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Comparative Assessment of the Proximate Composition and Safety of Commonly Consumed Fish Stock in Ado Ekiti, Ekiti State, Nigeria. Nutritional and Quality Evaluation of Three Economically Important Fish Species in Ado Ekiti

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Abstract

Fish are bioindicators of pollution in an aquatic environment. Fish stock are basically consumed based on taste preference and affordability as against the nutritional composition. This study investigated the quality of three commonly consumed fish species in Ado Ekiti through proximate analysis of the frozen samples, heavy metal assessment and microbial count of the smoked samples using standard procedures. Fish samples collected from two popular markets were identified as *Scomber scombrus* (local name: Titus), *Merluccius merluccius* (local name: Panla), *Clupea harengus* (local name: Shawa). The nutritional composition of the fish samples showed that Scomber scombrus had the highest quantity of fat while M.merluccius had the significantly highest ($p<0.05$) fibre and Clupea harengus had the highest protein. Protein composition in the three fish species ranged from (19.723±0.122) % to (26.165±0.166) % across both markets. The smoked fish samples examined for microbial count which include total bacterial count, total bacterial count(TBC), total fungi count (TFC) and total coliform count (TCC) showed that all the samples were laden with bacteria as TBC across all the species ranged from (4.190±0.015)105CFU/g- (9.466±0.020)105CFU/g were higher than the permissible limit recommended by WHO. Heavy metals were evident in the smoked fish in values within WHO permissible limits. This suggest that the nutrient composition of these fish is species- dependent regardless of the location, also the smoked fish can pose health danger if consumed directly due to the presence of microbes which may be airborne or be introduced during fish processing. Hence there is a need for appropriate bodies to ensure adequate hygiene on the part of vendors who market smoked fish in the community.

Keywords: Fish; Proximate; Smoked; Heavy metals; Contamination; Pollution; Microbe; Nutrition; Quality; Safety

Introduction

Historically, fish has been an important component of the diet of human beings for the past 30,000 years. Fish generally encompass all seafoods, including fined fishes, Crustaceans with chitinous exoskeleton such as Lobsters, Crabs, Shrimps, Muscle cockles, and oysters [1]. It is a high protein, low-fat food and an important source of essential nutrients required for supplementing both infants and adult diets and provides a range of health benefits [2]. In general, nearly 20% of animal protein sources are provided by fish [3] and it is a rich source of protein for the poor and wealthy [4]. Although, meat and poultry products are probably more sought after by consumers, they are also far more expensive. The relatively low price of fish is undoubtedly the main reason that it is eaten in much greater quantities than the other forms of animal protein [5]. This further explains the reason fish is often referred to as the cheapest and most important source of animal protein [6]. Aside being used as staple source of proteins and source of income, in recent time, fish is preferred more by people as a healthy alternative to red meat [7]. Fish is easily digestible and it contains less fats and high proportion of poly or mono unsaturated fatty acids (Omega – 3 fatty acid) and is thought to be associated with reducing the risk of human cardiovascular disease. Omega 3 fatty acids have been found to have cholesterol lowering properties as well as decreasing blood clotting activity [8].

Fish are marketed in different forms in Nigeria which include fresh fish, frozen fish, smoked or sundried fish [9]. Fresh fish is mostly available to people in the production areas while Fish is imported by the industrial sector. Nigeria is a leading importer of Fish in the world [10]. The bulk of the fish consumed in the country is frozen and are imported. Fish demand in Nigeria is about 2 million metric tonnes, while the current domestic fish production is about 0.9 million metric tonnes. The shortfall between demand and supply is made up by importation of more than 600,000 metric tonnes of fish, thus making frozen imported fish the bulk of fish consumed in Nigeria. Fish, being a highly perishable commodity, requires continuous reassurance of its quality [11]. Fish can be contaminated right from their aquatic habitat before catch or during the process of harvesting or process of preservation. Pollution due to heavy metals in fish is a global concern because they are not utilizable by the organisms and most of them are toxic to organisms [12]. Heavy metals amongst other environmental pollutants, are of serious public and other animal health concern. This is due to their potential toxicity and ability to bioaccumulate in aquatic ecosystems [13]. Heavy metals could also bind to cell membranes, thereby interfering with transportation processes in the body. Other vulnerable sites of attack include the protein, carboxylic acid (- COOH) and amino (- NH_3) groups [14]. Other impacts of heavy metals including lead poisoning causes damages to the central nervous system especially among children causing mental impairment. Exposure of humans to lead for a long term can cause coma or death [15]. Cadmium (Cd), known to be a heavy metal, can cause cancer by interfering with the body metabolic processes. Cadmium being one of most harmful heavy metals is capable of causing renal, hepatic and testicular injury. Some adverse effects of acute cadmium toxicity include kidney damage, testicular tissue damage, high blood pressure and red blood cells destruction [15]. Weak immune system, likely disease of lung cancer, ulcer and liver damages may result from exposure and consumption of chromium (Cd) containing substances. Long - term exposure to chromium can cause kidney, liver and nerve tissue damage. Many chromium compounds are also known to cause cancer, thus, making them carcinogenic [15]. Commonly consumed fish species in Nigeria include *Scomber scombrus* (local name Titus), *Clupea harengus* (local name shawa fish), *Merluccius capensis* (local name Panla fish), *Trachurus trachurus* (local name Kote fish), *Clarias gariepinus* (local name Eja aaro). Fish being a major faunal component of aquatic environments and are usually used as excellent environmental bioindicator of the health of aquatic systems [16]. Generally smoked fish are consumed due to its preserved state and its unique and palatable taste in meals. However, it is suspected that the procedure for smoking commercially consumed smoked fish may introduce some harmful substance into the fish samples [17]. In addition, it is also noted that individuals consume the smoked fish directly without any further cooking. Hence there is need to assess the quality of the different forms of fish consumed in Ado Ekiti, Nigeria.

Materials and Method

Study area

The study was carried out in selected markets in Ado-Ekiti, Ekiti state, Nigeria. Ekiti State was created on October 1st 1996 out of the old Ondo State. The town lies on latitude 7^0 49^1 North of the equator and longitude 5^0 27^1 East of the Greenwich Meridian about 250 metres above sea level. The people in Ado-Ekiti are mainly of Yoruba ethnic group. Fish samples were purchased from two major markets in Ekiti State, Nigeria. Iworoko market is a popular market usually being patronized by student of Ekiti state university while Oja Oba is a very big and popular market in Ekiti state usually patronized by the inhabitants of Ado metropolis.

Sample collection

Raw and smoked forms of *Scomber scombrus* ('Titus" Fish), *Clupea harengus* ("Shawa") and *Merluccius merlucius* ("Panla") was purchased from each market (i.e., Iworoko and Oja Oba market). They were packaged in a polythene bag and were labelled appropriately.

Proximate analysis of fish samples

The fish samples were evaluated for moisture content, crude fat, fibre, protein and carbohydrate using the standard procedure by [18].

Microbial count of smoked fish samples

Culture media preparation

Nutrient agar was used to determine the total bacteria loads of the sample. The media was prepared according to the manufacturer's specification and autoclaved for 15 minutes at 121°C

Serial dilution of Samples

Each sample was weighed and homogenized in 90mL of sterile distilled water, then blended in a sterile blender, and 1mL of the homogenate was constituted in 9mL of sterile peptone water. Then, using the pour plate technique, 0.1mL of the last dilutions (10^5) was inoculated in triplicate on properly prepared media. The plates were then incubated for 24 hours at 37°C and colonies

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developed were counted using colony counter and recorded as colony forming unit.

Total fungi count

One gram of each sample was carefully cut with the aid of a sterile scalpel and enriched in sterile sabouraud dextrose broth for twenty-four hours. Tenfold serial dilutions of the samples were thereafter carried out. The pour plate method was used. One milliliter of the serially-diluted sample (10^6) was dispensed into a conical flask containing sterile sabouraud dextrose agar (SDA) and two percent chloramphenicol to inhibit bacterial growth. The contents were properly mixed and dispensed aseptically into sterile petri-dishes. Incubation was carried out at 28^0C for five days. The colonies developed were counted and recorded as spore forming unit.

Coliform count

MacConkey broth was prepared according to the manufacturer specification and 25ml of the broth was put into the test tube. Inverted vials were introduced into the tubes, plugged with cotton wool and wrapped with aluminium foil. It was sterilized in autoclave at 121^0C for 15minutes. Serial dilution was carried out on the samples, the last three set of dilutions were inoculated with 1ml of the inoculums, each tube was plugged with cotton wool and incubated at 37^0C for 24hours. Fermentation and the formation of gas were observed after 24 hours. The tubes where fermentation or formation of gas occurred were taken as positive and non-fermented tubes were taken as negative. The positive tubes were subjected to confirmatory test by sub-culturing each tube into a fresh medium of MacConkey broth. A sterilized wire loop was used to take a loopful of culture to the freshly prepared medium, plugged with cotton-wool and incubated at 37^0C for 24 hours. The density of coliform bacteria was computed using Most Probable Number (MPN) and the results were recorded as MPN/ml.

Heavy metal analysis

The ash of each sample obtained was digested by adding 5ml of 2M HCl to the ash in the crucible and heated to dryness on a heating mantle. 5ml of 2M HCl was added again, heated to boil and filtered through what man No. 1 filter paper and washed into 100ml volumetric flask with deionized or distilled water and made up to mark. These diluents were aspirated into the Buck 211 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

Statistical analysis

All values were evaluated in triplicates per sample, means of each sample were measured and compared using ANOVA. Data were analysed using descriptive statistical tools such as mean and standard error alongside Duncan Multiple Range test. IBM SPSS Statistic version 21 was the software used for the analysis. One-way ANOVA post hoc test of multiple comparisons (Duncan) was used to determine significant differences among sets of means.

Results

Proximate analysis of frozen (Raw) fish samples

Table 1a & 1b shows the proximate composition of the raw fish samples collected from the two markets. Moisture content values ranged from 54.106% to 64.93% with *Clupea harengus* having the significantly highest moisture (P<0.05) and *Merluccius merlucius* having the lowest moisture content. Also, there was a similar trend in the composition of the fish species from both markets. *Merluccius merlucius* had the highest ash composition while *Scomber scombrus* had the lowest ash composition. *Clupea harengus* had the lowest fat composition while *Scomber scombrus* had the highest fat composition. *Scomber scombrus* had the lowest fibre while *M.merluccius* had the highest fibre. Protein composition in the three fish species ranged from 19.723% to 26.165% across both markets, however results from both markets showed that *C. harengus* had the highest protein composition. Similarly, *M. merluccius* had the highest carbohydrate composition from both markets with *S. scombrus* having the lowest CHO composition as shown in Table 1a & 1b. The results showed similar trend in the nutrient composition of the different species.

Table 1a: Proximate analysis of fish samples from Market A.

Sample Codes	MOISTURE	ASH	FAT	FIBRE	PROTEIN	CHO
	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error
PA	58.429±0.439^a	1.361±0.021^c	11.055±0.222^b	0.312±0.005^c	20.980±0.185^a	7.860±0.255^c
SA	64.930±0.252^c	1.018±0.047^b	6.179±0.062^a	0.225±0.006^b	24.859±0.105^c	2.786±0.133^b
TA	61.223±0.205^b	0.780±0.009^a	12.652±0.154^c	0.181±0.005^a	23.108±0.197^b	2.052±0.200^a

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Table 1b: Proximate analysis of fish samples from Market B.

Sample Codes	MOISTURE	ASH	FAT	FIBRE	PROTEIN	CHO
	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error
PB	54.106±0.175[a]	1.255±0.179[b]	9.483±0.176[b]	0.275±0.005[c]	19.723±0.122[a]	15.154±0.381[b]
SB	61.608±0.140[b]	0.900±0.018[a]	7.082±0.066[a]	0.193±0.002[b]	26.165±0.166[c]	4.049±0.094[a]
TB	61.863±0.408[b]	0.842±0.069[a]	11.602±0.237[c]	0.145±0.003[a]	22.004±0.086[b]	3.542±0.566[a]

Data were means of three triplicates and standard error each. Data with different superscript are significantly different from one another at (P<0.05)

Description of the sample code:

PA-Panla (*Merluccius merlucius*) collected from iworoko market.

PB- Panla (*Merluccius merlucius*) collected from Oja Oba Ado market.

SA- Shawa (*Clupea harengus*) collected from iworoko market.

SB- Shawa (*Clupea harengus*) collected from Oja Oba Ado market.

TA- Titus (*Scomber scombrus*) collected from iworoko market.

TB- Titus (*Scomber scombrus*) collected from Oja Oba Ado market.

Microbial analysis of smoked samples

Table 2 shows the bacterial count (TBC) in fish samples collected. Bacteria were present in all the fish species. The bacterial count ranged between 4.19x10^5(CFU/g)- 9.47x10^5(CFU/g) with *Scomber scombrus* (TA) having the highest bacterial count 9.47x10^5(CFU/g) and *Clupea harengus* (SB) had the lowest bacterial count 4.19x 10^5(CFU/g) as illustrated in Table 1. All the fish samples had bacteria count which were above the WHO permissible limit. The fungi count (TFC) in smoked fish samples collected ranged between 2.35x10^6(SFU/g) - 5.49x10^6(SFU/g) with *Scomber scombrus* (TA) having the highest fungi count 5.49x10^6(SFU/g) and *Merluccius merluccius* (PA) had the lowest fungi count 2.35x10^6(SFU/g). The Coliform count (TCC) in *Merluccius merluccius* (PA and PB) and *Scomber scombrus (TA* and TB) ranged between 1.00(MPN/g) – 5.67(MPN/g) with *Merluccius merluccius* (PA) having the highest coliform count 1.00(MPN/g) and *Scomber scombrus* (TB) had the lowest coliform count. However, *Clupea harengus* (SA and SB) had no coliform count.

Table 2: Microbial count of smoked fish sold at Ado-Ekiti.

Sample Code	Total Bacterial Count (CFU/g) x (10^5)	Total Fungi Count (SFU/g) x (10^6)	Coliform (MPN/g)
	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error
PA	5.630±0.2082[c]	2.3500±0.015[a]	5.67±0.333[d]
PB	6.050±0.076[d]	2.7533±0.020[b]	2.33±0.333[c]
SA	4.396±0.003[b]	3.1300±0.011[c]	0.00±0.00[a]
SB	4.190±0.015[a]	3.6267±0.008[d]	0.00±0.00[a]
TA	9.466±0.020[f]	5.4933±0.017[f]	1.33±0.333[b]
TB	8.840±0.030[e]	4.7767±0.012[e]	1.00±0.00[b]

Data were means of three triplicates and standard error each. Data with different superscript are significantly different from one another at (P<0.05)

CFU: Colony Forming Unit; SFU: Spore Forming Unit; **MPN –**Most Probable Number.

Heavy metal analysis of the smoked fish samples

Lead (Pb) was found in *M. merluccius*; *C. harengus*; *S. scombrus* ranging from 0.004ppm-0.015ppm with *Merluccius merlucccius* (PB) having the highest lead content 0.015ppm and *Scomber scombrus* (TA) had the lowest lead content 0.004ppm as shown in Table 3. All values detected were below the WHO permissible limit. Iron was found in all samples ranging from 0.492ppm -1.348ppm with *Clupea harengus (SA)* having the highest iron content 1.348ppm and *Scomber scombrus (TA)* had the lowest iron content 0.492ppm as shown in table. All values detected were within the WHO permissible limit. Cadmium was found in all samples ranging from 0.001ppm-0.011ppm with *Merluccius merluccius (PB)* having the highest cadmium content 0.011ppm and *Scomber scombrus* (TA) had the lowest cadmium content 0.001ppm as shown in Table 3. However, *Clupea harengus* (SA) had no cadmium content as shown in Table 3. Only *M. merluccius* had values higher than the permissible limit while the other

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species had values within the permissible limit as shown in Table 3. Nickel was not detected in all the fish samples as shown in Table 3. Zinc was found in all the fish species ranging from 1.184ppm-3.111ppm with *Merluccius merlucius* (PB) having the highest zinc content 3.111ppm and *Scomber scombrus* (TB) had the lowest zinc content 1.184ppm as shown in Table 3. All the values detected were within the WHO permissible limit except for *Merluccius merluccius* (PB) whose value was slightly above the WHO permissible limit as shown in Table 3.

Table 3: Heavy Metal Analysis of smoked fish samples.

Sample Code	Cd(ppm)	Fe(ppm)	Ni(ppm)	Pb(ppm)	Zn(ppm)
	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error	Mean ± Std Error
PA	0.0053±0.0003[c]	0.78167±0.0020[c]	0.00±0.00	0.0120±0.0005[d]	2.6566±0.0020[e]
PB	0.0100±0.0005[d]	0.91300±0.0020[d]	0.00±0.00	0.0146±0.0006[e]	3.1110±0.0020[f]
SA	0.0000±0.0000[a]	1.34800±0.0026[f]	0.00±0.00	0.0000±0.0000[a]	2.1040±0.0052[c]
SB	0.0023±0.0003[b]	1.28467±0.0035[e]	0.00±0.00	0.0060±0.0005[c]	2.2476±0.0023[d]
TA	0.0010±0.0000[a]	0.49233±0.0014[a]	0.00±0.00	0.0036±0.0003[b]	1.3143±0.0029[b]
TB	0.0026±0.0003[b]	0.53867±0.0014[b]	0.00±0.00	0.0056±0.0003[c]	1.1836±0.0014[a]
WHO LIMIT (2018)	0.05	0.30	0.005	5.0	3.0

Data were means of three triplicates and standard error each. Data with different superscript are significantly different from one another at (P<0.05)

ND- Not detected.

Discussion

Quality assessment of food entails the examination of the nutritional component, level of contamination and its relative health safety. Fish is commonly known to be a bioindicator of an aquatic environment. Comparing three commonly consumed fish species in Ado Ekiti, the proximate composition showed that *Merluccius merlucius* (commonly known as hake, local name 'Panla') had the lowest moisture content which could be the reason it has higher shelf life compared to other species particularly in smoked form. Also, *Scomber scombrus* and *Clupea harengus* had moisture content that ranged from 61.22%-64.93% which is in line with the report of Aberomand, [19] who reported that most fish species have moisture content ranges of 60%-80%. *Scomber scombrus* have the lowest carbohydrate composition with the value of 2.05% and *Mercluccius mercluccius* have the highest carbohydrate composition of 15.13%. Generally, CHO in fish is expected to be comparatively low as fish are known for being rich sources of protein rather than carbohydrates [20]. Ash content which reflects the difference in the mineral composition of the fish varies across the different species with *M. merluccius* having the highest ash composition across both markets. Fish fat content plays a vital role in providing essential fatty acids in the diet [21]. In this study, fat content ranged from 6.19% to 12.65%. *Scomber scombrus* from Iworoko Market (TA) displayed the highest fat content, while *Clupea harengus* from Iworoko Market (SA) exhibited the lowest. These variations could be attributed to factors such as species-specific metabolism, diet, and environmental conditions, as certain fish species accumulate more fats under specific circumstances [22]. This also suggest that *S. scombrus* (locally known as Titus fish) contains more fat than the other fish species. Fiber Content, Fish is not traditionally considered a significant source of dietary fiber [23]. In this study, the fiber content of the fish samples was generally low, ranging from 0.15% to 0.31%. *Merluccius merlucius* from Iworoko Market (PA) displayed the highest fiber content, while *Scomber scombrus* from Oja Oba Ado Market (TB) exhibited the lowest. These findings emphasize that fish is not a primary source of dietary fiber, but minor variations in fiber content may be influenced by factors such as diet and age. The protein content of the fish samples in this study ranged from 19.72% to 26.17%, with (*Clupea harengus*) from Oja Oba Ado Market (SB) exhibiting the highest protein content at 26.17% *Merluccius merlucius* from Iworoko Market (PA) displayed the lowest protein content at 19.72%. These findings align with previous research suggesting that most fish species fall within the protein content range of 15%-20% [19]. This also suggest that *C.harengus* is more proteinous compared to the other species of fish examined in this study.

The smoked fish samples examined for microbial count which include total bacterial count, total fungi count and total coliform count showed that all the samples were laden with bacteria as TBC across all the species ranged from $4.19x10^5$CFU/g-$5.63x10^5$CFU/g were higher than the permissible limit recommended by WHO with *S.scombrus* having the highest value. This high bacteria count could be as a result of contamination or inadequate hygiene during preparation or after smoking as most bacteria are airborne as fish samples are mostly exposed to attract interested consumers. Similarly, TFC and TCC were significantly highest in *S.scombrus* than the other fish species as illustrated in Table 3. A

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high presence of microbial contaminants in *S.scombrus* could be attributed to high moisture in the fish, as this supports microbial growth hence predisposing the Fish to a having low shelf life compared to the other species.

Heavy metals occur as natural constituents of the earth crust and are persistent environmental contaminants since they cannot be degraded or destroyed. Although these elements are lacking in abundance they are not lacking in significance [24]. In this study, concentrations of five heavy metals were determined in *Merluccius merluccius*, *Clupea harengus* and *Scomber Scrombrus* sold in Ado-Ekiti. The concentrations of contamination of cadmium and zinc, as seen in this study are within permissible limit in the environment as recommended by the WHO [25]. For instance, the WHO pollution threshold of cadmium and zinc are 0.005ppm and 3.0ppm respectively. Although all the fishes studied contained relatively low Pb levels ranging from ND to 0.06ppm, it is imperative to note that these metals even at low quantity can be harmful to health. The findings in this study are not comparable to that reported in Minna metropolis, Niger State of Nigeria by Ako & Salihu [26] who reported lower concentrations of lead in both smoked and oven-dried fish specimens with mean values ranging from 0.46 to 1.16mg/kg and 0.54 to 0.76mg/kg, respectively. Contamination of water bodies by Pb and other heavy metals could be from diverse sources. For instance, these metals can be carried away or blown by the wind from land surface to nearby river used by man for various activities. No matter the source of the metal, the final repositories are usually the aquatic systems. Lead from automobile exhaust systems could be transported in the form of aerosols to surface waters and as atmospheric fallout on land surfaces, which will eventually be washed into the aquatic system by water runoffs. These metals can also be deposited directly on the fish when exposed to the air in the market where they are being sold to consumers. Generally, the study showed the market location does not have any significant influence on the results observed as both markets recorded similar value ranges for all parameters measured

Conclusion

The proximate analysis revealed significant variations in moisture, carbohydrate, protein, ash, fat, and fibre content among the sampled fish species. Protein content exhibited notable diversity reflecting the species-specific metabolism and environmental factors influencing the nutritional composition. Fibre content, though generally low, displayed minor variations possibly linked to diet and age. Also, smoked fishes sold in these markets were contaminated with heavy metals (cadmium, iron, lead, and zinc) and microbial load. Therefore, presenting a potential risk of eating contaminated smoked fishes especially for people who consume smoked fish directly after purchase without additional cooking, improperly cooked or processed fish especially the species studied that are sold at Ado-Ekiti markets in Ekiti State and beyond. Generally, this study of the nutritional composition of the three fish species provides information that helps consumers to make informed decision on their choice of fish.

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