Assessment of Nitrate, Nitrite and N-Nitrosamines of some Roasted Food Materials in Nigeria.

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ABSTRACT
The nitrate, nitrite and N – Nitrosamines concentration in twelve roasted food materials from western Nigeria were determined. The mean concentration (mg/kg) of nitrate in Oshogbo ranged from 29.00±0.00 Zea may to 103.00±3.61 Discorea rotunda, nitrite ranged from 60.00±1.00 Zea may to 163.00±1.00 pork meat, and nitrosamines ranged from 0.07±0.01 Zea may to 0.23±0.01 Discorea rotunda. The mean concentration (mg/kg) of nitrate in Ado-Ekiti ranged from 22.00±0.00 Zea may to 96.00±2.00 Discorea rotunda, nitrite ranged from 49.00±0.00 Zea may to 188.67±12.53 Tilapia, and nitrosamines ranged from 0.05±0.01 Zea may to 0.19±0.02 Tilapia. The mean concentration (mg/kg) of nitrate in Ibadan ranged from 26.00±0.00 Zea may to 114.00±2.00 pork meat, nitrite ranged from 60.00±0.37 Zea may to 182.00±1.72 Discorea rotunda, and nitrosamines ranged from 0.06±0.00 Zea may to 0.34±0.01 Tilapia. The mean concentration (mg/kg) of nitrate in Akure ranged from 22.00±1.12 Zea may to 101.00±0.58 pork meat, nitrite ranged from 62.33±0.33 Zea may to 178.33±2.40 pork meat, and nitrosamines ranged from 0.07±0.00 white maize Zea may to 0.28±0.01 Tilapia. The mean concentration (mg/kg) of nitrate in Abeokuta ranged from 24.00±1.12 Zea may to 109.01±0.52 pork meat, nitrite ranged from 69.23±0.21 Zea may to 173.00±1.72 Tilapia, and nitrosamines ranged from 0.06±0.00 Zea may to 0.29±0.01 Tilapia. The results show the presence of high concentration of nitrite and N – nitrosamine toxic and carcinogenic contaminants, hence roasted foods should be discouraged.


INTRODUCTION
Nitrates, nitrites and nitrosamines have been considered as an environmental problem since the early 1960. This is as a result of many literature reports on the hazard of nitrates and nitrites in food as precursors of carcinogenic nitrosamine (1)

Nitrite is known to be a precursor for toxic and carcinogenic N – nitrosamines (2) and induces concern in experimental animals (3). After ingestion, residual nitrite can form traces of certain N – nitroso compounds in stomach (where the PHV 7) on reacting with secondary amines which might also be present in the ingested food (4). Nitrate can also interact with hemoglobin forming methaemoglobin by oxidation of ferrous iron (Fe2+) to ferric state (Fe3+) presenting or reducing the ability of blood to transport oxygen, a condition described as methaemoglobinæmia (5, 6). Nitrate on the other hand can be reduced to nitrite in vivo. The ingestion of 8 – 15g nitrate can cause abdominal pain, blood in stool and urine, weakness and collapse (7). Chronic ingestion of small dose can cause dyspepsia, mental depression and headache (8).

The potential sources of nitrates and nitrites in our food include soil organic matter, the use of nitrogenous fertilizers and herbicides in industrial agriculture livestock and human excrement and other organics wastes from chemical industries, domestic waste and septic tank effluents (9, 10, 11).

Nitrosamines which are one of the groups of nitroso compounds are formed when primary, secondary, tertiary amines react with nitrogen oxides or nitrites obtained from nitrite salts or nitrous acids, the conversion of nitrite or nitrate to nitrosamine as ozone or metal ions and the H of the medium used (12).

Most N – Nitrosamine have been shown to be carcinogenic in laboratory animals (13) and there is concern about health hazard to man who consumes precursors of Nitrosamine.

This study is therefore aimed at measuring the concentration of nitrates, nitrites and nitrosamines of some roasted local food materials that are distributed in some parts of South-West Nigeria.
MATERIALS AND METHODS

Samples of roasted food materials fish(Tilapia), cashew nut(Anacardium occidentale), white yam (Discorea rotunda), plantain (Musa sapientum), groundnut (Arachis hypogaea), cocoyam (Colocasia esculenta), bush meat, pig meat, coconut (Cocos nucifera), white maize (Zea may), yellow maize (Zea may) were purchased from Ado-Ekiti, Akure, Abeokuta, Osogbo and Ibadan in South Western Nigeria. Ten each of the roasted 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 Whatman 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 – naphthyl) 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 540nm against a blank of 45ml water, 2.4ml sulfanilamide reagent, and 2.5ml NED reagent (14). 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.

Determination of Nitrate

Another 10ml aliquot of the solution obtained after ion-exchange cleanup was mixed with 5ml NH4Cl buffer; pH9.6, prepared as follows: 20ml of HCl were diluted to 500ml with water, mixed with 50ml NH4OH, then brought to 1:1 with water. The buffer PH was adjusted to pH9.6 with HCl or NH4OH 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 (15).

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. About 100g of the resin (Dowex 1 – X1, 50 – 100 mesh, chloride form, strongly basic anion exchange resin, J.T Baker chemical Co. Phillipsburg, N.J) was allowed to soak in water overnight. 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. 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 and the material was 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 NH4OH 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.

The wash water was determined using a standard solution 1mg NaNO3/ml; (16). 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 aqueous 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 ethyl acetate and the organic phase was washed with water and then dried with (Na2SO4). 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 – naphthyl reagent was added. The absorbance of the test sample was measured at 540nm using spectrophotometer 20 (17).

RESULT AND DISCUSSION

The results from this study show that all the roasted food samples analyzed, market, distributed and consumed within South Western Nigeria contained detectable amounts of nitrates, nitrite and nitrosamine in varying concentration. For nitrates, the mean concentration in mg/kg ranged from 29.00 ± 0.00 white maize to 103.00 ± 3.61 white yam, 22.00 ± 0.00 white maize to 96.00 ± 2.00 white yam, 26.00 ± 0.00 white maize to 114.00 ± 2.00 pork meat, 22.00 ± 1.12 white maize to 101.00 ± 0.58 pork meat and 24.00 ± 1.12 white maize to 109.01 ± 0.52 pork meat for roasted food materials in Osogbo, Ado-Ekiti, Ibadan, Akure and Abeokuta respectively (see Table 1 - 5). From the results, nitrate occurred in much lower amount than nitrite which is consistent with the relative stability of these ions.

The nitrite results from Abeokuta, Ibadan, Osogbo, Ado-Ekiti and Akure showed that the roasted food materials values ranged from 69.23 ± 0.21 white maize to 173.00 ± 1.72 fish, 60.00 ± 0.37 white maize to 182.00 ± 1.72 white yam, 60.00 ± 0.37 white maize to 182.00 ± 1.72 white yam.
± 1.00 white maize to 163.00 ± 1.00 pork, 49.00 ± 0.00 white maize to 188.67 ± 1.53 fish, 62.33 ± 0.33 white maize to 178.33 ± 2.40 mg/kg NO2 pork respectively (Table 1 - 5). These values exceeded the WHO’s (1978) recommended acceptable daily intake (ADI) of nitrite which is stipulated at 0.2mg/kg body weight for children under 10kg weight, while the levels of nitrate in the samples is higher than WHO’s ADI for nitrate which is set at 5mg/kg body weight. However, the risk to human with respect to methaemoglobinemia and conversion of nitrate to nitrite in oral cavity and stomach leading to the possible formation of nitrosamines cannot be ignored. Chronic ingestion of small dose of nitrate can also cause dyspepsia, mental depression and headache (Siddiqi et al, 1988).

For children at 15 to 20kg of body weight consuming one serving of these products suggest ingesting about 45.5 to 60.7% respectively of ADI (for least nitrite level) and 99.25 to 132.33% of ADI (for highest level of nitrite).

Nitrosamine was also detected in the roasted food samples together with nitrate and nitrite. The concentration of nitrosamines measured in samples from Abeokuta ranged from 0.06 ± 0.00 white maize to 0.29 ± 0.01 fish; Ibadan from 0.06 ± 0.00 white maize to 0.34 ± 0.01 fish; Akure from 0.07 ± 0.00 white maize to 0.28 ± 0.01 fish; Osogbo from 0.07 ± 0.01 white maize to 0.23 ± 0.01 white yams and Ado-Ekiti from 0.05 ± 0.01 yellow maize to 0.19 ± 0.02 mg/kg fish. N–Nitrosamines are known toxicants as well as chemical carcinogens. Investigators have also demonstrated nitrosamine formation mainly in attempt to determine the extent of formation under human gastric conditions (18,19).

An observation of interest is that those roasted food with the highest concentration of nitrate; nitrite and nitrosamine are produced in Ibadan and Abeokuta, cities where wood and wood coals were used predominantly for roasting.

The apparent difference in the nitrate contents between the food samples might be due to constitution and chemical interactions between the various components effect by heating on boiling during processing.
CONCLUSION

In this work we have demonstrated the presence of high concentration of nitrite in roasted food commonly consumed in Nigeria as well as N – nitrosamine contamination. However, a more analytical method such as thermal energy analyse (TEA) need to be used to ascertain the exert amount of the N – Nitrosamines and hence estimation of the potential toxic effect of such amount. It is important that the nature of the nitrosamine contained in those roasted foods be determined and the level of the volatility. It is only then, that the toxic and carcinogenic potential of these nitrosamines be ascertained.

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