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Determination of Fat Contents, Iodine Values, Trace and Toxic Metals in Commonly Consumed Frozen Fish in Nigeria

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ABSTRACT
In Nigeria, frozen fishes are extensively consumed. But there is paucity of knowledge about the essential nutrients and contamination status of the fishes. In this study, fat contents, iodine values, trace and toxic metals were determined in 20 frozen fish obtained from Lagos and Ibadan, south western Nigeria. Average fat contents in the analysed fish species ranged from 4.38 to 58.9%, while average iodine values of oil in the fish samples ranged from 50.4 to 120 mg/100 g. The following ranges were however observed for the average metal concentrations in the fish samples: Pb; 2.09-12.3 $\mu g\ g^{-1}$, Cd; <0.01 $\mu g\ mL^{-1}$ (less than the detection limit of AAS), Cu; 2.38-4.38 $\mu g\ g^{-1}$ and Zn; 22.9-32.2 $\mu g\ g^{-1}$. The high fat contents and their corresponding iodine values recorded for Atlantic horse mackerel (58.9±0.45%, 112±2.00 mg/100 g), Sardine (34.4±3.15%, 57.8±9.60 mg/100 g) and Chub mackerel (38.8±1.55%, 120±16.5 mg/100 g) suggest that consumption of these fish species could be recommended to people with cardiovascular disorder. Continuous monitoring program is also recommended to establish the high lead concentration (12.3 $\mu g\ g^{-1}$) as found in Chub mackerel.

Key words: Frozen fish, iodine values, fat content, toxic metals, trace metals

INTRODUCTION
Fish are of high nutritional benefit mainly due to high content of unsaturated fats (particularly the two kinds of omega-3 poly unsaturated fatty acids: Eicesa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid (DHA) in different fish species) (Clarkson, 2002), high-quality protein and also due to essential trace metals. The health benefits related to seafood consumption especially fish have been well documented. Omega-3 fatty acids (EPA) have proved to have protective effects in preventing coronary heart disease, reducing arrhytmias and thrombosis (Kinsella *et al.*, 1990) lowering plasma triglycerides level (Ismail, 2005) and reducing blood clotting tendency. Brain and eye development have also been attributed to DHA in fish lipids. Adeyeye (2002) and Zalloua *et al.* (2007) indicated that seafood including fish is rich in protein, contains low cholesterol level and high percentage of polyunsaturated fatty acids, liposoluble vitamins and essential minerals. However, the content of trace or toxic heavy metals in fish can augment or counteract the positive biological benefits effects of omega-3 fatty acids and protein contents.

Metal accumulation in fish is a global public health challenge, since seafoods are very susceptible to heavy metal bioaccumulation and biomagnification. These heavy metals originate in the environment via natural sources as well as human activities. They gain entrance into aquatic media in form of inorganic compounds through the agents of rain and wind. Once they enter the water body they cannot be degraded, they accumulate and become persistent in the water environment. On reaching the aquatic environment, they either dissolve in water or they settle as compounds in the sediment. The dissolved metal ions can be ingested directly by aquatic fish or swallowed along with their foods from the sediments.

Many of the metals (Co, Cu, Mn, Fe and Zn) are essential trace elements for both terrestrial and aquatic organisms and are involved in biochemical processes such as enzymes activation. Studies have shown that essential elements especially Zn and Cu when deficient in the body system can lead to organs malfunction, chronic diseases and untimely death (FAO/WHO, 2001); consequently, daily intake of these elements via seafood ingestion is vital to normal human development. Nevertheless, above certain levels, these elements can have toxic effects. For instance, Copper is highly toxic to aquatic organisms and may cause irreversible harm at concentration just over that required for growth and reproduction (Baldwin *et al.*, 2003). Eisler (2000) showed that sublethal effects to fish and the aquatic food chain can occur at concentrations less than 9 ppb Cu, copper can impair or destroy a fish's ability to smell (olfaction), which can be fatal. Baldwin *et al.* (2003) have equally shown that increase in Cu concentration from 1.0 to 20.0 ppb interfered with behaviours tied to olfaction in juvenile coho salmon. Zn, an essential element is one of the most common heavy metal pollutants. However, at high concentration, Zn exerts adverse effect in fish accruing structural damage which affects the growth, development and survival of fish (Tuurala and Soivio, 1982). Acute Zn intoxication manifest by nausea, vomiting and severe anaemia is reported to have occurred in a patient with renal failure who had been dialyzed with water stored in a galvanized tank. On the other hand, Studies have shown that Pb and Cd have no known biological functions and consequently harmful to essential life processes (Thomas and Williams, 2004).

The critical effect of long-term exposure to cadmium has been attributed to renal tubular dysfunction, characterized initially by an increased excretion of low molecular weight proteins in the urine. Lead has no known nutritional, biochemical or physiological function (Goyer, 1996). There is sufficient evidence for lead to be classified as an animal carcinogen. The toxic effects of lead are the same, irrespective of whether it is ingested or inhaled and blood levels as low as <10-100 $\mu g\ dL^{-1}$ in children and 10-100 $\mu g\ dL^{-1}$ in adults have been associated with a wide range of adverse effects. Health impacts include; nervous disorders, anaemia and decreased haemoglobin synthesis, cardiovascular disease and disorders in bone metabolism, renal function and reproduction.

Fatty acid composition as well as trace and toxic metals of fish can vary as a result of diet, geographical location, gender and environmental conditions (Gruger, 1967). In Nigeria, frozen fish is the cheapest source of protein consumed. Fish species are frequently at the apex of trophic level in the aquatic ecosystems which makes them repository for heavy metals. Thus, heavy metals concentrations in fish may be an indicator of what is subsequently passed on to man. Thus, this research is focused on assessment of nutritional and anti nutritional values of frozen fish commonly consumed in South-west, Nigeria.

MATERIALS AND METHODS

Sampling and samples preparation: Twenty frozen fish samples comprising five different species: Hake (*Merluccius merluccius*), Sardine (*Sardinella eba*), Chub mackerel (*Scomber japonicus*), Atlantic horse mackerel (*Trachurus trachurus*) and Croaker (*Pseudolithus elongatus*) were sampled. These groups of fish are not reared in Nigeria but are imported majorly from Europe

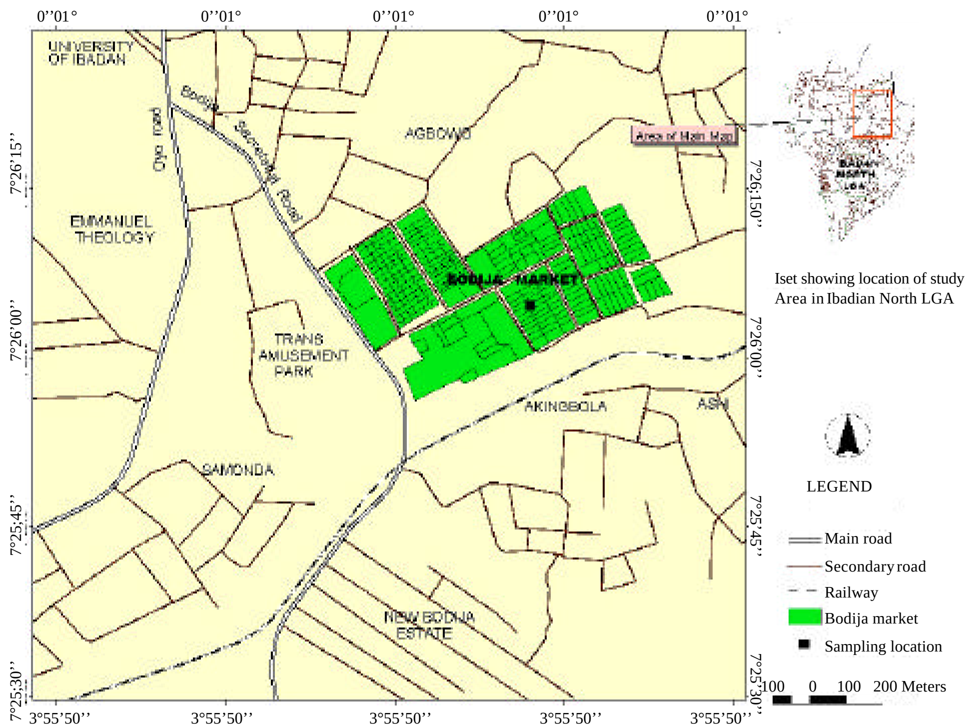

Fig. 1: Map showing sampling location in Ibadan

Table 1: English name, biological and common name of frozen fish obtained from Ibadan and Lagos

English name	Biological name	Common name
Hake	*Merluccius merluccius*	Panla osan
Sardine	*Sardinella eba*	Sawa
Chub mackerel	*Scomber jopanicus*	Alaran
Atlantic h. mackerel	*Trachurus trachurus*	Kote
Croaker	*Pseudolithus elongatus*	Apo, Ana

and North Africa. Two samples of each species were respectively purchased from two major markets in Ibadan and Lagos. The choice of the two sampling locations was informed by their population density and high commercial activities. The maps indicating the two sampling locations are shown in Fig. 1 and 2, respectively. The sampling locations were identified with the use of Geographical Positioning System (GPS). The samples were collected in polyethylene bags, kept in ice blocks and subsequently taken into the laboratory for analysis. The nomenclature of the frozen fish obtained from the two sampling locations is shown in Table 1.

Laboratory analysis: The fish samples were thoroughly washed with distilled water. They were then oven dried between 50-60°C for 24 h and were later transferred into a clean porcelain pestle and mortar where they were completely ground and homogenized. Grinding of dry samples was done using a porcelain mortar and pestle to avoid metal contamination from steel mills. 1.0 g of the homogenized sample was then subjected to wet digestion.

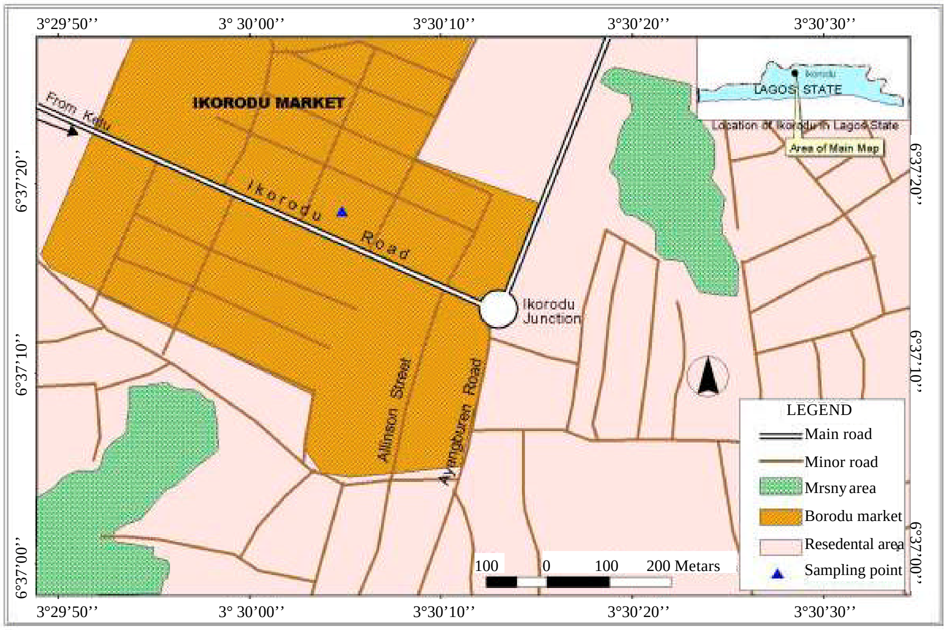

Fig. 2: Map showing sampling location in Lagos

Fat content determination using soxhlet extraction method: The fat extraction was carried out by soxhlet extraction method (AOAC, 1980). The 250 cm^3 of clean boiling flask were dried in an oven at 105-110°C for about 30 min. This was transferred into a desiccator and allowed to cool. About 5 g of the dried homogenized fish sample were accurately weighed into a labelled thimble. The cooled boiling flask was correspondingly weighed and labelled, 300 cm^3 of petroleum ether (boiling point 40-60°C) was measured into the boiling flask and the extraction thimble was plugged lightly with cotton wool. The soxhlet apparatus was assembled and allowed to reflux for about 6 h. The thimble was removed with care and the petroleum ether on the top of the container of the set-up was collected and drained into a container for reuse. The flask was removed and dried at (105-110°C) for one hour when it was almost free of petroleum ether. The flask and its content was finally transferred into a desiccator, allowed to cool and then weighed.

Calculation:

$$\text{Fat content (\%)} = \frac{W_2 - W_1}{W} \times 100$$

Where:

W_2 = Weight of flask and fat
W_1 = Weight of empty flask
W = Weight of sample

Determination of iodine value: The iodine value of oil, which measures the degree of unsaturation of the oil, is usually determined by Wijs' method (Pearson, 1970). In the method, the oil is treated with an excess of iodine monochloride (ICl). The unreacted iodine monochloride is treated with potassium iodide and the liberated iodine is titrated with standard thiosulphate solution.

Equations of reaction:

$$ICI + I_2 \rightarrow 3ICI \text{ (generated from Wijs' reagent)}$$
$$ICI + \text{oil} \rightarrow \text{halogenated fatty acid}$$
$$ICI + KI \rightarrow I_2 + KCI$$
$$I_2 + 2S_2O_3 \rightarrow S_4O_6^{2-} + 2I^-$$

The method was applied to determine the level of unsaturated fatty acids in the extracted fish oils. The Wijs'reagent was prepared by dissolving 8.0 g iodine trichloride (ICl_3) in 200 mL glacial acetic acid and 9.0 g iodine in 300 mL CCl_4. The two solutions were mixed and diluted to 1 L with glacial acetic acid. 0.3 g of fish oil was weighed and dissolved in carbon tetrachloride (CCl_4) and 25 mL of Wijs' reagent was added. The mixtures were thoroughly mixed and allowed to stand for 30 min in the dark. Twenty milliliter of 10% potassium iodide solution and 100 mL distilled water added. The iodine liberated was titrated with 0.1 M sodium thiosulphate solution using starch as the indicator just before the end point. A blank test containing the same quantities of reagents but without fish fat was equally carried out. The purpose of the blank experiment was to ascertain under the conditions of the determination, the weight of iodine equivalent to the halogens contained in 25 mL of Wijs solution in order to determine the amount of the iodine absorbed by the oil.

Calculation:

$$\text{Iodine value} = \frac{(V_1 - V_2) \times 1.269}{W}$$

where, V_1 is volume (cm^3) of thiosulphate used to titrate blank. V_2 is volume (cm^3) of thiosulphate used to titrate sample. W is weight (gram) of the oil. Iodine value is usually expressed as mg I_2/100 g oil.

Sample digestion for heavy metals determination: One gram of the dried and homogenized sample was accurately weighed into a digestion tube. 20 mL of 3 M Nitric acid (HNO_3) was subsequently added. This was allowed to cool and filtered into 25 mL volumetric flask. The digestion tube was washed with distilled water and again filtered into the volumetric flask and then made up to mark. Procedural blanks were also digested along with the samples. The sample digests were subsequently analysed for copper, zinc lead and cadmium contents using a flame atomic absorption spectrophotometer (Buck Scientific 200A model). All glasswares used were thoroughly cleaned by soaking overnight in 2 M nitric acid and then rinsing thoroughly with distilled water. The blank values were generally low and below the detection limits of the instrument for the metals. All the metal concentrations were expressed on a dry weight basis. Stock standard solutions for the atomic absorption analyses were obtained as the commercial BDH metal standards for atomic absorption spectroscopy. A recovery study of the analytical procedure was carried out by spiking and homogenising several already analysed solid with varied amounts of standard solutions of the metals. The spiked samples were then processed for organic matter destruction, as was initially done for the samples and then analysed. Average recoveries obtained were Pb, 92.8±1.8%, Zn, 103.3±0.33% and Cu, 100.6±0.47%.

RESULTS

Trace and toxic heavy metals in the frozen fish species: Table 2 shows that average lead levels ranged widely in the frozen fish samples from a low 2.09 μg g^{-1} in Hake (*Merluccius merluccius*) to a relatively high 12.3 μg g^{-1} in Chub mackerel(*Scomber japonicus*). Cadmium level in all the fish samples was below the detection limit of AAS. Average copper levels in the fish species ranged from 2.38 μg g^{-1} in Croaker (*Pseudolithus elongatus*) to 4.38 μg g^{-1} in Hake (*Merluccius merluccius*). Average zinc levels however ranged from 22.9 μg g^{-1} in Croaker to 32.2 μg g^{-1} in Atlantic horse. The following are the profiles of toxic and trace metals in the fish species analysed: Pb, Hake<Atlantic horse mackerel<Croaker<Sardine<Chub mackerel. Cu, Croaker<Chub mackerel = Atlantic horse mackerel<Hake<sardine. Zn, Croaker<Chub mackerel<Sardine<Hake<Atlantic horse mackerel.

Fat content and iodine values determination in frozen fish species: The results from Table 2 indicate that fat content in the frozen fish species ranged widely from a low 4.38% in Hake to a relatively high 58.9% in Atlantic horse mackerel.

The following profiles were obtained for fat content and iodine value in the fish samples respectively: Fat content; Hake<Croaker<Sardine<Chub mackerel<Atlantic horse mackerel. Iodine value; Hake<Sardine<Croaker<Chub mackerel<Atlantic horse mackerel. Table 2 equally shows that iodine values of oil obtained from frozen fish obtained from the two sampling locations ranged from 50.4 mg/100 g in Hake to 112 mg/100 g in Atlantic horse mackerel.

DISCUSSION

Trace and toxic heavy metals in the frozen fish species: The average lead levels found in Chub mackerel (12.3±0.68 μg g^{-1}) as shown in Table 2 was slightly lower than 12.6 μg g^{-1} obtained for the same fish species (Abou-Arab *et al*., 1996). Of all the fish samples analyzed from both locations only Chub mackerel bioaccumulates lead levels higher than values of lead reported for fish species in the literatures: 4.90-5.30 μg g^{-1} (Chale, 2002), 1.95-4.79 μg g^{-1} (Park and Presley, 1997), 2.0 μg g^{-1} (WHO, 1985; FEPA, 2003). Average lead level (2.09 μg g^{-1}) recorded for Hake was

Table 2: Mean levels±SD (μg g^{-1}, dry wt) of Pb, Cd, Cu, Zn, fat content (%) and iodine value (mg/100 g) of frozen fish obtained from Ibadan and Lagos

Sample name	No. of samples	Pb	Cd	Zn	Cu	Fat content	Iodine value
Hake	4	2.09±0.79	<0.01	30.9±0.63	4.38±0.21	4.38±0.21	50.4±9.75
Sardine	4	5.89±0.99	<0.01	28.8±0.09	4.25±0.21	34.4±3.15	57.8±9.60
Chub mackerel	4	12.3±0.68	<0.01	23.6±1.20	4.03±0.09	38.8±1.55	120±16.5
Atlantic horse mackerel	4	3.14±0.58	<0.01	32.2±0.20	4.03±0.38	58.9±0.45	112±2.00
Croaker	4	4.42±0.53	<0.01	22.9±4.80	2.38±0.21	15.6±11.1	76.3±8.90
Mean±SD		5.57±0.16	<0.01	25.7±1.75	3.56±0.12	30.5±4.04	63.3±4.60
Range		2.09-12.3		22.9-32.2	2.38-4.38	4.38-58.9	50.4-120

comparable to WHO and FEPA standard of 2.0 μg g^{-1} (WHO, 1985; FEPA, 2003). Thus, consumers of this fish species might not be exposed to lead-induced health hazards. Sardine, Atlantic horse mackerel and Croaker also bioaccumulate lead at varying degree of concentration. The average lead concentrations observed for Sardine (5.89 μg g^{-1}), Atlantic horse mackerel (3.14 μg g^{-1}) and Croaker (4.42 μg g^{-1}) were higher than WHO and FEPA standards of 2.0 μg g^{-1}, hence consumers of these fish species might be vulnerable to lead-induced health hazards. The absence of cadmium concentration in the fish species may be due to non-bioconcentration. Therefore, cadmium even though might be present in trace amount in the fish samples could not be said to be higher than the 2.0 μg g^{-1} maximum recommended limits of cadmium in fish (WHO, 1985; FEPA, 2003). Hence consumers of the fish species may not be vulnerable to cadmium-induced health hazards. Zinc levels were generally higher in the fish species from both locations than the corresponding lead and copper levels this may be due to the fact that zinc is a common heavy metal pollutant in the environment. All the fish species were not polluted with respect to zinc when compared with the WHO standards of 1000 μg g^{-1} (WHO, 1985) and FEPA standards of 75 μg g^{-1} (FEPA, 2003). Sorensen (1991) observed that zinc is an essential biological mineral which is regulated and maintained at certain concentrations in fish due to physiological requirements for survival, known as homeostatic regulation. Hence, consumers of these fish species will not be exposed to zinc-induced health hazards. Copper though essential in the diet can be harmful when large intake occurs. The harmful toxicity is largely attributed to its cupric (Cu^{2+}) form which is commonly found in fish (Ashraf, 2005). The average Cu levels (μg g^{-1}) recorded for Hake (4.14±0.01), Sardine (4.25±0.2), Chub mackerel (4.03±0.1) and Atlantic horse mackerel (4.03±0.38), were all higher than the 3.0 μg g^{-1} maximum recommended standards in food (WHO, 1985; FEPA, 2003). This is indicative of copper contamination of the fish species and as such consumers of the fish species may be vulnerable to copper-induced health hazards. The average Cu levels (μg g^{-1}) recorded for Croaker (2.38±0.21) was however lower than the 3.0 μg g^{-1} maximum recommended standards in food (WHO, 1985; FEPA, 2003). The copper levels in most of the analyzed fish samples were also found to be higher than the values obtained by Aucoin *et al.* (1999) (2.75-1.09 μg g^{-1}) and lower than 20 μg g^{-1} which is the maximum copper levels permitted for fishes according to Turkish food codex (Anonymous, 2002).

Fat content and iodine values determination in frozen fish species: Almost all the fish analysed for fat content were rich in fat when compared to the 5% fat content observed for marine fish by Bennion (1986). Rasoarahona *et al.* (2005) have observed that fat content of fish changes due to species, diet, gender, geographical origin and season.

Iodine value in food samples is a measure of the degree of unsaturation. The higher the iodine values the higher the level of unsaturation in the fish oil and the greater their stability to rancidity via oxidation. Therefore consumption of fish could reduce risk of cardiovascular disorder in humans.

This study has shown that oils obtained from Hake, Sardine and Croaker from the two sampling locations have iodine values below 100 mg/100 g which place them in the non-drying group of oil. However, oils obtained from Chub mackerel and Atlantic horse mackerel from the two sampling locations belong to the semi-drying group as their iodine value is greater than 100 mg/100 g. Hence, the high iodine value recorded for Chub mackerel and Atlantic horse mackerel from both locations suggests that the two fish species contain high level of unsaturated oil and as such could reduce the risk of heart diseases, high cholesterol, depression, anxiety, low immunity, cancer, eye disorders and ulcers in humans when consumed.

CONCLUSION

The results as obtained in this study show that trace and toxic metals can bioaccumulate in frozen fish species to varying degree of concentration. This suggests that aquatic environment, food and sediment in the coastal water can serve as anthropogenic sources of heavy metals contamination in marine environment. This study has also shown that the nutritional benefits i.e., high-quality protein, high content of the two kinds of omega-3 poly unsaturated fatty acids and high iodine values that fish possess can be jeopardized by the presence of elevated level of toxic metal especially lead as recorded for Chub mackerel and as such frequent consumption of this fish species should be discouraged as this could pose health risk to the consumer. It is therefore recommended that inspection of seafood for human consumption should be adequately looked into by the regulatory bodies charged with this responsibility in Nigeria. Frozen fish imported into the shore of Nigeria should equally be certified fit for human consumption by the government agency concerned.

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