Full Length Research Paper

Heavy metal, proximate and microbial profile of some selected commercial marine fish collected from two markets in south western Nigeria

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The study on the elemental, proximate and microbial composition of fresh samples of *Scomber scombrus*, *Gadus macrocephalus*, *Saclina pilchradus* and *Jack mackerel* was determined to gain the knowledge of the risk and benefits associated with indiscriminate consumption of marine fishes. Wet digestion was done for the samples and was analyzed for minerals, heavy metals and microorganisms. The species examined contained appreciable concentrations of moisture, protein, lipids and ash content suggesting that the fish species could be used as a good source of minerals. Heavy metals analyzed were above tolerable limits; therefore, it can be suggested that taste, size, freshness and other related external appearances should not be the only factor to be considered in making choice for marketing and consumption of marine and freshwater fishes in Nigeria.

Key words: *Scomber scombrus*, *Gadus macrocephalus*, *Saclina pilchradus*, *Jack mackerel*, elemental, proximate, microbial, heavy metals.

INTRODUCTION

Malnutrition (protein energy malnutrition, micronutrient deficiencies and over nutrition) is a public health problem in developing countries. Chronic malnutrition in Nigeria according to the 2008 Nepal Demographic and Health Survey Fact Sheet (NDHS) was 38% (NPC and ICF Macro, 2009). The nutrient composition of locally available foods/diets are used to estimate the adequacy of dietary intake of population groups, determine diet-disease relationships, health and nutritional status, and for achieving dietary intake goals (Ene-Obong et al., 2013).

Fish and fish products are highly nutritious with protein content of 15 to 20% and are particularly efficient in supplementing the cereal and tuber diets widely consumed in Africa (Fagbenro et al., 2005). It was further reported that in Nigeria, fish are regarded as a major food item contributing a total of 40% to dietary protein. It is

also a preferred and reliable source of animal protein with balanced amino-acids, vitamins and essential minerals for healthy human growth. Fish allows for protein improved nutrition in that it has a high biological value in terms of high protein retention in the body (Anthony and Akinwumi, 1991).

Fish is a highly proteinous food consumed by a larger percentage of populace because of its availability and palatability (Foran et al., 2005). Also, when compared to other protein sources like goat and chicken meat, it is safer, healthier and is also known to be an excellent source of protein from amino acid composition and protein digestibility (Astawan and Ikan, 2004). Fish is also one of the main sources of protein in developing countries (Louka et al., 2004). In Nigeria, fish is eaten fresh and smoked and form a much cherished delicacy that cut across socio-economic, age, religions and

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educational barriers (Adebayo et al., 2008).

According to the study of Adekoya and Miller (2004), fish and fish products constitute more than 60% of the total protein intake in adults especially in rural areas. The marine fish is generally cheaper and more abundant when compared with fresh water fishes, which are relatively more expensive in Nigeria. The major constituents of fish are moisture, protein and fat with minerals occurring in trace amount (Holland et al., 1993). All living organisms require these mineral elements and some of these biochemical attributes at moderate levels; but when they exceed metabolic demand or requirement, they tend to become accumulated in tissues of organisms such as fish which can only metabolize these substances to lesser extents because most of the heavy metals are non-biodegradable (Lenntech, 2006). Nutrient content varies with fish species. There are limited data on the nutritional composition of fish species which are commonly consumed by the poor in developing countries of Asia and sub-Saharan Africa (Oladipo and Bankole, 2013).

In a study by Adeniyi et al. (2012), the nutritional composition of three fish species namely catfish (*Clarias gariepinus*), electric fish (*Malapterurus electricus*) and tilapia fish (*Tilapia guineensis*) were analyzed. Oladipo and Bankole (2013) carried out a study on the nutritional and microbial quality of fresh and dried *C. gariepinus* and *Oreochromis niloticus.* Emmanuel et al. (2011) carried out a comparative analysis of the proximate composition of *Tarpon atlanticus* and *C. gariepinus* from culture systems in South-Western Nigeria. Fawole et al. (2007) carried out a study on the proximate and mineral composition in some selected fresh water fishes in Nigeria and discovered the presence of heavy metals (such as zinc, copper, lead, cadmium, nickel and arsenic) present in the order Zn > Ni > As > Cu > Pb > Cd). Olagunju et al. (2012) evaluated the nutrient composition of *Tilapia zilli*, *Hemisynodontis membrenacea*, *Clupea harengus* and *Scomber scombrus* consumed in Zaria. Omotosho (1995) did a comparative analysis of the chemical composition of *Dasyatis margarita* (Gunther) with *T. zilli* (Gervias) and *C. gariepinus* (Burchell) from Nigerian waters. Fish has the potential to be considered as a balanced food and can therefore be expected to provide relief from malnutrition; Sanker et al. (2013) investigated the chemical composition and nutritional value of Anchovy (*Stolephorus commersonii*) caught from Kerala coast, India. They reported that the proximate composition of anchovies compares well with the general composition of fish reported earlier by Gopakumar (1997), Ninan (2003), Devi (2006) and Mohan et al. (2008).

Fish mineral and metal contents may vary according to the surrounding environment (Ambedkar and Muniyan, 2011; Sen et al., 2011). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms

(Ashraj, 2005; Vosylene and Jankaite, 2006; Farombi et al., 2007). Heavy metals have long been recognized as serious pollutants of the aquatic system. The heavy metals that are toxic to many organisms at very low concentrations and are never beneficial to living beings are Hg, Cd and Pb (Dural et al., 2006). The fish is often exposed to various microorganisms. A number of these microorganisms are naturally present in the aquatic environment, and some of them enter water via animal excreta, agricultural runoff, industrial and human wastes (Adams and Thompson, 2006; Almedia et al., 2009; Altinok et al., 2008).

MATERIALS AND METHODS

Sample collection

The fish samples used for this study were collected from two different major markets in Ogbomoso, Oyo State, Nigeria.

Sample preparation

The fish samples were thoroughly washed with tap water and distilled water to remove any adhering contaminants and drained under folds of filter paper. The fish sample was dissected with a knife and the intestines, guts and bones were removed. The muscle samples were then homogenized into a fine mesh with an electric food blender and thereafter, stored in a deep freezer (- 18°C) prior to analysis.

Metal analysis

Three specimens from each species were used for the analysis. The tissues were oven dried at 70 to 73°C until constant weight was obtained. The specimens were then ground to fine powder and stored in desiccators in order to avoid moisture accumulation before digestion. The digestion procedure was carried out as described by Kotze et al. (2006). Twenty milliliter (20 ml) of concentrated nitric acid (55%) and 10 ml of perchloric acid (70%) was added to approximately 1 g tissue (dry mass) in a 100 ml Erlenmeyer flask. The digestion was done on a hotplate (200 to 250°C) until the solutions were clear. The solutions were then filtered through an acid resistant 0.45 mm filter paper and made up to 50 ml each with distilled water. The samples were stored in clean glass bottles prior to the determination of the metal concentration using an inductively coupled plasma mass spectrophotometer (ICP-MS). A standard sample, consisting of tuna homogenate (sample IAEA-350) from the International Atomic Energy Agency Marine Environment Laboratory, was prepared and used as a control in accordance with the afore-mentioned procedures with every set of samples, to ensure accuracy of data through comparison.

Analytical standards were prepared from Holpro stock solutions. Prior to use, all glassware were soaked in a 2% Contrad soap solution (Merck chemicals) for 24 h, rinsed in distilled water, acid-washed in 1 m HCL for another 24 h and rinsed again in distilled water.

Proximate analysis

Moisture content analysis: Moisture content of fish fillets was determined according to the method of AOAC (2000). The samples

Table 1. Mean and standard error of heavy metal composition of fish species collected from the two markets.

Sample	Fe	Pb	Ni	Cd	Zn	Cu
Scomber scombrus	3.1561±0.1230	0.4571±0.0163	0.0256±0.0116	0.4883±0.0123	0.2724±0.0048	0.2766±0.0030
Sardina pilcladus	5.6259±0.0052	0.1265±0.0029	0.3141±0.0036	0.1937±0.0001	0.3531±0.0001	0.2394±0.0004
Jack mackerel	0.8658±1.000	0.0141±0.0001	0.0135±0.0100	0.0234±1.0040	0.0964±0.0002	0.0161±5.7705
Gadus macrocephalus	2.3714±1.000	0.2024±0.0001	0.1011±1.000	0.0298±1.0000	0.3448±1.0000	0.1271±0.000

were dried in moisture dish in an oven at 105°C until constant weights was obtained.

Ash content analysis: Ash content of fish fillets was determined according to Association of Official Analytical Chemists. Pre-dried samples obtained from moisture content analysis were ashed in furnace at 550°C overnight.

Crude protein analysis: Crude protein content of fish fillets was determined according to the method of AOAC (2000). Briefly, 1 g of sample was weighed into digestion tubes. Two Kjeltabs Cu 3.5 (catalyst salts) were added into each tube. About 20 ml of concentrated sulphuric acid (H_2SO_4) was carefully added into the tube and then shaken gently. Digestion procedure was carried out. Digested samples were cooled for 10 to 20 min. Distillation procedure was then performed using distillation unit and the distillate was titrated with 0.025N sulphuric acid (H_2SO_4) until the end point changes from green to pink. Volume of acid required in the titration was recorded. Blank was prepared with the exclusion of sample. The percentage of protein content was calculated according to the following equation:

$$\% \text{ Nitrogen} = \frac{0.014 \times VD \times N \times 100 \times TV}{\text{Weight of sample} \times AD}$$

% Protein = %N × F

Where, VD is the Volume of digest; N is the normality of acid; TV is the titre value; AD is the aliquot of digest and F is the conversion factor for nitrogen to protein (6.25).

Fat content analysis: Crude fat was obtained by exhaustively extracting 2.0 g of each sample in a Soxhlet apparatus using petroleum ether (b.p. 40 to 60°C) as the extractant.

Carbohydrate content analysis: Carbohydrate content was calculated based on difference calculation:

Carbohydrate = 100% - (%moisture + %ash + %crude protein + %fat).

Microbial analysis

The microbial content of the fish samples were enumerated by standard plate count technique using 0.1 ml aliquots of appropriate dilution pour plated onto Nutrients agar, MacConkey, Mannitol salt agar and Salmonella-Shigella agar for bacteria. Potato dextrose agar (PDA) plus chloramphenicol was used for fungi isolation and enumeration. All plates for bacteria isolation were incubated at 37°C for 24 to 48 h while PDA plates were incubated at room temperature for 3 to 5 days. Individual colonies were purified and identified by morphological and biochemical techniques (Jott et al., 1994). In the case of fungal isolates, the microscopic and

macroscopic features of the hyphal mass, morphology of cells and spores, nature of the fruiting bodies, among other criteria were used for identification (Tsuneo, 2010).

Statistical analysis

Analysis of the data was carried out using the excel worksheet package.

RESULTS

Metal composition

The mean values for the heavy metal composition of the fish species is shown in Table 1. In *S. scombrus*, Fe had the highest concentration of 3.1561 while other metals were found in trace quantities with the lowest being Ni (0.0256). *Sardina pilcladus* had the highest metal concentration of 5.6259 for Fe and the lowest is 0.1265 for Pb. In *Jack mackerel*, the highest concentration was 0.8658 for Fe while the lowest value of 0.0135 was obtained for Ni.

For *Gadus macrocephalus*, Fe had the highest value of 2.3714 while Cd had the lowest value of 0.0298. The overall order of heavy metal concentration in all the fish species is Fe > Cd > Pb > Zn > Ni > Cu.

Moisture, protein, carbohydrate and lipid composition

Table 2 shows the mean values for the proximate composition of the fish species. The highest moisture content was found in *G. macrocephalus* with value of 81.4233 while the lowest was recorded for *S. pilcladus* with 57.5700. For the protein content of the fishes, *S. pilcladus* had the highest value of 26.3866 while the lowest value of 11.7233 was recorded for *G. macrocephalus. G. macrocephalus* had the highest ash content of 1.4866 among the four fish species while *S. scombrus* recorded the lowest value of 1.1133. Considering the lipid content of the fish species, *S. scombrus* had the highest value of 10.2133 while *G. macrocephalus* recorded the lowest value of 2.0866. For the carbohydrate content, *S. pilcladus* recorded the highest value of 7.1233 while *S. scombrus* had the lowest value of 2.5133.

Table 2. Mean and standard error of proximate composition of fish species collected from the two markets.

Sample	Moisture content (%)	Protein content (%)	Ash content (%)	Lipid content (%)	Carbohydrate content (%)
Scomber scombrus	63.3866±0.5398	23.0900±0.0100	1.1133±0.0057	10.2133±0.0057	2.5133±0.0152
Sardina pilcladus	57.5700±0.3732	26.3866±0.0057	1.3033±0.0057	7.8133±0.0057	7.1233±0.0057
Jack mackerel	72.8566±0.0208	16.7833±0.0057	1.4166±0.0115	6.0866±0.0057	2.8833±0.0057
Gadus macrocephalus	81.4233±0.0057	11.7233±0.0057	1.4866±0.0057	2.0866±0.0057	3.3033±0.0057

Table 3a. Bacterial count in fish species collected from the two markets.

Fish species	$\times\ 10^3$ cfu ml^{-1}
Scomber scombrus	4.0
Sardina pilcladus	3.0
Jack mackerel	7.0
Gadus macrocephalus	10.8

Table 3b. Fungal count in fish species collected from the two markets.

Fish species	$\times\ 10^3$ cfu ml^{-1}
Scomber scombrus	1.0
Sardina pilcladus	1.0
Jack mackerel	1.0
Gadus macrocephalus	1.0

Microbial analyses

The microbial profile of the four fish species reveal that *G. macrocephalus* had the highest bacterial concentration of 10.8×10^3 cfu ml^{-1} while *S. pilcadus* had the lowest bacterial count of 3.0×10^3 cfu ml^{-1}. All the fish species were found to have recorded the same values (1.0×10^3 cfu ml^{-1}) of fungal count (Table 3a and b). Table 4 shows the different microorganisms isolated from each fish species. *S. scombrus* was found to have *Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus plantarum* and *Pseudomonas florescence.* From *G. macrocephalus*, *Clostridium botulinum* and *S. aureus* were isolated. *S. aureus*, *Salmonella* species, *Shigella* species and *E. coli* were isolated from *Sardina pilcladus* while from *J. mackerel*, *P. florescence* and *Flavobacterium* species were both isolated. Table 5 shows the antibiotic resistivity of the bacterial isolates to chloramphenicol, ampicillin and tetracycline, respectively. The highest resistance to chloramphenicol (32 mm) was exhibited by *P. florescence* isolated from *J. mackerel* while the lowest (12 mm) was exhibited by *Shigella* species isolated from *S. pilcladus.*

For Ampicillin, the highest resistance (40 mm) was exhibited by *S. aureus* isolated from *S. scombrus* while the lowest (25 mm) was exhibited by *P. florescence* isolated from *S. scombrus.* The highest resistance to tetracycline (50 mm) was exhibited by *E. coli* isolated from *S. pilcladus* while the lowest (29 mm) was exhibited by *E. coli* isolated from *G. macrocephalus.*

DISCUSSION

In the present study, heavy metals were found to have bioaccumulated in the tissues of the fishes under study following different patterns. Such pattern has been observed in a number of other studies, covering several fish species (Dural et al., 2006; Storelli et al., 2006; Ploetz et al., 2007; Rashed, 2001; Pyle et al., 2006; Agah et al., 2009). Muscle has been considered to have metal accumulating potential (Erdogrul and Erbilir, 2007; Uysal et al., 2009; Bervoets and Blust, 2003; Dahunsi et al., 2012). Also, Kotze et al. (2006) and Senthil et al. (2008) reported significant bioaccumulation of metals in fish muscle. The high value recorded for Fe in the fish muscles may be due to its availability in the water and feeds they consume. This trend agrees with other studies where elevated amount of Fe was found in fish tissues (Yilmaz et al., 2007; Dural et al., 2006; Uysal et al., 2009; Dahunsi et al., 2012). The accumulation pattern of Cd and other metals studied conformed closely with the work done by Vinodhini and Navayanan (2008) where they carefully observed the trend of bioaccumulation of heavy metals in various organs of the fresh water fish *Cyprinus carpio* (common carp) exposed to heavy metal contaminated water system. Cadmium was found in this study to have low levels of bioaccumulation in the body muscle investigated. Cadmium is considered in other works to have the highest concentration in liver.

Ivan et al. (2011) reported that it reached 40 to 100 times greater concentrations in muscle than those found by Agusa et al. (2004) in five Caspian sturgeon species. An increased Cd levels in these fishes is worrying, especially considering the fact that it could be very hazardous for fish genetic material according to the study of Alibabic et al. (2007) and it is one of the most toxic heavy metals, even at relatively low concentrations (Dural et al., 2006; Fianko et al., 2007; Yilmaz et al., 2007).

Table 4. Isolated bacteria from fish species collected from the two markets.

Fish	Organism
Scomber scombrus	*Staphylococcus aureus*
	Escherichia coli
	Lactobacillus plantarum
	Pseudomonas florescence
Gadus macrocephalus	*Clostridium botulinum*
	Staphylococcus aureus
Sardina pilcladus	*Staphylococcus aureus*
	Salmonella species
	Shigella species
	Escherichia coli
Jack mackerel	*Pseudomonas florescence*
	Flavobacterium species

Table 5. Antibiotic sensitivity of bacteria isolates collected from the two markets.

Fish	Organism	Chl (mm)	Amp (mm)	Tet (mm)
Scomber scombrus	*Staphylococcus aureus*	23	40	45
	Escherichia coli	20	38	40
	Lactobacillus plantarum	26	35	38
	Pseudomonas florescence	20	25	35
Gadus macrocephalus	*Clostridium botulinum*	16	30	49
	Staphylococcus aureus	22	28	39
	Escherichia coli	19	26	29
Sardina pilcladus	*Staphylococcus aureus*	26	29	45
	Salmonella species	32	38	47
	Shigella species	12	26	40
	Escherichia coli	21	36	50
Jack mackerel	*Pseudomonas florescence*	32	37	49
	Flavobacterium species	25	34	40

Chl, Chloramphenicol; Amp, ampicillin; Tet, tetracycline.

These metals can remain in the body like other heavy metals for a long period of time and can bioaccumulate for many years after exposure to low levels (Groundwork, 2002).

A fish contaminated with these metals can find its way into man's food chain, resulting in biomagnifications of such heavy metal and this becomes harmful to man's health. Generally, virtually all the heavy metals analyzed were found to exceed National Environmental Standard and Regulation Enforcement Agency (NESREA) (2007) and WHO (2011) standards. For the proximate composition of the fishes, moisture of a given samplesimply refers to the water content of that sample. The fish moisture content indicates that the percentage moisture in fish muscles was within the acceptable level (60 to 80%) in all the samples which could be due to the stable water levels in the environmental location where the fish were raised. The percentage of water is also a good indicator of its relative content of energy, protein and lipid. The high moisture content is a disadvantage in that it increases the fishes' susceptibility to microbial spoilage, oxidative degradation of polyunsaturated fatty

acids and consequently decreases in the quality of the fishes for longer preservation time in agreement with Omolara and Omotayo (2008).

The protein levels shows that all the fish species are good sources of protein. The relatively high to moderate percentage crude protein may be attributed to the fact that fishes are good source of pure protein, but the differences observed in values obtained could also be as a result of fish consumption or absorption capability and conversion potentials of essential nutrients from their diets or their local environment into such biochemical attributes needed by the organisms body (Adewoye and Omotosho, 1997). The observed range of ash content in the fishes indicates that the species is a good source of minerals such as calcium, potassium, zinc, iron and magnesium.

Ash is a measure of the mineral content of food item. It is the inorganic residue that remains after the organic matter has been burnt off. Generally, lipids are soluble in ether; hence, they are ether extractable. They serve as source of energy during starvation and fasting. According to the study of Ackman (1989), generally, fish can be grouped into four categories according to their fat content: lean fish (< 2%), low fat (2 to 4%), medium fat (4 to 8%) and high fat (> 8%). These marine fishes had a high lipid content; hence, their classification as high fat fishes. This indicates that the fishes are better sources of lipid in the body when consumed (Osibona et al., 2009). The appreciable values of carbohydrate could be due to the presence of elements like calcium and potassium in their diets. The microorganisms isolated from the fishes under study are an indication of the nature of the aquatic environment where they were reared. This conforms with previous studies that a number of these microorganisms are naturally present in the aquatic environment, and some of them enter water via animal excreta, agricultural runoff, industrial and human wastes (Adams and Thompson, 2006; Almedia et al., 2009; Altinok et al., 2008).

Also, it has been reported that the spread of antibiotic resistant microorganisms in the environment is recognized as an important public health issue which has attracted the attention of physicians concerning their future ability to treat infectious diseases (Mukherjee and Chakraborty, 2007).

Conclusion

This study therefore, showed that marine fishes are good sources of minerals. Thus, it is right to say that, the mineral elemental contents of each species is a function of the availability of these elements in their local environment, diet absorptive capability and as well as their preferential accumulation. However, it was discovered that, some hazardous heavy metals and pathogenic microbes recorded high values which may pose danger to the humans consuming such fishes. Also, this present work has elucidated more on the importance of marine fishes as good sources of protein and minerals.

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