Effects of Smoking Methods on Quality, Microbial, and Chemical Safety of Traditional Smoked Silver Catfish (*Chrysichthys nigrodigitatus*).

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ABSTRACT

The effects of smoking methods on quality, microbial and chemical safety of traditional smoked silver catfish *(Chrysichthys nigrodigitatus)* from twenty processing centers from Lagos State, Nigeria were investigated. Fresh silver catfish (100 samples) were obtained from processing centers and samples were divided into two batches, one batch was smoked with a drum kiln and the second batch was smoked with a convective smoking kiln at the Fish Hatchery Unit, Federal University of Agriculture, Abeokuta and smoked samples were analyzed in the laboratory.

Laboratory analyses were carried out on the two batches for proximate, rancidity indices (PV, TBA, TVBN, TMA, and pH), heavy metals and microbiological (Coliform, Fungi, *Listeria monocytogenes, Salmonella paratyphi* and *Staphylococcus aureus*). The results revealed that the proximate, quality, microbiological, polycyclic aromatic hydrocarbon and heavy metal concentrations of the smoked samples were significantly ($p<0.05$) different. The concentrations of the six major PAHs in the drum smoked fish samples exceeded the EU maximum level of 5.0g/kg for BαP permissible in smoked fish. While convective smoked fish samples showed levels below 3.51g BαP/kg.

The study also showed that the levels of the four heavy metals investigated in the smoked fish samples are below the maximum permissible levels set by WHO. LC count of fresh silver catfish was 1.9×10^2 cfu/g and that of drum smoked samples was 4.0 x 10 cfu/g while convective smoked fish samples contain no strain of *Listeria monocytogenes*. The study concluded that traditional drum smoked fish contained high PAHs and contamination by *Listeria monocytogenes* and this may expose consumers to chemical and microbial risks.

(Keywords: silver catfish, smoking, quality, PAHs, heavy metals)

INTRODUCTION

Fish provides between 30% and 80% of the total animal protein intake of the coastal people of West Africa (NCBI, 2012). In Nigeria, fish has an edge over meat because it is cheaper and relatively more abundant (Eyo, 2001) and constitutes about 40 % of the animal protein intake (Eyo, 2001; Abolagba and Melle, 2008). Fish is a cheap source of animal protein with little or no religious rejection of it, which gives it an advantage over pork or beef.

Fish is a rich source of lysine suitable for supplementing high carbohydrate diet. It is also a valuable source of vitamins A, B, and E, iodine and oils containing polyunsaturated fatty acids (Eyo, 2001, da Silva, 2002, Abolagba and Melle, 2008). In Nigeria, fish smoking is the most practiced preservation method. Practically all species of fish available in the country can be smoked and it has been estimated that 70-80 percent of the domestic marine and freshwater catch is consumed in smoked form (Akinyemi *et al.,* 2011). Smoked fish constitutes a major source of animal protein for a vast majority of the population in Nigeria, particularly the rural population (Eyo, 1992).

Traditional smoking techniques involve treating of pre-salted, whole, or filleted fish with wood smoke in which smoke from

incomplete wood burning comes into direct contact with the product, this had been found to contaminate smoked fish with polycyclic aromatic hydrocarbons (PAHs) if the process is not adequately controlled or if very intense smoking procedures are employed (Estaca *et al.,* 2011).

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds, containing 2 or more fused aromatic rings made up of carbon and hydrogen atoms and smoked fish is one source of PAH (Guillen *et al.,* 1997). When fish is smoked, roasted, barbecued, or grilled; PAHs are formed as a result of incomplete combustion or thermal decomposition of the organic materials (WHO, 2006). Pyrolysis of the fats in the meat/fish generates PAH that become deposited on the meat/fish. PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat/fish and the proximity of the food to the heat source (Phillips, 1999; Kazerouni *et al*., 2001).

Several analyses of charcoal roasted/grilled common fish have proven the presence of PAHs such as benzo[a]pyrene, anthracene, chrysene, benzo [α] anthracene, indeno[1,2,3)c,d]pyrene (Ogbadu and Ogbadu, 1989; Akpan *et al.,* 1994; Duke and Albert, 2007; Akpambang *et al.,*2009; Linda *et al.,* 2011). Most of these PAHs have been found to be carcinogenic while some are not (Fritz and Soos, 1980; Bababunmi *et al.,* 1982; Alonge, 1988; Lijinsky, 1999; Borokovcova *et al.,* 2005; Adeyeye, *et al.*, 2015; Adeyeye, *et al.*, 2016).

Emerole (1980) screened for the presence of PAH in local foodstuffs available in Nigerian market. They discovered that appreciable amounts of benzo [α] anthracene and benzo [α] pyrene were found present in three varieties of smoked fish and smoked meat (suya) purchased from a popular market in Ibadan, Nigeria. In a recent study carried out by Olabemiwo *et al.* 2011 to assess the PAHs content of two smoked fish species available in Western Nigeria; it was found out that the sum of all PAHs in the smoked fish *(Claria gariepinus* and *Tilapia guineensis*) ranged from 0.497 to 0.814 1g/kg and 0.519 to 0.772 1g/kg, respectively.

Adeyeye, *et al*., 2015 and Adeyeye, *et al.*, 2016 also discovered similar results for smoked West African ilisha and traditional drum smoked fish from Lagos State, Nigeria. High levels of PAHs have been reported to be associated with the dark colorations in intensively heated products. This was supported by Ova *et al*. (1998) who reported that the PAH levels were significantly higher in the fish skins than in the edible parts.

This study was embarked on to investigate the effects of smoking methods on quality, microbial and chemical safety of traditional smoked silver catfish *(Chrysichthys nigrodigitatus)* from Lagos State, Nigeria.

MATERIALS AND METHODS

Materials

Sample Collection: Fresh silver catfish (100) samples were collected from twenty major processing centers from Lagos State, Nigeria by purposive sampling method in frozen sterile containers (Ziploc). The samples were divided into two batches, one batch was smoked with drum kiln and the second batch was smoked with convective smoking kiln at the Fish hatchery unit, Federal University of Agriculture, Abeokuta and smoked samples were analyzed in the laboratory.

Drum Smoking Process

Fresh silver catfish samples were carefully cleaned. The samples were not eviscerated, cut into uniform pieces (fillet) before smoking. Fresh samples were smoking with drum kiln in open air at varied temperature. Smoking was done for 36 hours until the fish is fully dried.

Convective Smoking Process

Smoked silver catfish was prepared using the modified method of by Crapo (2011). Fresh silver catfish were carefully cleaned. The samples were eviscerated, leaving the skin on the fish. The samples were also cut into uniform pieces (fillet) before smoking. The internal temperature of the silver catfish was checked during smoking using a thermometer. Hands, utensils and work surfaces were cleaned when transferring fish from smoker to oven to cool down to avoid cross-contamination. Smoking was done for 16 hours at 80^{o}C until the fish is fully dried.

Proximate Analysis

The proximate composition of all the samples were carried out in triplicates according to the standard method AOAC (2000).

Physico-Chemical Analysis

Kent pH meter (model 7020, Kent Ind. Measurement Ltd., Surrey, U. K) equipped with a glass electrode was used to measure the pH of the flesh in triplicates, employing 10 g of smoked fish homogenized in 10 ml of distilled water. The rancidity (quality) indices of all the samples were carried out in triplicates according to the standard method AOAC (2000). All chemicals used in this study were of the analytical grade unless stated otherwise.

Microbiological Studies

Microbiological analysis was carried out to determine the presence of the following pathogenic microorganisms: *Listeria monocytogenes, Salmonella paratyphi*, *Escherichia coli*, *Staphylococcus aureus,* and *Fungal count.* The microbiological procedures recommended in the International Commission on Microbiological Specification for Foods (ICMSF, 1986) were applied. Culture media were those of Oxoid, Biolife and Difco. For each sample, 25g were weighed out and transferred to a sterile blender with 225 ml of 0.1% peptone and mixed thoroughly for 2 minutes to prepare fish homogenate. The samples were then analyzed as follows.

Total Viable Bacterial Counts

Appropriate dilutions of the fish homogenate were prepared and inoculated onto sterile petri dishes. Plate count agar (Oxoid) media were then poured. Plates were incubated at 37 °C for 48 hours and colonies were then counted and reported as total colony count/ml. A second set of plates was incubated at 37 °C for 48 hours in a carbon dioxide incubator or under anaerobic conditions using a gas pack anaerobic jar. Colonies were then counted and reported as anaerobic total bacterial count. In case of spore formers count, the food homogenate was boiled first at 80 °C and then rapidly cooled. Appropriate serial dilutions were prepared and inoculated onto the surface of sterile and dried plate count agar media. These were incubated finally at 37 °C for 48 hours.

Detection of *Escherichia coli*

One ml of each of the decimal dilutions of the smoked fish homogenate was plated on poured Eosine Methylene Blue Agar (Oxoid) and then incubated at 37 °C for 24 hours. Counts were calculated from the number of growth on the plates. The colonies with green metallic sheen were counted as *Escherichia* co*li*.

Detection of *Staphylococcus aureus*

A sample of 0.1 ml of the smoked fish homogenate and dilutions was inoculated on Baird-Parker (Difco) agar plates and incubated at 37 °C for 48 hours. Colonies appearing to be black and shiny with narrow white margins and surrounded by clear zones were identified by coagulase test reactions.

The coagulase test was carried out by first inoculating typical colonies in brain heart infusion broth (Difco) and incubating at 37 °C for 24 hours. From the resulting cultures, 0.1 ml was then added to 0.3 ml of rabbit plasma in sterile tubes and incubated at 37 °C for 4 hours. The formation of a distinct clot was evidence of coagulase activity.

Detection of *Salmonella paratyphi*.

Samples of smoked fish homogenate and dilutions were inoculated in Salmonella-shigella agar (Oxoid) and incubated at 37 °C for 24 hours. For identification, 2–3 suspected colonies were inoculated into tryptone broth for indole test, triple sugar iron agar slant (Oxoid), urea broth and lysine iron agar. These were incubated at 37 °C for 24 hours. Salmonella species is indole negative, on triple sugar iron it produces acid (yellow) and alkaline (red) with or without gas and hydrogen sulfide, is urea negative, and on lysine iron agar shows an alkaline (purple) reaction throughout the medium. Serological tests were then carried out.

Detection of Listeria monocytogenes.

A sample of 0.1 ml of the smoked fish homogenate and dilutions was inoculated on Brilliant Listeria Agar (Oxoid) plates and incubated at 37 °C for 24 hours. Colonies appearing were counted and reported as *Listeria monocytogenes*.

Enumeration of Fungi

Appropriate dilutions of Sabouraud dextrose agar plates (Oxoid) were poured over 1 ml of the smoked fish homogenate and dilutions. Plates were incubated at 25 °C for 3 days and then colonies were counted and reported as fungal count/m

Determination of PAHs by Gas Chromatography (GC)

To start the cold extraction, 2 g of each sample was weighed into a clean extraction container and 10 ml of dichloromethane was added, thoroughly mixed and allowed to settle. The mixture was carefully filtered into clean solvent and rinsed into extraction bottle, using filter paper fitted into Buchner funnel. The extract was concentrated to 2 ml and then transferred for clean-up and separation (this involved further purification of the extract prior to gas chromatographic analysis). To achieve this, 1 cm of moderately packed glass wool was placed at the bottom of 10 mm ID × 250 mm long chromatographic column. Slurry of 2 g activated silica in 10 ml dichloromethane was prepared and placed into the chromatographic column. To the top of the column 0.5 cm of sodium sulfite was added. The column was rinsed with additional 10 ml of dichloromethane. The column was pre-eluted with 20 ml of dichloromethane and this was allowed to flow through the column at a rate of about 2 minutes until the liquid in the column was just above the sulfite layer. Immediately, 1 ml of the extracted sample was transferred into the column. The extraction bottle was rinsed with 1 ml of dichloromethane and added to the column as well. The stop-cork of the column was open and the eluant was collected with a 10 ml graduated cylinder. Prior to exposure of the sodium sulfite layer to air, dichloromethane was added to the column in 1-2 ml increments.

Accurately measured 10 ml of the eluant was collected and labeled aliphatic. The concentrated aliphatic fractions were transferred into labeled glass vials with rubber crimps caps for GC analysis. 1 µL of the concentrated sample was injected by means of syringe through a rubber septum into the column. Separation occurs as the vapor constituents' partition between the gas and liquid phases. The sample components were automatically detected as they emerge from the column (at a constant flow rate) by the Flame Ionization Detection (FID) detector whose response was dependent upon the composition of the vapor.

Heavy Metal Analysis

Heavy metal, such as Cu, Cd, Hg, and Pb in fresh and smoked fish samples were determined by AOAC (2000) method using atomic absorption spectrophotometer. All chemicals used in this study were of the analytical grade unless stated otherwise.

DATA ANALYSIS

The data obtained were subjected to descriptive statistics using IBM SPSS version 21.0 software. One way analysis of variance (ANOVA) was performed followed by Duncan's Multiple Range Test ($p<0.05$) to find the difference between means. Significant level was set at $P<0.05$.

RESULTS AND DISCUSION

The result of the study revealed that the mean moisture content of smoked silver catfish samples (Table 1) obtained using local drum kiln and convective smoking kiln ranged from 11.18%-14.77% and 8.48%–10.43%, respectively. Moisture content of fish is of great importance as a number of biochemical reactions and physiological changes in fish depend on moisture content. Of greater significant is the effect of moisture on the stability and quality of fish. High moisture content also promotes microbial growth. In the present study, the protein content of smoked fish samples (Table 1) ranged from 52.96% – 58.36% and 56.81% – 61.42% using local drum kiln and convective smoking kiln, respectively.

Table 1: Proximate Composition of Smoked Silver Catfish (*Chrysichthys nigrodigitatus*) from 20 Different Processing Centers using Local Drum Kiln and Convective Smoking Kiln.

Locations	Moisture %		Protein %		Fat %		Crude fibre %		Ash %		Carbohydrate %	
	Local	Convect	Local	Convect	Local	Convect	Local	Convect	Local	Convect	Local	Convect
Agbalata	11.86c	8.91d	54.80e	57.45c	19.32l	20.32l	2.04de	2.56ef	1.34de	1.62de	10.64bcd	9.14k
Ajido	14.32k	9.42fg	53.46b	58.78g	17.68f	18.68e	2.21gh	2.47cd	1.31cde	1.56cd	10.99bcd	9.09k
Asakpo	14.06j	10.12j	56.06h	59.13h	18.04g	19.04f	1.93bc	2.23b	1.42def	1.67ef	8.49bc	7.81c
Boguru	13.11g	9.47g	54.51de	57.74d	19.33l	20.13j	2.27hi	2.52def	1.36def	1.86ij	9.42bcd	8.08d
Fvanoveh	11.83c	8.79bc	58.13j	61.34l	16.54b	17.54a	2.16fg	2.43c	1.39efg	1.82hi	8.90bcd	8.28ef
Gberefun	14.91n	10.23k	54.03de	57.47c	18.11gh	19.11fg	1.89ab	2.13a	1.14a	1.56cd	9.92bcd	9.50m
Gbetrome	14.30k	9.56h	57.24i	60.63k	17.17c	18.17c	2.37j	2.59f	1.28bcd	1.61de	7.64ab	7.44b
Ilaje	12.73e	8.83c	58.36j	61.42m	16.52b	17.52a	2.14fg	2.48cde	1.16a	1.34a	9.09bcd	8.41h
Kofegameh	12.18d	8.74b	53.62b	57.89e	20.41o	20.41m	1.99cd	2.31b	1.41efg	1.74fgh	10.39bcd	8.91j
Pako	14.36l	10.43l	54.29cd	57.93e	18.23i	19.23h	1.83a	2.67g	1.45fg	1.81ghi	9.84bcd	8.23e
Afuye	13.73ij	9.92i	55.49g	58.78g	18.06g	20.06j	2.15fg	2.54def	1.36def	1.58d	9.21bcd	7.12a
Bodin Yawa	14.21k	10.43m	54.12c	57.45c	17.28d	20.28kl	2.12efg	2.43c	1.21abc	1.46b	11.06bcd	8.95j
Idale	11.18a	8.75b	54.47cde	57.73d	19.22k	20.22k	1.99cd	2.30b	1.48g	1.73fg	11.66cd	9.27l
Igbodun	11.47b	8.48a	58.11j	60.36j	16.05a	18.05b	2.33ij	2.71g	1.31cde	1.63de	10.73bcd	8.77i
Ilogun	13.19gh	9.34e	53.64b	56.92b	18.34j	19.34i	2.11ef	2.42c	1.37def	1.49bc	11.35bcd	10.49o
Mejona	15.23n	10.13j	54.33cd	57.66d	17.56e	18.56d	1.93bc	2.12a	1.33de	1.57cd	9.62bcd	9.96n
Oluwo	12.97f	9.98i	52.96a	56.81a	18.22i	19.22h	2.25hi	2.56ef	1.45fg	1.80ghi	12.15cd	10.63p
Okorisan	14.77m	10.18jk	54.29cd	59.53i	18.18hi	19.18gh	1.96bcd	2.24b	1.21abc	1.45b	9.59bcd	7.42b
Orita	13.27h	9.36ef	55.13f	58.64f	20.22n	18.22c	2.12efg	2.43c	1.40efg	1.92j	15.78e	9.43m
Orogoro	15.39o	10.06j	54.22cd	57.39c	19.57m	20.57n	2.04de	2.31b	1.18ab	1.31a	12.70de	8.36gh

Local = Local drum kiln
Convect = Convectional smoke kiln
Data are means of 3 replicates
Data with the same subscript are not significantly different at $p<0.05$.

An inverse relationship was observed between the moisture and protein content in the smoked silver catfish. This finding agrees with the works of Goktepe, 1996 and da Silva, 2002. Fat content of smoked silver catfish samples (Table 1) ranged from 16.52% – 20.41% and 17.52% – 20.57% using local drum kiln and convective smoking kiln respectively. Crude fiber content of smoked silver catfish samples (Table 1) ranged from 1.83% – 2.37% and 2.12% – 2.71% using local drum kiln and convective smoking kiln, respectively.

Ash content of smoked silver catfish samples (Table 1) ranged from 1.14% – 1.48% and 1.31% – 1.92% using local drum kiln and convective smoking kiln respectively. The increase in mineral content, ash and crude fiber can be attributed to an increase in the dry matter content per unit weight following sample dehydration and during the smoking process (da Silva, 2002). da Silva, 2002 and da Silva *et al., 2008.* In this study carbohydrate content was obtained by difference that is the percentage of water, protein, fat and ash subtracted from 100. Carbohydrate content of smoked silver catfish samples (Table 1) ranged from 8.49% – 15.78% and 7.12% – 10.63% using local drum kiln and convective smoking kiln, respectively.

Carbohydrate content of smoked silver catfish samples is low because it is proteinous food. The quality indices of the fresh and smoked silver catfish were studied. Fats undergo changes during storage which result in production of an unpleasant taste and odour which is commonly referred to as rancidity (FAO, 1992; da Silva, 2002; da Silva, *et al.,* 2008). The peroxide value (PV) results are similar in pattern to TBA. In this study, PV of 8.24–9.39mgeq.peroxide/kg and 7.13-8.67mgeq.peroxide/kg were obtained for smoked silver catfish samples using local drum kiln and convective smoking kiln (Table 2).

Table 2: Quality Indices of Smoked Silver Catfish (*Chrysichthys nigrodigitatus*) from 20 Different Locations using Local Drum kiln and Convective smoking kiln.

Locations	Peroxide value (PV) (mEq,peroxide/kg)		Free fatty acid(FFA) %		Thiobarbituric acid (TBA) (mg Mol/kg)		Total volatile base-nitrogen (TVB-N) (mgN/100g		Trimethyl amine value (TMA)(mgN/kg)		pH	
	Local	Convect	Local	Convect	Local	Convect	Local	Convect	Local	Convect	Local	Convect
Agbalata	9.11efg	8.36gh	1.21de	1.13ef	1.10a	1.08b	19.69k	17.86i	2.52abc	2.36bcd	6.43abcde	6.51a
Ajido	9.19ghi	8.31efgh	1.13abcd	1.07abcdef	1.01a	0.99a	18.38c	16.61d	2.63abcd	2.41cde	6.48bcdef	6.73ef
Asakpo	9.24i	8.46i	1.11abcd	1.05abcdef	1.08a	1.06ab	18.56de	16.78e	2.58abcd	2.29b	6.67gh	6.62bcd
Boguru	9.17ghi	8.41hi	1.26e	1.23g	1.14a	1.12bc	19.27hi	17.36g	2.72a	2.53h	6.62fgh	6.66cdef
Fvanuveh	8.99cde	7.84d	1.12abcd	1.10bcdef	1.11a	1.08b	19.15gh	17.62h	2.56abcd	2.38bcd	6.54cdefg	6.61def
Gberefun	9.03def	8.23e	1.09abcd	1.04abcde	1.02a	1.00a	18.44cd	16.89f	2.91cd	2.67ij	6.41abcd	6.90g
Gbetrome	9.14fghi	8.67j	1.07abc	1.01a	1.03a	1.01a	19.13g	17.41g	2.84cd	2.62i	6.73h	6.68def
Ilaje	8.94cd	7.89d	1.18cde	1.13ef	1.06a	1.04a	18.53de	16.67de	2.74bcd	2.50efg	6.39abc	6.73ef
Kofegameh	9.08efg	8.26efg	1.20de	1.14f	1.15a	1.11bc	18.78f	16.34c	2.93cd	2.69ij	6.46bcdef	6.86g
Pako	9.21hi	8.34fgh	1.16bcde	1.11cdef	1.13a	1.06ab	19.31ij	17.64h	2.71bcd	2.43def	6.55cdefg	6.75f
Afuye	9.39j	8.63j	1.05ab	1.02abc	1.05a	1.01a	18.11b	16.32c	2.75bcd	2.48efg	6.42abcde	6.57abc
BodinYawa	9.08efg	8.25ef	1.09abcd	1.03abcd	1.01a	0.98a	17.63a	15.56a	2.79cd	2.51gh	6.57cdefgh	6.69cde
Idale	8.79b	7.91d	1.06ab	1.00a	1.02a	0.99a	18.18b	16.23c	2.84cd	2.63ij	6.43abcde	6.52a
Igbodun	9.15fghi	8.36gh	1.13abcd	1.11cdef	1.10a	1.07ab	19.43j	17.92i	2.78cd	2.52gh	6.41abcd	6.63cde
Ilogun	8.24a	7.13a	1.07abc	1.05abcdef	1.04a	1.02a	17.59a	15.67b	2.87cd	2.68ij	6.54cdefg	6.74f
Mejona	9.12fghi	8.32efgh	1.16bcde	1.12def	1.12a	1.10bc	19.15gh	17.32g	2.36ab	2.11a	6.60efgh	6.65cdef
Oluwo	8.69b	7.51c	1.04ab	1.02abc	1.10a	1.06ab	19.30ij	17.81i	2.63abcd	2.49efg	6.63fgh	6.53ab
Okorisan	8.26a	7.42b	1.02a	1.01ab	1.09a	1.07ab	18.19b	16.23c	2.72bcd	2.53h	6.58defgh	6.84g
Orita	8.91c	8.23e	1.03a	1.01ab	1.02a	1.00a	19.26ghi	17.53h	2.96cd	2.72j	6.27a	6.59abcd
Orogoro	9.16ghi	8.47i	1.12abcd	1.09abcdef	1.10a	1.05a	18.64ef	16.75e	2.54abcd	2.33bc	6.32ab	6.73ef

Local = Local drum kiln
Convect = Convectional smoke kiln
Data are means of 3 replicates
Data with the same subscript are not significantly different at $p<0.05$.

These values are below the recommended value of between 20 and 40mgeq.peroxide/kg for rancid taste to begin. Free fatty acid value (FFA) is the number of mg of potassium hydroxide required to neutralize the free acid in g of the sample. The result is often expressed as percentage of free acidity.

The acid value measures the extent to which the glycerides in the oil have been decomposed by lipase action. The decomposition is accelerated by heat and light. As rancidity is usually accompanied by free fatty acid formation, the determination is often used as a general indication of the condition and edibility of oil or oil and convective smoking kiln. These values are within the range of legislative standard for TVB-N which is 20mgN/100g for fresh fish. This suggests that the level of protein decomposition or breakdown in all the samples is low. The spoilage of fish is due to bacterial and enzyme action, which results in the production of various volatile compounds such as trimethylamine (TMA), dimethylamine (DMA), ammonia and volatile acids. Trimethylamine (TMA) is a reduction product of trimethylamine oxide during spoilage and ammonia is mainly formed as a product of protein breakdown. Trimethylamine (TMA) is one of the volatile amines plus ammonia which can be used as an index of spoilage (da Silva, 2002). In this study, the trimethylamine value (TMA) of 2.36 – 2.96 mgN/kg and 2.11 – 2.72 mgN/kg were obtained for smoked silver catfish samples using local drum kiln and convective smoking kiln (Table 2).

The trimethylamine value (TMA) of 2.36 – 2.96 mgN/kg and 2.11 – 2.72 mgN/kg were obtained for smoked silver catfish samples (Table 2) and are within the range of < 3 mgN/100g for fresh fish, >8 mgN/100g for spoiled fish and > 5 mgN/100g for doubtful quality specified U.S.F.D.A (da Silva, *et al.,* 2008).

pH is the most critical factors affecting microbial growth and spoilage of foods. The pH value of smoked silver catfish samples ranged from 6.27 – 6.86 and 6.5 – 6.86 using local drum kiln and convective smoking kiln

(Table 2). The pH values of the fresh fish samples was high, this may be due to biochemical reactions and enzyme action as a result of delay in reaching the shore from the sea because most of the fishermen had no cooling system in their boats or canoes. It was observed that the pH in fish tissues drops due to smoking. This agrees with the findings of da Silva, 2002 and da Silva *et al.,* 2008).

The result microbiological analysis indicated the predominance of *Listeria monocytogenes, Staphyloccoccus aureus, Salmonella paratyphi* and *Escherichia coli* in the fresh and smoked silver catfish samples. The results of the microbiological study on fresh silver catfish samples. (Table 3) indicated that Total Viable Count (TVC) of fresh fish samples increased significantly ($p<0.05$). Total plate count (TVC) of fresh silver catfish samples was 6.6×10^6– 8.8×10^8 cfu/g and TVC of samples of smoked silver catfish were 2.0×10^4- 8.6×10^4 cfu/g and 1.0×10^3-5.4×10^3 cfu/g using local drum kiln and convective smoking kiln. The TVC values obtained for the smoked silver catfish samples were within the range of specified microbiological limits recommended by ICMSF (1986) for fish and fishery products, the maximum recommended bacterial counts for good quality products (m) is 5×10^5(5.7 log10 cfu/g).

Listeria monocytogenes count of fresh silver catfish samples was 1.8×10^2-2.5×10^2 cfu/g and that of samples of smoked silver catfish from different processing centres (local drum kiln) ranged from 1.3×10^1– 13.2×10^1 cfu/g. Although the *Listeria monocytogenes* count values obtained for the smoked silver catfish samples were low, the range of specified microbiological limits recommended by ICMSF (1986) for *Listeria monocytogenes* for fish and fishery products is the presence of the organism, that is zero tolerance so most of the samples from local drum kiln do not meet the ICMSF recommended microbial specification. Therefore, the smoked silver catfish samples from all processing centres need to be cooked before consumption in order to destroy *Listeria monocytogenes* that is present in the smoked silver catfish to prevent possibility of food poison by listeriosis.

All the smoked silver catfish samples of convention smoke kiln tested negative for *Listeria monocytogenes* while the fresh fish samples contained *L. monocytogenes*. Goktepe and Moody (1998) reported that *Listeria spp*. counts of raw catfish fillets were 4.37 log cfu/g; after brining, the count decreased slightly to 3.24 log cfu/g and no *Listeria spp*. were detected in samples after hot smoking.

Staphylococcal count of fresh silver catfish samples ranged from 5.0×10^3 - 6.4×10^3 cfu/g and that of samples of smoked silver catfish from different processing centres (local drum kiln) ranged from 5.6×10^2- 60.4×10^2 cfu/g and 1.0×10^2- 3.5×10^2 cfu/g for convective smoking kiln. The Staphylococcal count values obtained for the smoked silver catfish were low below the specified recommended value for all fish.

The *Staphylococcus aureus* safety level is equal to or greater than 10^4/g and in many cases, these levels represent the point at or above which the agency will take legal action to remove products from the market (FDA, 2001). In addition, smoking also reduced Staphylococcal and fungal counts. The isolation of Staphylococcus in smoked samples can be attributed to post processing contamination. *Salmonella paratyphi* was not detected in smoked silver catfish samples obtained using local drum kiln and conventional smoke kiln and this conformed with the specified microbiological limits recommended by ICMSF (1986) for *Salmonella paratyphi* count for fish and fishery products which is the presence of the organism, that is zero tolerance. In all cases, this suggests Good Manufacturing Practices (GMP) and no fecal contamination of the products as *S. paratyphi* and *E. coli* serve as indicator organisms for faecal contamination of foods. In this study, fungal count of samples of smoked silver catfish from different processing centers (local drum kiln) ranged from 1.1×10^1 - 10.0×10^1 cfu/g.

The populations of fungi in the samples were all below 5×10^5cfu/g specified microbiological limits recommended by ICMSF (1986) for fungi, except for the samples from convective smoking kiln that had no fungi count.

Table 3: Microbial Quality (cfu/g) and pH of Smoked Silver Catfish (*Chrysichthys nigrodigitatus*) from 20 Different Processing Centers using Local Drum Kiln and Convective Smoking Kiln.

Locations	*Listeria Monocytogenes*		*Salmonella paratyphi*		*E.coli*		Staphylococcal count		Fungal count		T.V.C.		pH	
	Local	Conv	Local	Conv	Local	Conv	Local	Conv	Local	Conv	Local	Conv	Local	Conv
Agbalata	4.0 x $10^{1}{}_{c}$	-	-	-	-	-	23.4 x$10^{2}{}_{g}$	2.5 x$10^{1}{}_{cd}$	-	-	4.6 x$10^{4}{}_{c}$	4.4 x$10^{3}{}_{f}$	6.43abcde	6.51a
Ajido	1.4 x $10^{1}{}_{a}$	-	-	-	-	-	17.1 x$10^{2}{}_{d}$	3.5 x$10^{1}{}_{ef}$	-	-	6.1 x$10^{4}{}_{f}$	5.3 x$10^{3}{}_{g}$	6.48bcdef	6.73ef
Asakpo	6.1 x $10^{1}{}_{d}$	-	-	-	-	-	39.0 x $10^{2}{}_{k}$	-	-	-	5.4x$10^{4}{}_{de}$	1.0 x$10^{3}{}_{a}$	6.67gh	6.62bcd
Boguru	7.5 x $10^{1}{}_{ef}$	-	-	-	-	-	60.2 x $10^{2}{}_{n}$	-	-	-	5.0 x$10^{4}{}_{d}$	1.1 x$10^{3}{}_{a}$	6.62fgh	6.66cdef
Fvanoveh	11.0 x $10^{1}{}_{g}$						30.5 x $10^{2}{}_{i}$	1.4 x$10^{1}{}_{ab}$			2.5 x$10^{4}{}_{a}$	1.8x$10^{3}{}_{cd}$	6.54cdefg	6.61def
Gberefun	5.4 x $10^{1}{}_{cd}$	-	-	-	-	-	14.3 x$10^{2}{}_{c}$	-	-	-	4.3 x$10^{4}{}_{c}$	1.5x$10^{3}{}_{bc}$	6.41abcd	6.90g
Gbetrome	8.2 x $10^{1}{}_{f}$	-	-	-	-	-	18.6 x$10^{2}{}_{e}$	2.4 x$10^{1}{}_{cd}$	-	-	2.6 x$10^{4}{}_{a}$	1.0 x$10^{3}{}_{a}$	6.73h	6.68def
Ilaje	16.0 x 10^{1}	-	-	-	-	-	25.0 x $10^{2}{}_{h}$	3.2 x $10^{1}{}_{e}$	-	-	2.0 x$10^{4}{}_{a}$	1.2 x$10^{3}{}_{a}$	6.39abc	6.73ef
Kofegameh	12.3 x $10^{1}{}_{h}$	-	-	-	-	-	34.2 x $10^{2}{}_{j}$	-	-	-	5.5x$10^{4}{}_{de}$	1.3x$10^{3}{}_{ab}$	6.46bcdef	6.86g
Pako	2.6 x $10^{1}{}_{b}$	-	-	-	-	-	19.5 x$10^{2}{}_{f}$	3.1 x $10^{1}{}_{e}$	-	-	3.3 x$10^{4}{}_{b}$	1.1 x$10^{3}{}_{a}$	6.55cdefg	6.75f
Afuye	2.5 x $10^{1}{}_{b}$	-	-	-	-	-	5.6 x $10^{2}{}_{a}$	-	-	-	2.4 x$10^{4}{}_{a}$	1.0 x$10^{3}{}_{a}$	6.42abcde	6.57abc
BodinYawa	4.1 x $10^{1}{}_{c}$	-	-	-	-	-	18.0 x$10^{2}{}_{e}$	3.2 x $10^{1}{}_{e}$	-	-	4.1 x$10^{4}{}_{c}$	3.5x$10^{3}{}_{e}$	6.57cdefgh	6.69cde
Idale	1.3 x $10^{1}{}_{a}$	-	-	-	-	-	18.1 x$10^{2}{}_{e}$	1.2 x $10^{1}{}_{a}$	-	-	4.5 x$10^{4}{}_{c}$	1.4x$10^{3}{}_{ab}$	6.43abcde	6.52a
Igbodun	5.1 x $10^{1}{}_{cd}$	-	-	-	-	-	56.5 x $10^{2}{}_{m}$	-	8.0 x$10^{1}{}_{c}$	-	6.3 x$10^{4}{}_{f}$	1.2 x$10^{3}{}_{a}$	6.41abcd	6.63cde
Ilogun	7.0 x $10^{1}{}_{e}$	-	-	-	-	-	39.2 x $10^{2}{}_{k}$	1.3 x$10^{1}{}_{ab}$	8.2 x$10^{1}{}_{c}$	-	3.1 x$10^{4}{}_{b}$	1.3x$10^{3}{}_{ab}$	6.54cdefg	6.74f
Mejona	8.5 x $10^{1}{}_{f}$	-	-	-	-	-	45.0 x $10^{2}{}_{l}$	-	4.1 x$10^{1}{}_{a}$	-	3.3 x$10^{4}{}_{b}$	5.4 x10^{3}	6.60efgh	6.65cdef
Oluwo	6.0 x $10^{1}{}_{d}$	-	-	-	-	-	60.4 x $10^{2}{}_{n}$	-	8.0 x$10^{1}{}_{c}$	-	5.0 x$10^{4}{}_{d}$	1.1 x$10^{3}{}_{a}$	6.63fgh	6.53ab
Okorisan	13.2 x $10^{1}{}_{i}$	-	-	-	-	-	-	2.1 x $10^{1}{}_{c}$	-	-	2.2 x$10^{4}{}_{a}$	1.4x$10^{3}{}_{ab}$	6.58defgh	6.84g
Orita	16.0 x $10^{1}{}_{j}$	-	-	-	-	-	30.2 x $10^{2}{}_{i}$	-	-	-	4.5 x$10^{4}{}_{c}$	2.0x$10^{3}{}_{cd}$	6.27a	6.59abcd
Orogoro	11.4 x $10^{1}{}_{g}$	-	-	-	-	-	8.5 x$10^{2}{}_{b}$	1.0 x$10^{1}{}_{a}$	7.0 x$10^{1}{}_{b}$	-	5.1 x$10^{4}{}_{d}$	1.4x$10^{3}{}_{ab}$	6.32ab	6.73ef

Data are means of 3 replicates.
Data with different subscripts in the same column indicate significant difference at p<0.05.
T.V.C = Total viable count
- = no count.

From the results polycyclic aromatic hydrocarbons, most of the fresh samples do not contain polycyclic aromatic hydrocarbons and the few samples that showed the presence of polycyclic aromatic hydrocarbons were negligible in the range of 0.02 – 0.08 1g/kg for BαP (Table 4). However, the following compounds, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, benzo [α] anthracene, chrysene, benzo[β] fluoranthene, benzo [k] fluoranthene, benzo [α] pyrene, and benzo [ghi] perylene were detected in smoked fish samples (Table 5).

No significant difference (P < 0.05) was observed between smoked fish samples in the concentrations of six PAHs using the same smoking method but significant differences (P < 0.05) were found using different smoking method forfluorene, anthracene, fluoranthene, benzo [α] anthracene, and benzo [ghi] perylene. All smoked fish samples smoked with local drum smoking kiln showed Benzo (α) pyrene (BαP) levels exceeding 5.01g/kg. In drum kiln smoked fish, the concentrations of six PAHs fluorene, anthracene, benzo [β] fluoranthene, benzo [α] anthracene, benzo [α] pyrene and benzo [ghi] perylene for Silver catfish, 4.22, 5.96, 5.94, 5.16, 5.65, and 5.36 0 1g/kg, respectively.

In convective kiln smoked fish, the concentrations of six PAHs fluorene, anthracene, benzo [β] fluoranthene, benzo [α] anthracene, benzo [α] pyrene and benzo [ghi] perylene for Silver catfish, 3.79, 4.52, 2.87, 3.65, 2.23 and 2.73 0 1g/kg respectively. The concentrations of the six major PAHs (fluorene, anthracene, benzo [β] fluoranthene, benzo [α] anthracene, benzo [α] pyrene and benzo [ghi] perylene) in the drum smoked fish exceeded the EU maximum level of 5.0 1g/kg for BαP permissible in smoked fish. However, in Table 4, it can be seen that there are some of smoked fish samples produced by convective smoking kiln that still have low or moderate Benzo (α) pyrene (BαP) levels.

Table 4: Concentration (µg /kg) of Polycyclic Aromatic Hydrocarbons in Smoked Silver Catfish *(Chrysichthys nigrodigitatus)* samples from 20 Different Locations using Local Drum Kiln and Convective Smoking Kiln.

Type of PAH	Drum smoked fish	Convective smoked fish
Naphthalene	4.63_b	3.06_b
Acenaphthylene	3.69_a	2.33_a
1,2-Benzanthracene	5.19_c	3.87_b
Acenaphthene	3.78_a	2.34_a
Fluorene	5.22_c	3.79_b
Phenanthrene	7.91_d	5.83_d
Anthracene	5.96_c	4.52_c
Fluoranthene	7.13_d	4.17_c
Pyrene	3.66_a	2.84_a
Benzo(a)anthracene	5.16_c	3.65_b
Chrysene	5.23_c	4.81_c
Benzo(b)fluoranthene	5.94_c	2.87_a
Benzo(a)pyrene	5.65_c	2.23_a
benzo [ghi] perylene	5.36_c	2.73_a
Indeno(1,2,3-cd)pyrene	5.16_c	2.98_a
Dibenzo(a,h)anthracene	5.11_c	2.07_a

Data are means of 3 replicates
Data with the same subscript are not significantly different at ($p<0.05$)

Table 5: Heavy Metals Profile (Concentration (µg/g)) of Smoked Silver catfish *(Chrysichthys nigrodigitatus)* Samples from 20 Different Locations using Local Drum Kiln and Convective Smoking Kiln.

Processing centers	Pb Drum	Conv	Cd Drum	Conv	Hg Drum	Conv	Cr Drum	Conv
Agbalata	0.0015_b	0.0013_a	0.0013_a	0.0010_a	0.0018_c	0.0019_d	0.0794_b	0.0767_b
Ajido	0.0011_a	0.0010_a	0.0010_a	0.0011_a	0.0016_b	0.0015_c	0.0756_b	0.0783_b
Asakpo	0.0013_a	0.0011_a	0.0015_b	0.0013_a	0.0011_a	0.0013_b	0.0783_d	0.0752_b
Boguru	0.0016_b	0.0014_b	0.0012_a	0.0013_b	0.0014_b	0.0016_c	0.0893_c	0.0819_c
Fvanoveh	0.0013_a	0.0013_a	0.0017_a	0.0012_a	0.0013_a	0.0012_a	0.0789_b	0.0795_b
Gberefun/Yovoyan	0.0011_a	0.0012_a	0.0015_b	0.0014_b	0.0016_b	0.0015_c	0.0754_b	0.0743_b
Gbetrome	0.0017_c	0.0018_d	0.0011_a	0.0013_a	0.0013_a	0.0011_a	0.0776_a	0.0798_b
Ilaje	0.0012_a	0.0013_a	0.0016_b	0.0015_b	0.0011_a	0.0012_a	0.0698_a	0.0714_b
Kofegameh	0.0015_b	0.0012_a	0.0013_a	0.0014_b	0.0014_b	0.0015_c	0.0847_c	0.0841_c
Pako	0.0013_a	0.0012_a	0.0012_b	0.0011_a	0.0017_c	0.0014_b	0.0745_b	0.0736_b
Afuye	0.0016_b	0.0017_c	0.0016_b	0.0015_b	0.0013_a	0.0016_c	0.0696_a	0.0684_a
Bodin Yawa	0.0013_a	0.0014_b	0.0013_a	0.0012_a	0.0016_b	0.0015_c	0.0789_b	0.0773_b
Idale	0.0013_a	0.0011_a	0.0015_b	0.0016_c	0.0014_b	0.0013_b	0.0762_b	0.0726_b
Igbodun	0.0012_a	0.0015_b	0.0016_b	0.0015_b	0.0015_b	0.0017_c	0.0711_b	0.0733_b
Ilogun	0.0015_a	0.0016_c	0.0017_c	0.0018_d	0.0020_b	0.0019_d	0.0796_b	0.0791_b
Mejona	0.0013_a	0.0010_a	0.0019_c	0.0017_c	0.0018_b	0.0016_c	0.0645_a	0.0652_a
Oluwo	0.0016_b	0.0018_c	0.0018_c	0.0019_d	0.0016_a	0.0014_b	0.0736_b	0.0698_a
Okorisan	0.0017_a	0.0016_c	0.0015_a	0.0017_c	0.0015_b	0.0013_b	0.0732_b	0.0741_b
Orita	0.0019_d	0.0017_c	0.0021_d	0.0020_d	0.0020_b	0.0018_d	0.0774_b	0.0773_b

Data are means of 3 replicates
Data with the same subscript are not significantly different at ($p<0.05$)

While samples processed by convective smoking kiln showedlevels below 3.5g BαP/kg.

The results of the mean concentrations of Hg, Pb, Cd, and Cr in the fresh and smoked fish samples analyzed are presented in Table 5. Concentration of Hg varied from 0.0019 to 0.0011g/g in the drum smoked fish samples and convective smoked fish samples respectively. Concentration of Pb varied from 0.0021 to 0.0010g/g in the drum smoked fish samples and convective smoked fish samples respectively.

Concentration of Cd varied from 0.0020 to 0.0011g/g in the drum smoked fish samples and convective smoked fish samples, respectively. Concentration of Cr varied from 0.0893 to 0.0645g/g in the drum smoked fish samples and convective smoked fish samples, respectively. Levels of the four heavy metals investigated in all the smoked fish samples are generally below the maximum permissible levels set by World Health Organization (Brain and Allen, 1993) for Pb (0.3 ppm); Cd (0.2 ppm), Hg (0.2ppm) and Cr (0.5ppm)and hence pose no risk to consumers.

CONCLUSION

This research work revealed that the quality of smoked fish from both smoking methods were within the quality standards set by USFDA and WHO. Majority of traditional drum smoked samples had high BaP and PAH levels exceeding the EU maximum permissible level of 5.0g/kg for BaP. However the use of convective smoking kiln drastically reduced the amount of PAHs generated.

The study also showed that the levels of four heavy metals investigated in the smoked fish are generally below the maximum permissible levels set by World Health Organization. However, *Listeria monocytogenes* was detected in drum smoked fish samples which poses risk to smoked fish consumers. This study revealed that traditional drum smoked fish samples may pose high (chemical and microbial) risks to smoked fish consumers as traditional drum smoked silver catfish samples contain high amount of polycyclic aromatic hydrocarbon concentrations and *Listeria monocytogenes*.

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