PROXIMATE ANALYSIS AND SPECTROPHOTOMETRIC DETERMINATION OF SODIUM NITRITE IN CURED MEAT PRODUCTS SAMPLED IN NIGERIA

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ABSTRACT

Cured meat products which are mostly imported from other countries form part of the diet of a large percentage of the Nigerian population. Elevated sodium nitrite concentration frequently found in cured meat products has been found to have deleterious effects on humans, raising public health concerns. Sodium nitrite concentration was determined for 13 cured meat products randomly sampled and purchased from supermarkets and open markets in Nigeria using a Jenway 640517 UV/Vis spectrophotometer. The calibration curve was linear over the range of 0.0-1.2 ppm and the coefficient of determination (r^2) was 0.999. Nitrite concentrations within the range of 7.81 ± 0.8061 and 179.83 ± 0.2687 mg/kg were determined. Further, moisture content (%), ash value (%), and pH values were found to be within the ranges of 27.51 - 74.42; 1.18 - 3.31, and 4.65 - 6.45 respectively. A comparison of results with permissible values given by the World Health Organisation (WHO) and Food and Agricultural Organisation (FAO) revealed that 76.9% (n=10) of the samples conformed with the recommended nitrite concentration of 10-125 mg/kg whilst 23.1% (n=3) did not. This highlights the need for stringent regulatory control of nitrite concentrations in cured meat products to avoid nitrite toxicity.

Keywords: Cured meat products; nitrite toxicity; UV-Spectrophotometer; public health; regulatory control.
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INTRODUCTION

Cured meat products which are mostly imported from other countries form part of the diet of a large percentage of the Nigerian population. Primarily, meat is cured with sodium nitrite to prevent the growth of spore-forming bacteria *Clostridium botulinum* which causes food poisoning [1]. Sodium nitrite, aside from stabilizing red meat colour consequent of nitrosomyoglobin formation, and aiding flavour development in the preserved meat, also delays the development of oxidative rancidity [2-4]. The latter is by preventing the formation of toxic malonaldehyde consequently inhibiting food spoilage. The process of smoking meat eliminates oxygen [5]. Nitrite existing in two forms may be obtained as the pure chemical sodium nitrite (i.e., as dry powder which is mixed or ground into the meat) or as curing salt or pre-blend in which the sodium nitrite is distributed in and diluted by ordinary salt (sodium chloride) [6]. Foods to which sodium nitrite is added include hot dogs, ham, corned beef, bacon, hamburger, sausages, luncheon meats, salami, and other smoked or cured meat products [7].

Paradoxically, sodium nitrite is a toxic substance, and it is toxic to humans at an estimated lethal dose of one (1 g) in 70 kg adults (about 14 mg/kg) [8]. Its toxicity is manifested firstly, via the relaxation of smooth muscle, especially in the small blood vessels leading to reduced blood pressure. Secondly, oxidation of haemoglobin to methaemoglobin renders haemoglobin incapable of carrying oxygen thereby resulting in methaemoglobinaemia [9]. Thirdly, sodium nitrite has been linked to triggering migraines. Further, some studies have found a link between high processed meat consumption and colon cancer, possibly due to preservatives such as sodium nitrite [10, 11]. Frequent ingestion of meat cured with nitrite has also been linked to chronic obstructive pulmonary disease (COPD) [9]. Symptoms of nitrite poisoning include cyanosis, nausea, dizziness, vomiting, vertigo, collapse, gastroenteritis, tachypnoea, coma, convulsion, and death due to circulatory collapse [12]. Additionally, sodium nitrite has been reported to react with co-administered proteins [13] or endogenous amines and amides to form two classes of potent carcinogenic N-nitroso compounds namely: nitrosamines and nitrosamide-type compounds which include N-nitrosoureas, N-nitrosocarbamates, and N-nitrosoguanidines. These compounds are known to be carcinogenic, mutagenic, and teratogenic in experimental animals [14].

The total nitrosamines levels in several consumer products in Nigeria are considered high, compared to accepted standards [15, 16]. In a bid to guarantee the beneficial effect of sodium nitrite for meat curing only, its concentrations must be determined by suitable methods. Hence, various methods including colorimetric, potentiometric, and ultraviolet (UV) spectrophotometric derivatization in combination with high-performance liquid chromatography (HPLC) using oxidative amperometric detection have been used to determine nitrate and nitrite concentrations in meat [17-19], food grains [20]; potable water [21] and other products [22]. Other chromatographic techniques such as reverse phase - HPLC methods with UV absorbance electrochemical detection and ion chromatography have also been reported for the determination of nitrates and nitrites [23-25]. In Nigeria, the National

Agency for Food and Drug Administration and Control (NAFDAC) is the Federal agency responsible for testing or validating scientific data related to human safety of food additives.

However, no standard or permissible limit on sodium nitrite has been established in Nigeria and no information on the safety of meat products cured with sodium nitrite is currently available to the best of our knowledge. European legislation (EU Directive 95/2) [26] imposes a maximum residue of 50 ppm nitrite in cured meat products. Few dietary intakes estimate for nitrite has been published such as for local cereal food in Nigeria [27], hence, our study is one of the pioneer studies for the purpose of setting standards for nitrite levels in cured meat products in Nigeria. Consequently, this study focused on the chemical analyses for sodium nitrite concentrations in 13 cured meat products of diverse origins marketed in Southwest Nigeria. Additionally, the moisture content, ash, and pH of the meat samples were determined as part of the proximate composition.

MATERIALS AND METHODS

Materials and Reagents

Flasks and glassware were rigorously washed and thoroughly rinsed with deionized water. All reagents and chemicals were of analytical grade unless where otherwise stated. Concentrated HCl (Analar®); potassium ferrocyanide trihydrate, sulphanilamide, zinc acetate dihydrate, and disodium tetraborate decahydrate were from British Drug House (BDH) Chemicals Ltd, Poole England while sodium nitrite, glacial acetic acid, and N-naphthylethylenediamine dihydrochloride were from JT. Baker Ltd, England.

Sampling of cured meat

Samples were randomly purchased from supermarkets, fast-food restaurants, and open markets in Lagos, Ogun, and Oyo States, Nigeria. The meat products investigated included corned beef, ham, bacon, luncheon meat, and sausages. They were serially coded samples A-M and their labelled information including batch numbers were recorded prior to their storage at - 20°C.

Sample preparation and procedure

All solutions were prepared with deionized water. The stock solution (1000) ppm, intermediate solution (100 ppm), and working solution (1 ppm) were prepared by diluting to appropriate concentrations. A series of standard solutions were then made from the stock solution which contained 0.2, 0.4, 0.6, 0.8, and 1.0 ppm sodium nitrite respectively. A calibration curve was prepared from the absorbance of these standard solutions when plotted against the different concentrations of sodium nitrite. The solubility of sodium nitrite allows for its extraction from the meat samples with hot water. Precipitation of the proteins with potassium ferrocyanide, zinc acetate, and borax solutions occurred after extraction, and this was followed by filtration. The filtered nitrite was then reacted with sulphanilamide and naphthylethylenediamine (NEDA) to produce an amount of purple-coloured dye that is indicative of the concentration of nitrite ion present depending on the colour intensity. Sodium nitrite concentration for each sample was determined by photoelectric measurement of the absorbance at 538 nm using a Jenway 6405 UV/Vis spectrophotometer [28].

Nitrite content expressed as $NaNO_2$ was then calculated in accordance with the methods of the Association of Official Analytical Chemists (A.O.A.C.) [29]. Further, the moisture content (%w/w) and ash value (%; g/100 g DW) were determined by official AOAC methods [30]. The pH measurements were made using a digital pH–meter calibrated with pH 4 and 7 buffers.

Statistical analysis

Descriptive statistics such as percentages, mean values, and standard deviation (SD) of the mean were used to present results for each parameter. Ranges were given to express variations for the minimum to maximum determinations. The calibration plot and regression coefficient were computed using Microsoft Office Excel worksheet version 2016.

RESULTS AND DISCUSSION

A plot of absorbance readings against standard nitrite concentrations produced a calibration curve that was linear over the concentration range of 0.0-1.2 ppm employed. The linear regression equation was calculated to be $y = 0.538x - 0.013$ and regression coefficient (r^2) was 0.999. This compares favourably with a reported HPLC method for nitrite [31]. Table 1 gives the labelled information and concentrations of nitrite for extracted, sampled cured meat products.

Table 1: Labelled information and nitrite concentrations of sampled cured meat products

Sample	Description	Batch No.	Origin	Mean Conc.	Mean Conc.± S.D.
A	Corned Beef	311790L3	Argentina	61.58	61.58 ± 0.0007
B	Corned beef	DIPOA0078/76	England	61.40	61.40 ± 0.0000
C	Corned beef	FR65286002CE	France	14.87	14.87 ± 0.0007
D	Ham	NL129EG	Holland	14.86	14.86 ± 0.0021
E	Beef-Luncheon	NSCECI	Brazil	14.49	14.49 ± 0.0007
F	Hot-Dogs	FRIBOI-85467-1	UK	18.94	18.94 ± 0.0007
G	Cocktail-sausages	NL153EG	Holland	15.24	15.24 ± 0.0007
H	Minced beef	UKRJ021EC	UK	8.19	8.19 ± 0.0007
I	Bacon	NL129EG	Holland	179.83	179.83 ± 0.0007
J	Luncheon-Meat	CICNCI	Holland	34.51	34.51 ± 0.0007
K	Sausage-Roll	15/60-0P-026	Nigeria	7.81	7.81 ± 0.0014
L	Meaty-Sausage	FT-1514	Nigeria	30.07	30.07 ± 0.0007
M	Hamburger	Nil	Nigeria	10.05	10.05 ± 0.0007

SD=standard deviation

An elemental analysis part of this study has been published previously [32]. However, in the present study, the mean values of sodium nitrite were found to range from a minimum of 7.81±0.0014 mg/kg in sample K to a maximum, of 179.83±0.0007 mg/kg in sample I. Samples A and E from Argentina and Brazil (South America) had a mean nitrite concentration of 38.0 mg/kg; samples from Holland D, G, I and J had a mean of 61.1 mg/kg and samples from the UK and France (Europe) had a mean of 22.2 mg/kg. The increasing order of mean nitrite concentration for cured meat samples in relation to their country of origin was Nigeria<Brazil<France<UK<Argentina<Holland as shown in Table 2.

Table 2: Comparative levels of Sodium Nitrite concentrations in cured meat products in relation to their country of manufacture

Sample Code	Sample Origin (Continent)	^{a}N	Mean ± SD	Minimum	Maximum
A	Argentina (South America)	2	61.58 ± 0.0007	61.58	61.58
E	Brazil (South America)	2	14.49 ± 0.0007	14.49	14.49
D, G, I & J	Holland (Europe)	8	61.10 ± 0.0011	14.86	179.83
B, F, H	UK (Europe)	6	29.50 ± 0.0007	8.19	61.40
C	France (Europe)	2	14.87 ± 0.0007	14.87	14.87
K, L, M	Nigeria (Africa)	6	13.40 ± 0.0011	7.81	30.07

^{a}Each sample was determined in duplicates.

Consequently, samples indigenously produced in Nigeria (K, L, M) had the least mean nitrite concentration (13.4 mg/kg) which compared favourably with concentrations derived for meat products (15 ppm $NaNO_2$) from the United States [33], while the highest singular nitrite concentration was seen among the Holland samples. Three (D, G, and J) out of the four products from Holland had mean nitrite concentrations within allowable limits of 14.86 ± 0.0021 mg/kg, 15.24 ± 0.0007 mg/kg, 34.51 ± 0.0007 mg/kg while one (sample I) exceeded the limit with a nitrite value of 179.83 ± 0.0007 mg/kg.

A comparison of results in this study with the permissible values given by the World Health Organisation (WHO) and the Food and Agricultural Organisation (FAO) [12] however revealed that (n=10, 76.9%) of the samples conformed to the recommended sodium nitrite concentration of 10-125 mg/kg. However, (n=1, 7.69%) of the samples had sodium nitrite concentrations above the recommended levels, and (n=2, 15.4%) of the samples had concentrations below the recommended levels. Three samples (A, B, and I) with nitrite concentrations of 61.58 ± 0.0007, 61.40 ± 0.0000, and 179.83 ± 0.0007 had values above the EU recommended level of 50 mg/kg or 50 ppm. Levels of nitrite in the cured meat products marketed in Nigeria (range: 7.81-34.51 mg/kg) were comparable (in terms of falling within specifications) with those obtained for similar samples previously in a New Zealand study (range: <5 - 24.51 mg/kg) and other overseas studies except for samples A, B, and I [33]. The average level of nitrite (as $NaNO_2$) detected in sampled cured meat products in this study was 36. 3 mg/kg or ppm (range: 7.81-179.83; *n* = 13) and is comparable with results obtained for Canadian cured meat products in various studies between 1972 to 1996. Results from that study was 28 ppm (0−252 ppm; *n* = 197); 44 ppm (0−275 ppm; *n* = 659); 31 ppm (1−145 ppm; *n* = 76); and 28 ppm (4−68 ppm; *n* = 35) but less than the value reported in a US study with a mean of 10 ppm residual nitrite ion (equivalent to 15 ppm of $NaNO_2$) in cured meats [34].

Although sodium nitrite is added to meat for its antimicrobial activity, "cosmetic" effect, and ability to delay the process of oxidative rancidity, low levels may result in food poisoning due to botulinum toxin caused by the presence of the spore-forming bacteria, *Clostridium botulinum* which may also cause food spoilage [7]. High levels on the other hand may induce the disorder known as methaemoglobinaemia which could be fatal. In methaemoglobinaemia, the ferrous ion, Fe^{2+} in haemoglobin is oxidized by nitrite to ferric ion, Fe^{3+} converting the haemoglobin to methaemoglobin which does not transport oxygen as efficiently as haemoglobin. Also, large amounts of sodium nitrite in the diet can react with other substances found in meat such as amines or amides to form nitrosamines which are potentially carcinogenic [35]. Results for selected proximate composition and pH are given in Table 3.

Table 3: Selected proximate composition and pH of sampled cured meat products

Sample code	Description	*pH	*Moisture (%)	*Ash (%)
A	Corned beef	6.09	58.1	2.61
B	Corned beef	6.12	59.91	2.84
C	Corned beef	6.09	67.52	3.01
D	Ham	6.42	66.83	3.31
E	Beef-Luncheon	6.14	60.12	3.16
F	Hot-Dogs	6.12	74.42	1.45
G	Sausages	6.27	69.26	2.89
H	Minced beef	5.31	70.55	1.18
I	Bacon	6.37	67.52	2.76
J	Luncheon-meat	6.45	36.43	3.08
K	Sausage-Roll	5.31	38.49	3.31
L	Meaty-Sausage	4.65	36.71	3.10
M	Hamburger	5.37	27.51	2.99

** Values are mean of two readings or determinations as applicable*

The determination of ash and moisture content for the samples provide information on the mineral component (inorganic residue) and stability of the products respectively; while the measurement of pH of the samples provides information on the taste, freshness, technical processing characteristics, and degree of preservation [36-38]. Inhibition of microorganisms by pH depends on temperature, water activity, and the presence of preservatives [33]. The ideal pH range of meat products is 5.3 to 6.7 [39]. In this study, the pH range of analysed meat products is 4.65-6.45. Only one (7.69%) of the samples had a pH value below the ideal pH range (< 5.3 - 6.7) thus indicating a low degree of preservation. Ash content of the samples ranged from 1.18 - 3.31 %. Sample H had the least mineral composition while samples D and K had the highest amount of inorganic residue. The moisture content of samples in our study ranged from 27.51-74.42%; sample F was the most stable and sample M was the least stable. Thus, sample M is most prone to microbial degradation and spoilage since it is known that the higher the moisture content of a product, the less stable it is since moisture is an essential requirement for microbial activity [39]. It suggests that nitrite levels, pH, moisture content, and ash value aside from other physicochemical characteristics should be strictly controlled for meat products.

This study established that the sodium nitrite concentration of 10 out of the 13 cured meat samples conformed to the WHO and FAO standards [12] and that the indigenously produced cured meat products had the permissible values of nitrite for human consumption. However, the low concentration of sodium nitrite in samples H and K may be insufficient to prevent botulism and oxidative rancidity while the high level of sodium nitrite in the sample I may result in methaemoglobinaemia or nitrosamine formation which may predispose consumers to cancer. Our study, therefore, calls for the need for stringent regulatory control of nitrite concentration in cured meat products imported into or produced indigenously in Nigeria to minimize public health risks. This study will also contribute to upcoming studies for setting standards for sodium nitrite concentration in food products.

CONCLUSIONS

This study established that the sodium nitrite concentration of 10 out of the 13 cured meat samples conformed to the WHO and FAO standards. This has implications for public health and calls for stringent regulatory control of sodium nitrite concentration in cured meat products circulating in developing countries like Nigeria.

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DISCLOSURE OF INTEREST

The authors declare no conflict of interest in the study.

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