100.00	(+)25.00	0.12	0.23	(+)91.67
	Groundnut	60.00	113.00	(+)88.33	32.00	50.00	(+)56.25	0.07	0.12	(+)71.43
	cocoyam	60.00	88.00	(+)46.67	23.67	38.00	(+)60.54	0.05	0.10	(+)100.00
Mean		76.00	125.33	-	45.22	62.67	-	0.08	0.15	-
Grains:	Yellow maize	47.00	100.00	(+)112.77	20.00	37.00	(+)85.00	0.02	0.11	(+)450
	White maize	38.00	63.00	(+)65.79	10.00	26.00	(+)160.00	nd	0.04	(+)0
	Cashew nut	62.00	120.00	(+)93.55	32.00	58.00	(+)81.25	0.05	0.15	(+)200
	Coconut	82.00	113.00	(+)37.81	52.00	63.00	(+)21.16	0.04	0.12	(+)200
Mean		57.25	114.75	-	28.5	46.00	-	0.03	0.11	-
Animals:	Fish	86.00	162.00	(+)88.37	63.33	87.00	(+)37.38	0.4	0.21	(+)50
	Bush meat	89.33	138.00	(+)54.48	58.00	78.00	(+)34.48	0.09	0.18	(+)100
	Cow meat	47.67	87.33	(+)83.20	25.00	51.00	(+)104.00	0.12	0.16	(+)33.33
	Pork	89.00	188.67	(+)111.99	48.00	112.00	(+)133.3	0.09	0.18	(+)100
Mean		78.00	144.00	-	48.58	82.00	-	0.11	0.1	-

Means of two independent determinations; (+) increase

Table 2: Estimated daily nitrate, nitrite and nitrosamine intake from roasted tubers, grains

		Daily consumption (g)	Estimated daily intakes		
			Nitrate (mg)	Nitrite (mg)	Nitrosamine (mg)
Tuber:	yam	18.50	11.203	0.041	0.018
	Groundnut	39.30	0.104	0.006	0.001
	cocoyam	7.20	5.013	0.023	0.014
Total		65.00	16.320	0.070	0.033
Grains:	maize	0.60	0.138	0.039	0.012
	Cashew nut	3.11	0.093	0.006	0.001
	Coconut	2.21	0.062	0.002	0.001
Total		5,92	0.293	0.047	0.014
Meats :	Fish	1.27	0.027	0.349	0.222
	Bush meat	0.45	6.225	0.618	0.011
	Cow meat	0.81	10.325	1.608	0.022
	Pork	0.20	5.849	1.314	0.324
Total		2.75	23.426	3.889	0.579

CONCLUSION

From the standpoint of nitrite and nitrosamine toxicity based on its levels before ingestion, the food grains; tubers and meats in this study will make very little contribution to nitrate, nitrite and nitrosamine intake by adults or infants and thus pose no hazard. However, it must also be noted that the food grains, tubers and meats are roasted with coals before consumption and are most often, consumed with other food crops. The nitrogen oxide from the coal that react with the amine in the food sample during roasting and nitrate, nitrite and nitrosamine of the other items than make up the diet add up to the total intake. The finding in the study therefore need further verification by more detailed studies, which would include a wider volume of food materials as well as roasting methods. Due to the high sensitivity of young infants, nitrates, nitrites and nitrosamine would be determined more often particularly in plant foods and the method of processing.

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