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Determination of Protein, Nitrite and Nitrate in Animal Protein Sources in Nigeria

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The protein, nitrite and nitrate levels were determined in the body parts of twelve different types of fish, snake, prawn, amphibian and camel, they all serve as animal protein sources. The parts examined for these parameters were: head, gills, internal organs, muscle, bone and skin. All the samples yielded high levels of protein with values ranging between 21.71 g/100 g– 98.80 g /100 g . The nitrite levels ranged between 4.2 mg /kg – 112.06 mg /kg and the nitrate levels ranged between 168.46mg/kg - 4580.67 mg/kg. Nitrate is sparingly used in Nigeria to cure fish and meat. Hence the results of the nitrite and the nitrate might have been due to their levels in the samples. Most of the coefficient of variation values were low in the various sample body parts showing almost homogenous distribution of protein, nitrite and nitrate levels. While the nitrite levels were within the maximum permitted levels, many of the nitrate levels exceeded the maximum permitted levels.

Key words: Determination of protein, Nitrite, Nitrate, Animal protein.

Introduction

Foods can be preserved by fermentation, refrigeration, heat treatment, moisture removal, irradiation and chemical additives. Chemical preservatives are one group of chemicals that are either added intentionally to foods, or appear in foods as a result of processing or storage. The conditions of preservatives, in accord with good manufacturing practices have been enumerated (Ihekoronye and Ngoddy 1985).

Combinations of the salts of nitrites and nitrates have been used in curing solutions and curing mixtures for meats. Nitrites decompose to nitric acid and forms nitrosylheamoglobin, nitrosylmyoglobin and dinitrosylhaemochrome when it reacts with the haem pigments in meats and thereby forms the stable red and pink colors of cured meats (Fox 1966; Giddings 1977). This activity also prevents iron (Fe^{3+}) from catalysing the oxidation of lipids (Zubillaga *et al* 1984). Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic. They are currently added as nitrites and nitrates of sodium or potassium. Recent work has emphasized the inhibitory property of nitrites toward *Clostridium botulinum* in meat products, particularly in bacon and canned or processed hams (Frazier and Westhoff 1978). The inhibitory mechanisms by which nitrite inhibits *C. botulinum* have been enumerated (Benedict 1980). Nitrates have a limited effect on a limited number of organisms and would not be considered a good chemical preservative.

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Smoking is a very ancient preservation technique. The smoking of foods usually have two main purposes, adding desired flavors and preservation. The smoking helps preservation by impregnating the food near the surface with chemical preservatives from the smoke or by combined action of the heat and preservatives and by the drying effect, especially at the surface. Intensive smoking does prolong shelf life but it has detrimental effects (Bender 1992).

In view of the biological significance of nitrosamine poisoning it seems desirable to evaluate the extent of contamination of some Nigerian animal protein sources by nitrosamine precursors; the conditions under which these various substances are formed in the food materials and the roles which various factors, which are known to enhance the presence of nitrosamines and their precursors, play in the N-nitrosation reaction *in situ* and under various processing conditions. Inhibition of conversion of nitrites to nitrosamines is also discussed.

Materials and Methods

Samples were purchased at Oba market (Ado-Ekiti, Ekiti State, Nigeria). The samples were identified and then grouped under their major headings: Fish (A – F), Camel (G), Snake (H – I), Prawn (J – K) and Amphibian (L). The samples were further separately divided into their various organs giving a total number of forty samples for analysis. The details of these groupings are shown in Tables 1 and 2.

The separated body parts of the major samples were homogenised in Kenwood major blender.They were transferred into plastic containers, labelled and kept in the freezer for pending analysis. All samples were in dry state after being smoke dried (as purchased).

Determination of moisture and protein. Moisture was determined by the method of the Association of Official Analytical Chemists (AOAC 1990). The dry matter values were calculated by taking moisture content from 100% of the corresponding sample. All proteins were determined by the micro-Kjeldahl method as described by Pearson (1976).

Determination of nitrite and nitrate. Nitrite. The nitrite was extracted from the samples by buffer solution (pH 9.8) and alumina cream. To the sample extract was added 1ml each of sulphanilic acid, 1-naphthylamine and sodium acetate, respectively. The mixture was kept in the dark for color development. The Griess pigment formed was quantitatively measured spectrophotometrically at 540nm (Kamm *et al* 1965).

Nitrate. 25ml of sample extract and buffer solution (pH 9.8) were run through the prepared nitrate reduction column of spongy cadmium and determination of nitrite by the formation of a diazo compound as detailed above. All determinations were in duplicate. The difference between the original nitrite and new nitrite of the same sample gave the value of nitrate.

Nitrite levels have been determined both by HPLC spectrophotometrically. Agreement between the two methods was reasonable (Wootton *et al* 1985). The U V spectrophotometer used was Pharmacia (Pharmacia LKB, Biochrom 4060 UV Visible Spectrophotometer).

Statistical analyses. All the moisture and dry matter (DM) values were reported as g/100g, while all the protein values were reported as g /100g on dry matter basis. Both the nitrite and nitrate levels were reported as mg/ kg on dry matter basis. Mean, standard deviation and coefficients of variation were also calculated (Steel and Torrie 1960). Students T-tests were also calculated to determine the level of significance at P = 0.05 level of the parameters in each group.

Results and Discussion

The common, scientific, vernacular and family names of all the major samples for analysis are shown in Table 1. The sample alphabet representative and sample part; moisture, dry matter and protein values are all shown in Table 2.

The moisture content varied between 5.34 – 35.89 g /100g. The low value of moisture would ensure a longer shelf life for most of the samples. The protein content g/100g ranged between 21.71 – 98.80 on dry matter (DM) basis. These two extremes were shared by the organs of *Malapterurus electricus* whose skin was 21.71g /100g, while the internal organs gave the value of 98.80g /100g DM. The protein levels compared favorably with many unconventional sources of protein in Nigeria. The protein levels of three different types of land snails found in Nigeria ranged between 72.20- 88.83g/ 100g DM (Adeyeye 1996) 54.90 in *Zonocerus variegatus* (Olaofe *et al* 1998) 49–61 in locusts and 45g/ 100g in termites (Mayhew and Macmillan 1988). About 25g of meat will

Table 1

Scientific and vernacular names of the animal protein sources

Major food group		Common name	Vernacular name [a] (Y)	Scientific name	Family
Fish	(A)	Grass-eater	Agbodo	*Distichodus rostradus*	Distichodonida (scaly)
	(B)	Tilapia	Epiya	*Oreochromis niloticus*	Cichlidae (scaly)
	(C)	Trunkfish	Osan	*Gymnarchus niloticus*	Gymnarchidae (scaly)
	(D)	Electric cartfish	Ojiji	*Malapterurus electricus*	Malapteruridae (Non-scaly)
	(E)	Mudfish	Abori	*Clarias anguillaris*	Clarlidae (Non-scaly)
	(F)	Sole	Abo	*Cynoglossus senegalensis*	Cynoglossidae
Camel	(G)	The bactrian camel	Rakunmi	*Camelus bactriamus*	Camelidae
Snake	(H)	Diamond snake	Oka	*Python sp*	Boidae
	(I)	Sunbeam snake	Monamona	*Xenopeltis unicolor*	Xenopeltidae
Prawn	(J)	African river prawn	Ede pupa	*Macrobrachium vollenhovenii*	Palaemonidae
	(K)	Caramote prawn	Ede funfun	*Penacus kerathurus*	Penaeidae
Amphibian	(L)	African bullfrog	Konko	*Rana adspersa*	Ranidae

[a]Y, Yoruba

Table 2
Moisture, dry matter and crude protein levels of the samples

S. No	Sample number	Animal body part	Moisture g/100g	Dry matter g/100g	Protein g/100g
1	A_1	Head	6.90	93.09	67.83
2	A_2	Gills	7.51	92.48	49.64
3	A_3	Internal organs	22.63	77.36	33.20
4	A_4	Muscle	7.14	92.85	61.95
5	A_5	Bone	21.00	79.00	35.10
6	A_6	Skin	6.32	93.67	71.88
7	B_1	Head	13.76	86.23	47.40
8	B_2	Muscle	9.97	90.02	68.54
9	B_3	Bone	10.29	89.70	33.19
10	B_4	Skin	12.79	87.20	72.24
11	C_1	Head	7.37	93.62	44.95
12	C_2	Internal organs	10.64	89.35	84.99
13	C_3	Muscle	10.18	89.81	74.50
14	C_4	Bone	16.07	83.92	51.32
15	C_5	Skin	6.03	93.96	77.43
16	D_1	Head	10.88	89.11	36.19
17	D_2	Internal organs	8.04	92.95	98.80
18	D_3	Muscle	9.13	90.86	21.71
19	D_4	Bone	14.53	85.46	96.97
20	D_5	Skin	8.45	91.54	39.94
21	E_1	Head	6.63	93.36	87.03
22	E_2	Muscle	9.60	90.39	71.62
23	E_3	Bone	5.90	94.09	70.85
24	F_1	Head	10.84	89.15	45.88
25	F_2	Internal organs	5.45	94.54	79.92
26	F_3	Internal	7.18	92.81	67.56
27	F_4	Bone	5.34	94.65	35.80
28	F_5	Skin	8.62	91.37	74.19
29	G_1	Muscle	8.79	91.20	86.89
30	H_1	Flesh + bone	10.96	89.03	26.26
31	I_1	Muscle	8.23	91.76	87.82
32	I_2	Bone	5.69	94.30	64.28
33	J_1	Flesh	18.36	81.63	89.75
34	J_2	Exoske-leton	34.11	65.88	78.13
35	K_1	Whole organism	18.89	81.10	78.66
36	L_1	Skin	16.84	83.15	95.63
37	L_2	Muscle	8.53	91.46	92.42
38	L_3	Bone	5.52	94.47	33.80
39	L_4	Head	6.47	93.52	64.87
40	L_5	Internal	35.89	64.11	90.86

a, All determinations were on dry matter basis

Table 3
Nitrite and nitrate levels of the samples

S.No.	Sample	Nitrite[a,b]	Nitrate [a,b]
1	A_1	34.27	2105.60
2	A_2	12.11	1544.98
3	A_3	32.19	1622.41
4	A_4	12.82	1538.07
5	A_5	18.73	1196.46
6	A_6	42.81	3586.60
7	B_1	41.86	1442.54
8	B_2	28.99	1943.90
9	B_3	65.66	2529.65
10	B_4	36.70	2440.37
11	C_1	75.84	2914.98
12	C_2	13.43	2592.05
13	C_3	37.75	1031.18
14	C_4	47.66	1096.40
15	C_5	34.06	4580.67
16	D_1	43.21	2650.10
17	D_2	51.75	2530.29
18	D_3	37.53	2947.28
19	D_4	51.60	2466.53
20	D_5	4.26	2617.54
21	E_1	4.82	2737.25
22	E_2	4.31	2996.02
23	E_3	4.78	3064.62
24	F_1	40.49	2481.10
25	F_2	88.96	2610.43
26	F_3	54.95	2703.37
27	F_4	76.60	1960.38
28	F_5	111.63	3740.83
29	G_1	112.06	3265.13
30	H_1	81.43	2794.00
31	I_1	39.23	300.78
32	I_2	24.28	501.70
33	J_1	19.60	558.62
34	J_2	25.80	675.47
35	K_1	28.24	415.66
36	L_1	43.30	331.93
37	L_2	34.11	508.20
38	L_3	38.11	969.62
39	L_4	26.63	503.74
40	L_5	49.91	168.46

[a]All determinations were on dry matter basis; [b]Unit, mg/kg

supply 45% of a child's daily need for protein. The addition of 100g of meat to the average Zambian diet would increase the protein by 50% (Jensen 1981). The amino acids in the protein complement, the cereal sources of protein by making good their relative deficiency of lysine (Bender 1992).

Information on the nitrate and nitrite contents of Nigerian foodstuffs is very scanty. Fafunso and Maduagwu (1980) showed that the nitrate and nitrite levels in seven common edible Nigerian leafy vegetables on a fresh weight basis, ranged from 48mg/kg – 270mg/kg NO_3^- and from 0.024 – 0.064 mg/kg NO_2^-. Maduagwu (1976) and Bassir and Maduagwu (1978) reported the presence of nitrates and nitrites in some Nigerian indigenous beverages, namely: palm wine, *pito*, *burukutu*, *ogogoro* and *nono*. Their findings showed that these constituents were widely distributed in beverages

Table 4
Various statistical analysis values for the various analytical results

Sample	$\overline{X}$[a]	SD[b]	CV[c]	T[cd]	Tt[e]
A:A_1-A_6 Moisture	11.92	7.65	64.51	32.98*	2.78
Dry matter	88.08	7.69	8.73	0.29	2.78
Protein	53.27	16.61	31.18	0.02	2.78
Nitrite	25.49	12.71	49.86	0.05	2.78
Nitrate	1932.35	861.36	44.58	0.001	2.78
B:B_1-B_4 Moisture	11.70	1.86	15.90	12.06*	4.303
Dry matter	88.29	1.86	2.11	0.06	4.303
Protein	55.34	18.38	33.21	0.01	4.303
Nitrite	43.30	15.82	36.54	0.02	4.303
Nitrate	2089.12	502.19	24.04	0.003	4.303
C:C_1-C_5 Moisture	10.06	3.87	38.47	0.76	3.18
Dry matter	90.13	4.07	4.52	0.62	3.18
Protein	66.64	17.46	26.20	0.15	3.18
Nitrite	41.75	22.77	54.54	0.01	3.18
Nitrate	24443.06	1468.09	60.09	0.001	3.18
D:D_1-D_5 Moisture	10.21	2.65	25.95	1.88	3.18
Dry matter	89.98	2.88	3.20	0.84	3.18
protein	58.72	36.40	61.99	0.01	3.18
Nitrite	37.67	19.62	52.08	0.02	3.18
Nitrate	2642.35	185.15	7.01	0.02	3.18
E:E_1-E_3 Moisture	7.38	1.96	26.56	-	-f
Dry matter	92.61	1.96	2.12	-	-f
Protein	76.50	9.13	11.93	-	-f
Nitrite	4.64	0.28	6.03	-	-f
Nitrate	2932.63	172.65	5.89	-	-f
F:F_1-F_5 Moisture	7.49	2.31	30.84	2.68	3.18
Dry matter	92.50	2.31	2.50	1.68	3.18
Protein	60.67	18.96	31.25	0.17	3.18
Nitrite	74.53	27.98	37.54	0.003	3.18
Nitrate	2699.22	649.29	24.05	0.002	3.18
G : G_1	-	-	-	-	-g
H : H_1	-	-	-	-	-g
I : I_1-I_2 Moisture	6.98	1.80	25.79	-	-f
Dry matter	93.03	1.80	1.93	-	-f
Protein	76.05	16.65	21.89	-	-f
Nitrite	31.76	10.57	33.28	-	-f
Nitrate	401.24	142.07	35.41	-	-f
J:J_1J_2 Moisture	26.24	11.14	42.45	-	-f
Dry matter	73.76	11.14	15.10	-	-f
Protein	83.94	8.22	9.79	-	-f
Nitrite	22.70	4.38	19.30	-	-f
Nitrate	617.05	82.63	13.39	-	-f
K:K_1	-	-	-	-	-g
L:L_1-L_5 Moisture	14.69	12.69	86.39	0.34	3.18
Dry matter	85.34	12.68	14.86	0.03	3.18
Protein	75.52	26.36	34.90	0.06	3.18
Nitrite	38.41	8.85	23.04	0.50	3.18
Nitrat	496.39	299.47	60.33	0.01	3.18

[a]$\overline{X}$ =Mean; [b]SD = Standard deviation; [c]CV = Coefficient of variation percent; [d]T = T-test calculated value, [e]T = T-test table value; f = Not calculated; g, See Tables 2 and 3 *Significant at P<0.05.

being hawked for sale in various parts of Nigeria. Nitrate concentrations ranged between 6.60 and 91.30 mg/kg while 0.00–2.90 mg/kg nitrite was detected in these drinks. Amoo (1998) reported levels of nitrate in weaning foods to be 27.00–198.00 mg/kg; weaning food components, 83.70–155.70 mg/kg; vegetables, 263.00– 437.40 mg /kg and alcoholic and non-alcoholic beverages, 0.23–12.96 mg/kg; and the nitrite levels was 2.25 – 5.63 mg/kg for weaning food components, 1.80 – 5.85 mg/kg for vegetables and 5.40–7.20 for alcoholic and non-alcoholic beverages. All Nigerian foodstuffs presumably contain nitrates since nitrates are known to be natural constituents of plant and animal materials.

Our nitrate and nitrite levels in the animal protein sources are shown in Table 3. The nitrite levels ranged from 4.26–112.06 mg/kg, while the levels of nitrate ranged from 168.46– 4580.67 mg/kg ; all determinations were on dry matter basis. The values were greater than the literature values (mostly from plant sources) cited above. The skin of *Malapterurus electricus* (D_5) had the lowest nitrite (4.26 mg/kg) but the levels of nitrite in *Clarias anguillaris* organs were also low with values of 4.82 (E_1), 4.31 (E_2) and 4.78 (E_3). The concentration of nitrites in different body organs appeared to be dependent on the type of animal/fish, body organ, mode of preparation and the likely level of smoking before dryness was achieved. Among the samples available for internal organs investigation, three samples (D_2 = 51.75, F_2 = 88.96, L_5 = 49.91mg/kg) or 60% recorded highest levels of nitrite, while A_3 (32.19) and C_2 (13.43) occupied positions three and five respectively in their groups and made up 40% of the five samples. The head region nitrite levels were highest for E_1 (4.82) and C_1 (75.84); second positions for A_1 (34.27) and B_1(41.86); third position for D_1 (43.21) while both F_1 (40.49) and L_4 (26.63) occupied fifth position each in their groups. For the bones, B_3 (65.66) occupied the highest level in its group; second positions were occupied by C_4 (47.66), D_4 (51.60) and E_3 (4.78); third positions were shared by F_4 (76.60) and L_3 (38.11), while A_5 (18.73) occupied position five in its group. For the skin samples or flesh, A_6(42.81), F_5 (111.63), I_1 (39.23) and J_2 (25.80) all occupied first positions in the nitrite levels in their various groups, while L_1 (43.30) and B_4 (36.70) occupied second and third positions respectively in their groups. G_1 (112.06) was the highest of all the nitrite levels. The sample is usually prepared by first parboiling the muscle of camel and then drying before it is sold. Internal organs of fish were hardly consumed in Nigeria but this will depend on the size and the age of the fish concerned. The levels of nitrite in the head of the samples (fish and amphibian) are of major concern because the fish head is now becoming a delicacy (Okoye 1991) in Nigeria. When fishes are smoked their bones become soft thereby making them suitable for consumption, hence high levels of nitrites in the samples are sources of serious concern. *Oreochromis niloticus* has good values of beneficial minerals (Adeyeye *et al* 1996) but the nitrite levels in it might demand a second thought in its consumption. Both tilapia and the prawns (both red and white) have been recommended for the preparation of fortified cornflour feed as infant formulae substitute (Bamiro *et al* 1994) because of their high levels of proteins and beneficial minerals (Adeyeye 2000). However, the levels of nitrites in the prawns were also high.

The nitrate levels were as varied as the nitrites but the pattern of distribution did not follow the trend of the nitrites. In the skin the nitrates distribution was A_6, F_5 and J_2 in position one in their groups, B_4 and I_1 were in second positions, while L_1 was in the fourth position. In the internal organs, samples A_3, C_2 and F_2 were in the third positions, while D_2 and L_5 were in fourth and fifth positions, respectively. For the head E_1 was in first position, A_1, C_1 and D_1 were in the second positions, F_1 and L_4 were in the third positions, while B_1 was in the fourth position in its group. For bones B_3, E_3 and L_3 were in first positions, C_4 was in position four, D_4 and F_4 were in position five while A_5 was in the sixth position in its group for nitrate level. The following samples: *Xenopeltis unicolor*, *Macrobrachium vollenhovenii*, *Rana adspersa* and *Penaeus kerathurus* have nitrate levels below 1000 mg/kg but all other sample values were higher than 1000 mg/kg.

The values of nitrite reported by Wootton *et al* (1985) in cured meat products were generally higher than the values under discussion but the values reported for nitrates were generally lower than our values. Maximum levels of nitrites and nitrates in foods are sodium nitrate (500 ppm), sodium nitrite (200ppm) (Christain and Greger 1990), residual nitrite (70ppm) in meat products and fish meat sausage (in Japan) and 120ppm in bacon (in US) (Kada 1974); in New South Wales they are 125ppm (nitrite) and 500ppm (nitrate) respectively as the sodium salts (NSW 1908). Our results should be seen in the context of maximum permitted levels of nitrite and nitrate incured products in other countries of the world. It is obvious that while nitrite levels are satisfactory, many of the nitrate levels exceeded the maximum permitted levels.

In 1981 the Committee on Nitrite and Alternative Curing Agents in Food of the National Academy of Sciences reviewed the scientific literature on the links between nitrites, nitrates, nitrosamines and cancer. They concluded that circumstantial evidence from epidemiological studies has implicated foods containing high levels of nitrate, nitrite and nitrosamines in the development of cancer, particularly of the stomach and oesophagus. The ingestion of large amounts of nitrate is sometimes a health concern for another reason. It can cause a condition called methaemoglobinemia, which involves the production of abnormal haemoglobin unable to carry the usual amount of oxygen. This condition is most likely to occur in infants, who may become cyanotic (turn blue from lack of oxygen) if they consume food with high levels of nitrate. This effect may be cumulative, since the methaemoglobin is reduced only at a slow rate (Burden 1961).

There is a special delicacy in Nigeria called "pepper soup" prepared mainly from fish or meat, water, salt and pepper. The pungent taste of red pepper is due to capsaicin ($C_{18}H_{27}NO_3$), while the pungent taste of black and white pepper is due to the alkaloid piperine ($C_{17}H_{19}NO_3$). The piperine content of pepper is as high as 5%. Formation of N-nitroso piperidine, a mutagen by the reaction of nitrite with piperine in an acid solution (human stomach is acidic) has already been reported (Rao *et al* 1981). Other identified nitrosating species and their resources include: NOSCN (from saliva) (Boyland and William 1971), C_6H_4OHNO (from smoke which has many phenolic compounds) and NOCl (from gastric juice, curing salts) (Sebranek and Fox Jr 1985). All these might add to the level of nitrites ingested from meat sources.

The conversion of nitrite to nitrosamine is blocked by ascorbic acid. In this way vitamin C tends to inhibit the formation of carcinogen. The presence of α–tocopherol and erythorbic acid are also effective in reducing nitrosamine formation from nitrites. Currently, the food industry in the United States uses smaller amounts of nitrite to cure foods and through the use of antioxidant keeps the formation of nitrosamines at minimal levels (IFT 1987).

The statistical results for all the samples are shown in Table 4. The groups were A(A_1-A_6), B ($B_1 – B_4$), C ($C_1 – C_5$), D ($D_1 – D_5$), E ($E_1 – E_3$), F ($F_1 – F_5$), G (G_1), H (H_1), I ($I_1 – I_2$), J ($J_1 – J_2$), K (K_1) and L ($L_1 – L_5$). The highest CV values were observed for moisture in $A_1 – A_6$, $E_1 – E_3$, $J_1 – J_2$ and $L_1 – L_5$. The moisture level ranged between 15.90 – 86.39%. The moisture content values were significantly different in $A_1 – A_6$ and $B_1 – B_4$. The CV values was high in protein for $D_1 – D_5$ (61.90) but less than 50.00 in the other sample groups. Nitrite CV levels were greater than 50.00 in $C_1 – C_5$ and $D_1 – D_5$ but less than 50.0 in the rest of the samples. CV for the nitrate levels were high in $C_1 – C_5$ (60.09) and $L_1 – L_5$ (60.33) but less than 50.00 in the rest. The low levels of CV in many of the sample groups was an indication of almost homogenous distribution of the parameters under discussion.

The control of ingoing nitrite levels, use of reductants (ascorbate or erythorbate) and adherence to good processing practices will substantially reduce the problem of nitrosamine formation from nitrites (Dudley 1979). Based on established facts, we are suggesting, that consumption of fruits high in vitamin C should be encouraged, where as of smoke to dry/preserve meat and fish, and consumption of pepper soup should be lessened and great caution should be taken in consuming smoke dried bush meat.

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