NUTRITIONAL QUALITIES, CHEMICAL COMPOSITION AND POLYCYCLIC AROMATIC HYDROCARBON LEVELS IN SALTED AND UNSALTED *Clarias gariepinus* SMOKED WITH *Lophira alata* WOOD

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Abstract: The study investigated the effects of smoking *Clariasgariepinus* with *Lophiraalata* wood on the nutritional qualities, chemical composition and Polycyclic Aromatic Hydrocarbon levels (PAHs) when stored for 28 days. For organoleptic qualities, only the taste values of the salted samples scored significantly higher ($p<0.05$) than the unsalted sample till day 14. All the proximate composition except fibre and carbohydrate of the smoked samples were significantly different from the fresh sample. Storage resulted in no significant change in all the proximate composition of the smoked samples except carbohydrate which increased significantly with storage. Mineral composition varied significantly between fresh and smoked samples except calcium. Storage did not significantly affect the concentrations of the minerals except sodium which decreased. There were differences in the values of concentrations of sodium, potassium and calcium in the salted and unsalted samples during storage. Mould was observed on day 14 in salted and unsalted samples with no significant difference ($p>0.05$). Microbial infestation occurred from first week in both samples and it increased significantly from the 21st day of storage. No insect infestation was observed. The microorganisms detected included *Aspergillus spp., Fusarium spp., Saccharomyces spp., Penicillium spp., Staphylococci aureus, Staphylococci lentus, Klebsiella pneumoniae, Escherichia coli* and *Kocuriarosea*. Forty three PAHs were detected in salted samples and 23 in unsalted samples, only in salted samples was Anthracene, a carcinogenic PAH, detected at low level of 0.57 µg kg^{-1} which poses no health risk to consumers. Thus, this study provided a possible application of *L. alata* as safe wood for smoking fish.

Keywords: *Clariasgariepinus, Lophiraalata,* microorganisms, nutritional, PAHs, smoked

Introduction

Fish is the most important source of protein available in the world today and it represents about 14% of all animal protein on global basis (Abolagba and Melle, 2008). Fish is regarded as healthier than meat due to the high content of long chain poly unsaturated fatty acids (LCPUFA's) which are associated with improving health and preventing diseases of old age (Kabahenda *et al*., 2009). In Nigeria, fish is eaten fresh, preserved or processed (Adebayo-Tayo *et al*., 2008). They are widely accepted on the menu card and form a much cherished delicacy that cut across socio-economic, age, religious and educational barriers (Adeleye, 1992). Fish is however susceptible to damage as soon as it is harvested. Immediately fish dies, it remains in first class quality only for a short time (Clucas and Ward, 1996). Haruna (2003) reported that fish is a low-acid food that supports the growth of pathogens if not carefully handled and rapidly processed after harvesting. Preservation of fish generally slows down or prevents spoilage. Preservation methods are applied with an intention to making the fish safer and extend its shelf-life (Ghazala, 1994). The methods commonly used in Nigeria, are the traditional techniques such as salting/brining, sun-drying and smoking which prevents spoilage and increase fish availability to the consumers (Abolagba *et al.*, 1996).

According to FAO (2002), 45% of total fish caught in Nigeria are utilized as smoked fish. However, Bolaji (2005) reported that despite the rudimentary nature of process of traditional methods, lack of control over the drying rate sometimes resulted in under-drying or over-drying and this exposes the fish to unexpected winds, dust, dirt, insect infestation and contamination. Daramola *et al.* (2007) observed that smoked fish and shell fish products deteriorated in quality with storage time and temperature. Hence, another major concern is what happens to the nutritional quality and microbial composition of the fish after smoking and period of storage. Also, the presence of polycyclic aromatic hydrocarbons (PAHs) in smoked fish has been the subject of much concern in recent years due to their toxic potentials which can cause health hazards in humans.

Fuel wood is the main source of energy for fish smoking. According to Abolagba *et al.*, (2005), many wood types are used as fuel for fish smoking. Among the many factors influencing the choice of wood are local availability and cost. Thus, fish smoking is carried out most often by using a mixture of woods and even dry leaves. It is difficult, if not impossible to determine the effect of the different wood types on the quality of smoked fish. The purpose of this study is to determine how woods of different species affect the quality of smoked fish.

Lophiraalata (Family- *Ochnaceae*) occurs abundantly in wet evergreen forest in Western and Central Africa, from Sierra Leone to Ghana, Nigeria, Cameroon and Republic of Congo. It is a large tree of about 45 m tall. The timber is strong and resistant to fungi, dry wood borers and termites (Ken, 2014). The heartwood is dark red, chocolate brown, with no characteristic odour or taste. The wood is used for various construction works, so it is readily available; therefore, fish processors have access to the remains of wood and sawdust when the wood is cut for construction works, hence the use in this study as source of fuel for smoking *Clariasgariepinus*.

However, wood smoke contains appreciable amounts of carcinogenic PAHs, which are major cause of concern regarding their toxicity (Garcia-Falcon and Simal-Gandara, 2005). Against this background, this study was aimed at evaluating the effects of smoking salted and unsalted catfish (*Clariasgariepinus*) with *Lophiraalata* wood on its organoleptic qualities, chemical and mineral composition, storage stability, microbial composition and polycyclic aromatic hydrocarbons (PAHs) level.

Materials and Methods

Preparation and smoking of fish

Sixty-one (61) fresh catfish, *Clariasgariepinus* of average total weight of about 30 kg were purchased from local fishermen in the early morning immediately the fish were

brought to Makoko fish market, Yaba Local Government Area, Lagos, Nigeria. They were transported, in an ice chest, to the laboratory of the Department of Marine Sciences, University of Lagos, Nigeria. Each fish was, gutted and then washed with clean portable water. They were divided into three, two parts have thirty fishes each, one of the parts were salted and the other unsalted, they were smoked with the traditional cylindrical smoking drum for a duration of 8hours at the Fish Processing Unit, in the Botanical Garden of the University of Lagos. The wood fuel used was *Lophiraalata* (Yoruba name: Eki; Trade name: Azobe). The smoked samples were packed in labelled plastic bowls and transported for storage and observation in the laboratory. The fish samples were labeled as salted smoked fish (SSF) and unsalted smoked fish (USF) for ease of identification. The remaining unsalted and unsmoked fish was used as control. Organoleptic assessment, proximate, mineral composition analysis, microbial load and polycyclic aromatic hydrocarbons (PAHs) levels of the samples were determined.

Organoleptic assessment

The organoleptic assessment of the smoked samples was done using the method of Eyo (2001). The smoked fish was evaluated by ten semi-trained panelists drawn from students of the Department of Marine Sciences, University of Lagos. The organoleptic parameters that were evaluated included: texture, taste, appearance and aroma with provision for a score on overall acceptability. A 10 point hedonic scale was used with a score of 10 indicating "excellent", 8 "good"; 6 "fair", 4 "poor" and 2 "bad". A score below 2 was considered unacceptable. The organoleptic assessment was carried out for freshly smoked samples and then for stored smoked samples weekly for a period of 28 days.

Proximate and mineral composition analysis

The proximate composition analyzed included crude protein, moisture, ash, fibre, lipid and carbohydrate while the minerals analyzed were copper, zinc, sodium, potassium, magnesium and calcium. The moisture content was determined by oven drying about 5 g of each sample in a Gallenkamp oven at 800^0C for 18 h, crude protein was determined by the Kjedahl method, lipid determination was by the modified Bligh and Dyer procedure, ash content was by incineration in a carbolite Sheffield LMF3 muffle furnace at 550^0C for 24 h. Crude fibre was by acid base- digestion using 1.25% H_2SO_4 (W/V) and 1.25% NaOH (W/V) solution. Carbohydrate content was determined by calculating the difference between the sum total of proximate composition and 100 % (AOAC, 2005).

For the mineral analysis, the ash obtained from the proximate analysis was digested by dissolving in nitric and perchloric acid mixture (4 ml 60% perchloric acid plus 25 ml concentrated nitric acid). After digestion, Na and K contents were determined using a flame photometer, while Cu, Zn, Mg, and Ca contents were determined by flame atomic absorption spectrometry using a Perkins Elmer spectrophotometer, USA Model 205 series.

Insect and mould attack observation

During the storage period, insect and mould attacks were determined according to the method of Khan and Khan (2001) with modifications. Insect and mould attacks were determined on hedonic scale of 0-3. A numerical score of 0 meant there was no sign of infestation or damage, score of 1 meant occasional infestation, 2 meant noticeable consistent infestations and 3 meant heavy insect/mould infestations covering most or the whole fish sample. For insect infestation, a score of 3, led to the rejection of the fish and a score below 3 was the limit of acceptability for mould.

Microbial analysis

One gram of Nutrient Agar was weighed into a 250 ml conical flask and dissolved with about 50 ml deionised water and 9.75 g of Potato Dextrose Agar (PDA) was also weighed as well as 13 g of MacConkey Agar (MAC) and 15 g of Salmonella Shigella Agar (SSA). The various media were dissolved in separate 250 ml conical flasks. They were allowed to soak for 15 min. The Nutrient Agar (NA), Potato Dextrose Agar (PDA) and MacConkey Agar (MAC) were autoclaved at 121^0C for 15 min at a pressure of 0.15KPa. For Salmonella Shigella Agar (SSA), it was boiled for 20 min in a water bath at 100^0C. After sterilizing the media, they were allowed to cool to about 47^0C and then poured aseptically into Petri-dishes and left to solidify. One gram of the fish sample was weighed out and homogenized by pounding using a sterile mortar and pestle. After homogenizing, the homogenate sample was added into the sterile diluents labelled 10^{-1} per sample. Using a micro-pipette, 1 ml of the sample was transferred in diluents 10^{-1} to diluents 10^{-2}. It was shaken lightly to mix and the same procedure was repeated from 10^{-2} to 10^{-3} and so on till 10^{-8}. Suitable dilution factors were plated out on the media in the Petri-dishes and spread out using the glass spreader. After spreading, the plates were incubated in the 37^0C incubator for 24 h. The Potato Dextrose Agar (PDA) plates were put in 27^0C incubator (also known as Room temperature incubator) for 72 h.

After 24 h, the bacterial plates at the 37^0C were brought out and the Total Bacterial Count (TBC) was taken. The plates were sub-cultured in freshly made plates which were put in the incubator for another 24 h. After this, the pure isolates were subjected to testing using API 20E and 20NE kits to enumerate the type of micro-organisms present in the samples. After 24 h, the PDA plates were brought out and sub-cultured in fresh PDA plates. They were incubated for another 72 h after which they were characterized by the colour and shape of the colonies found (Harrigan and McCance (1976); Speck (1984) and Sneath *et al*., (1986).

Analysis of polycyclic aromatic hydrocarbons (PAHs)

Ten grams of sample were put into a separating funnel, 25 ml of dichloromethane (DCM) was added and the mixture was separated after shaking vigorously for 30 min. This process was repeated three times, and then the extract was concentrated on the water bath to the minimum volume of 20 ml. The remaining extract was treated with anhydrous $NaSO_4$ to eliminate the remaining water in the sample. Anhydrous $NaSO_4$ was poured on the cotton wool placed in the Pasteur pipette and the sample was then poured through the cotton wool which serves as a separating medium into a vial bottle which made the sample ready for Gas Chromatographic Analysis (GCA) (Garcia-Falcon and Simal-Gandara, 2005).

Data analysis

Data collected in this study were analyzed using Scientific Package of Social Science (SPSS) version 17.0. Analysis of variance was determined by one-way ANOVA procedures. T –test was also used to ascertain a comparative result among treatments (salted and unsalted). The level of significance was defined at $P<0.05$. Results presented are mean values of three determinations ± standard deviation (SD).

Results and Discussion

The sensory evaluation of the smoked salted and unsalted *Clariasgariepinus* stored for 28 days showed that there was no significant difference ($p> 0.05$) between the salted and unsalted values throughout the storage period except in taste up to the 14^{th} day (Table 1). This is in agreement with the finding of Bilgin *et al.* (2008). This indicates that, as expected, salting improves taste. The lack of effect on taste at and after the 21^{st} day may be due to the infestation of the sample by moulds and microorganisms (Tables 6 and 7).

Table 1: Mean ± SD for organoleptic evaluation of salted and unsalted smoked *Clariasgariepinus*, during 28 days storage period

Organoleptic Parameters	Days									
	0		7		14		21		28	
	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted
Texture	10.00 ± 0.00^{d}	10.0 ± 0.00^{d}	9.8 ± 0.63^{d}	9.8 ± 0.63^{d}	7.8 ± 0.63^{c}	7.8 ± 0.63^{c}	5.8 ± 1.47^{b}	5.8 ± 1.47^{b}	3.4 ± 0.97^{a}	3.4 ± 0.96^{a}
Taste	9.20 ± 1.03^{g}	6.6 ± 0.96^{f}	6.8 ± 1.03^{e}	4.4 ± 0.84^{d}	4.4 ± 1.26^{b}	2.8 ± 1.03^{c}	2.0 ± 0.00^{a}	2.0 ± 0.00^{a}	2.0 ± 0.00^{a}	2.0 ± 0.00^{a}
Appearance	10.00 ± 0.00^{d}	10.0 ± 0.00^{d}	9.8 ± 0.63^{d}	9.2 ± 1.03^{d}	7.2 ± 1.03^{c}	7.8 ± 0.63^{c}	3.4 ± 1.35^{b}	3.4 ± 1.34^{b}	2.2 ± 0.63^{a}	2.2 ± 0.63^{a}
Aroma	10.00 ± 0.00^{c}	10.0 ± 0.00^{c}	8.6 ± 1.89^{c}	8.6 ± 1.89^{c}	5.6 ± 2.63^{b}	5.6 ± 2.63^{b}	3.4 ± 1.35^{a}	3.4 ± 1.34^{a}	2.0 ± 0.00^{a}	2.0 ± 0.00^{a}

Data is expressed as means ± SD of three separate determinations.
a-g Means of salted and unsalted of the same day not followed by the same superscript letter are significantly different (P<0.05) within the row

Table 2 shows that smoking affected the proximate composition of the fish. Moisture and lipid contents were significantly reduced (p< 0.05) but crude protein and ash contents were significantly increased due to the effect of heating which resulted in loss of water and fish fat. Fibre and carbohydrates were unaffected by heating so there was no significant difference between the fresh and smoked samples. Salting had no significant effect on the proximate composition as the values for the salted samples were not significantly different from the unsalted values (Table 2). This is similar to other reports which indicated that smoking results in concentration of nutrients like protein and lipid (Gokoglu *et al*., 2004; Tao and Linchun, 2008).

Table 2: Mean ± SDof proximate composition of fresh and freshly smoked salted and unsalted *Clariasgariepinus*

Proximate composition (%)	Smoked Fish		
	Fresh	Salted (SSF)	Unsalted (USF)
Crude Protein	30.67±1.50^{a}	63.04±3.24^{b}	62.40±2.50^{b}
Moisture	53.19±2.00^{a}	20.65±1.00^{b}	22.30±1.40^{b}
Ash	1.99±0.40^{a}	3.98±0.70^{b}	3.32±1.08^{b}
Fibre	2.32±1.00^{a}	2.24±1.00^{a}	2.22±1.00^{a}
Lipid	0.93±0.20^{a}	0.30±0.08^{b}	0.32±0.05^{b}
Carbohydrate	10.20±1.00^{a}	9.79±1.30^{a}	9.44±1.20^{a}

a-b Means not followed by the same superscript letter are significantly different (P<0.05) within the row

Table 3: Proximate composition (%) of smoked salted and unsalted *Clariasgariepinus* during 28 days storage period

Proximate composition (%)	Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Crude protein	Salted	63.04 ± 3.00^{a}	62.98 ± 2.00^{a}	61.90 ± 1.00^{a}	61.12 ± 3.00^{a}	62.56 ± 2.00^{a}
	Unsalted	62.98 ± 2.00^{b}	60.99 ± 2.40^{ab}	60.99 ± 2.30^{ab}	60.40 ± 1.50^{ab}	57.98 ± 2.10^{a}
Moisture	Salted	20.65 ± 2.20^{a}	20.88 ± 1.40^{a}	21.65 ± 1.80^{a}	22.48 ± 1.60^{b}	23.03 ± 1.09^{b}
	Unsalted	22.30 ± 1.00^{a}	21.30 ± 1.50^{a}	21.78 ± 1.40^{a}	21.00 ± 2.00^{a}	21.20 ± 2.40^{a}
Ash	Salted	3.98 ± 1.60^{a}	3.98 ± 1.40^{a}	3.68 ± 1.00^{a}	3.98 ± 1.60^{a}	3.87 ± 1.10^{a}
	Unsalted	3.32 ± 1.40^{a}	3.22 ± 1.30^{a}	3.29 ± 0.90^{a}	3.02 ± 0.80^{a}	3.09 ± 1.20^{a}
Fibre	Salted	2.24 ± 1.20^{a}	2.26 ± 1.30^{a}	2.28 ± 1.11^{a}	2.16 ± 1.08^{a}	2.26 ± 1.20^{a}
	Unsalted	2.22 ± 1.30^{a}	2.28 ± 1.20^{a}	2.29 ± 1.30^{a}	2.29 ± 1.20^{a}	2.39 ± 1.30^{a}
Lipid	Salted	0.30 ± 0.04^{a}	0.28 ± 0.02^{a}	0.29 ± 0.05^{a}	0.29 ± 0.07^{a}	0.27 ± 0.05^{a}
	Unsalted	0.32 ± 0.05^{a}	0.28 ± 0.03^{a}	0.34 ± 0.02^{a}	0.32 ± 0.04^{a}	0.34 ± 0.02^{a}
Carbohydrate	Salted	9.79 ± 1.30^{a}	10.19 ± 1.20^{a}	10.37 ± 1.80^{a}	12.38 ± 2.50^{b}	12.68 ± 1.60^{b}
	Unsalted	9.44 ± 1.20^{a}	10.89 ± 1.40^{a}	10.31 ± 1.08^{a}	12.27 ±2 .02^{b}	12.50 ± 1.00^{b}

Data is expressed as Mean±SD of three separate determinations.
a-b Means not followed by the same superscript letter are significantly different (P<0.05) within the row

Table 3 shows that storage did not affect the proximate composition of both the salted and unsalted samples except the carbohydrate and moisture contents of the salted sample which significantly increased at the 21st day. For moisture, it may be due to high relative humidity in the storage environment, while for carbohydrate, it may be due to the effect of moulds and microorganisms, the growth of which had been facilitated by the moisture present at that period of storage (Tables 6 and 7).

Table 4 shows that mineral compositions in the smoked samples were significantly higher than in the fresh sample (p <0.05) except in calcium. With the removal of water by heat during smoking, the relative amounts of these minerals in the sample increased. Calcium, being mainly in the bones, is minimally affected by the heat. The values in the salted samples are only significantly higher than the unsalted in sodium and potassium, two elements that are abundant in the salt used.

Storage did not significantly affect the mineral composition in salted and unsalted smoked samples except sodium in salted sample which reduced with storage (Table 5). Moisture could have accumulated on the dried samples causing sodium chloride to dissolve and be lost resulting in a significant decrease in sodium during storage. Mbunda (2012) reported that the mineral composition in fresh water sardine (*Rastrineobolaargentea*) and marine pelagic fish (capelin) were constant during 42 days of storage.

The order of abundance of mineral concentrations (mg/g) in the fresh sample in this study (Table 4) was calcium (28.91); sodium 10.34; potassium (9.8); magnesium (8.56); copper (0.45) and zinc (0.29). This order is contrary to the finding of Effiong and Fakunle (2011), who reported the order of sodium (3.2), calcium (2.9); potassium (0.71); magnesium (0.28); and zinc (0.084). These significantly different results in the order and abundance of these elements may be due to the environment in which the fish samples developed. The order of mineral composition in the smoked salted samples is Na>K>Ca>Mg>Cu>Zn. This is in line with the report of Adewumi *et al*. (2014) who stated that Na, K, Mg and Ca form the major minerals for all the species examined in their studies.

Table 4: Mean (mg/g) ± SDof mineral composition of fresh and freshly smoked salted and unsalted *Clariasgariepinus*

Mineral	Smoked		
	Fresh	Salted (SSF)	Unsalted (USF)
Cu	0.45±0.08^{a}	0.81±0.01^{b}	0.90±0.01^{b}
Zn	0.29±0.08^{a}	0.49±0.09^{b}	0.51±0.01^{b}
Na	10.34±1.30^{a}	49.61±2.40^{b}	16.46±1.50^{c}
K	9.38±1.10^{a}	39.41±1.30^{c}	18.46±1.20^{b}
Mg	8.56±1.20^{a}	12.81±1.20^{b}	10.41±1.40^{b}
Ca	28.91±2.10^{a}	34.81±2.30^{a}	31.62±1.30^{a}

a-b Means not followed by the same superscript letter are significantly different (P<0.05) within the row

The sodium and potassium components in salted smoked samples were significantly higher ($p< 0.05$) than in unsalted sample seven with storage (Tables 4 and 5). These are the major elements in the salt used in the experiment.

Infestation of microbes started immediately in both salted and unsalted samples and their concentrations increased significantly ($p<0.05$) with storage. The microbes in the unsalted samples were significantly higher than in the salted (Table 6). This indicates that salting may somehow be effective in reducing microbial infestation. The increase in microbial load is obviously due to the multiplication of the microbes as observed by Bilgin *et al*. (2008). The method of storage probably facilitated high moisture with storage (Table 3) and provided favourable environment for the growth of the microbes.

Table 5: Mineral composition (mg/g) of smoked *Clarias gariepinus* during 28 days storage period

Mineral	Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Cu	Salted	0.11±0.01^a	0.11±0.10^a	0.13±0.10^a	0.13±0.36^a	0.13±0.04^a
	Unsalted	0.10±0.00^a	0.11±0.01^a	0.13±0.10^a	0.12±0.02^a	0.11±0.01^a
Zn	Salted	0.47±0.09^a	0.46±0.10^a	0.45±0.12^a	0.43±0.04^a	0.42±0.03^a
	Unsalted	0.48±0.01^a	0.49±0.04^a	0.46±0.01^a	0.45±0.08^a	0.47±0.03^a
Na	Salted	49.62±2.50^a	48.92±3.00^a	46.93±2.20^b	46.61±2.00^b	43.88±3.00^c
	Unsalted	16.96±1.00^a	16.86±1.00^a	16.86±1.00^a	16.62±1.00^a	16.77±1.00^a
K	Salted	39.41±1.50^a	39.01±1.30^a	40.47±1.00^a	40.96±1.20^a	40.80±1.40^a
	Unsalted	18.96±1.00^a	18.82±5.00^a	18.59±2.00^a	18.47±1.00^a	18.92±1.00^a
Mg	Salted	9.81±1.40^a	9.88±1.00^a	10.09±1.00^a	10.62±0.57^a	10.59±1.00^a
	Unsalted	8.41±1.20^a	8.31±1.00^a	8.12±1.00^a	8.08±1.00^a	8.54±1.30^a
Ca	Salted	38.81±1.30^a	38.99±1.70^a	38.09±1.00^a	37.96±1.80^a	38.69±1.20^a
	Unsalted	31.82±1.00^a	31.94±1.00^a	31.56±2.20^a	32.01±0.60^a	32.18±0.60^a

Data is expressed as Mean ± SD of three separate determinations.
a-c Means not followed by the same superscript letter are significantly different ($P<0.05$) within the row

Table 6: Microbial plate counts (CFUml^{-1}) of smoked salted and unsalted *Clarias gariepinus* during 28 days storage period

Culture medium	Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
NA	Salted	1.46±0.61^a	2.23±0.17^b	2.30±0.12^b	2.51±0.05^b	2.56±0.43^b
	Unsalted	1.36±0.54^a	2.22±0.16^b	2.89±0.84^b	3.73±0.41^c	4.26±0.60^d
MAC	Salted	0.76±0.23^a	1.20±0.03^a	1.64±0.57^a	2.02±0.51^b	3.00±0.76^c
	Unsalted	0.88±0.19^a	1.33±0.15^a	1.96±0.40^a	2.90±0.46^b	4.62±0.47^c
SSA	Salted	0.00±0.00^a	1.00±0.36^a	1.66±0.75^a	2.33±1.05^b	3.86±0.25^c
	Unsalted	0.83±0.12^a	1.66±0.75^b	2.10±0.27^b	3.33±1.05^c	5.00±1.24^d

a-d Means not followed by the same superscript letter are significantly different ($P<0.05$) within the row

Microbial populations for salted and unsalted smoked samples observed in this study over the storage period of 28 days included *Aspergillus spp., Fusarium spp., Saccharomyces spp., Penicillium spp., Staphylococci aureus, Staphylococci lentus, Klebsiella pneumoniae, Escherichia coli* and *Kocuriarosea*. This agrees with some of the micro-organisms isolated and identified by Omojowo and Ihuahi (2006), Abidemi-Iromini *et al.* (2011) and Adelaja *et al.* (2013), in their work with smoked *Clarias gariepinus* and *Chrysichtysnigrodigitatus*. The occurrence of the same bacteria and fungi on the salted and unsalted fish samples are indications that they were contaminated at the same point and time, probably during the display of the smoked dried fish samples in open trays during cooling of the samples immediately after smoking. The variations in the microbial counts during the storage period of 28 days may be a function of the efficiency of the storage and processing methods.

On the other hand, mould attack did not occur until day 14 in both salted and unsalted smoked samples. Although there was no significant difference ($p>0.05$) between the values in the salted and unsalted samples (Table 7), the salted samples recorded lower values for mould infestation (0.5±0.00 to 2.00±0.25) when compared to the unsalted samples (1.00±0.58 to 3.00±0.50). This supports the view that salting may reduce mould attack. However the increased shelf life up to 14 days may be due to the action of smoke and heat that reduced water activity and impaired the action of microbes as reported by Abolagba and Osifo (2004).

No insect attack was observed throughout the storage period in both smoked samples. It is not clear what the reason for this may be, but the smoke from the *Lophiraalata* wood may contain bioactive ingredients that repel insect infestation just as the timber is resistant to dry wood borers and termites (Ken, 2014).

Table 7: Mould attack of smoked salted and unsalted *Clarias gariepinus* over 28 days of storage

	Day 0	Day 7	Day 14	Day 21	Day 28
Salted	-	-	0.50±0.00	1.00±0.00	2.00±0.25
Unsalted	-	-	1.00±0.58	2.00±0.25	3.00±0.50
T-test (sig.)	-	-	0.184	0.225	0.225

Both the smoked unsalted and salted samples contain PAH components. While unsalted samples contained 23 PAHs andthe smoked salted sample contained 43 PAHs compounds, it was the smoked salted sample that contained the only carcinogenic PAH, anthracene at a low level of 0.57 ug kg^{-1} (Tables 8a & b). This shows that smoking with *Lophiraalata* produced fish safe for human consumption. The carcinogenic PAH levels in smoked fish largely depend on the smoke generated by combustion of wood, the fat level of fish and the procedure used for smoking (Simko, 2005; Reinik*et al*., 2007; Varlet *et al*., 2007).

Table 8a: Polycyclic aromatic hydrocarbons (ug kg^{-1}) in salted *Clariasgariepinus* smoked with *Lophiraalata* wood carcinogenic PAH is Bolden

S/N	Polycylic Aromatic Hydrocarbons	Concentration (µgkg^{-1})
1	**Anthracene**	**0.57**
2	Benzene, 1,3,5-trimethyl	1.12
3	Benzene, 1-ethyl-2,3-dimethyl-	0.89
4	Benzene, 1,2,3,4-tetramethyl-	0.38
5	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	0.48
6	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	0.15
7	2-Butyl-1-decene	0.58
8	Cholesterol	3.72
9	Cyclododecane	27.95
10	17-(1,5-Dimethylhexyl)-10,13-dimet hyl-2,3,4,7,8,9,10,11,12,13,14,15, 16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	3.53
11	Dodecane, 2,6,11-trimethyl-	0.16
12	Ethanone, 1-(3-methoxyphenyl	0.91
13	Hexadecane	0.44
14	Hexadecanoic acid, methyl ester	0.84
15	n-Hexadecanoic acid	2.80
16	Octadecane	0.23
17	13-Octadecenal, (Z)-	1.52
18	Octadecanal	0.93
19	9-Octadecenoic acid, methyl ester	0.67
20	Octadecanoic acid, methyl ester	0.26
21	6-Octadecenoic acid	4.64
22	9,17-Octadecadienal, (Z)-	0.68
23	1-Octadecene	0.95
24	9-Octadecenal, (Z)-	2.54
25	9-Octadecenoic acid, (E)-	0.66
26	Oleic Acid	1.77
27	Oxacyclotetradecan-2-one, 13-methyl-	0.25
28	Phenol, 2-methoxy-	1.51
29	Phenol, 2-methoxy-4-methyl-	0.63
30	Phenol, 4-ethyl-2-methoxy-	1.60
31	Phenol, 2,6-dimethoxy-	1.78
32	Phenol, 2-methoxy-4-(1-propenyl)-	2.85
33	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	1.04
34	4-Propyl-1,1'-diphenyl	0.99
35	Tridecane, 7-methylene-	1.04
36	Tridecane 3-methylene	0.69
37	Tetradecane	0.36
38	5-tert-Butylpyrogallol	2.74
39	Tetradecanal	0.55
40	1,9-Tetradecadiene	0.57
41	E-9-Tetradecenoic acid	0.46
42	2H-Pyran, 2-(8-dodecynyloxy)tetrahydro-	0.96
43	Squalene	1.81

European Union (EU) Maximum level for PAH (12.0 µg kg^{-1} as from 01/09/2014)

Table 8b: Polycyclic aromatic hydrocarbons (ug kg^{-1}) in unsalted *Clariasgariepinus* smoked with *Lophiraalata* wood

S/N	Polycyclic aromatic hydrocarbons (PAHs)	Concentration (µgkg^{-1})
1	Benzene, 1,2,3-trimethoxy-5-methyl	0.61
2	2-Butyl-1-decene	0.40
3	Cholesterol	7.49
4	Cyclopropa[5,6]stigmast-22-en-3-one,	1.06
5	Decanamide, N-(2-hydroxyethyl)-	0.57
6	(1,5-Dimethylhexyl)-10,13-dimethyl- tetradecahydro-1H-cyclopenta [a]phenanthren-3-ol	13.84
7	3-(2-Ethyl-piperidin-1-ylmethyl)-8 htho[2,3-b]furan-2-one	1.50
8	n-Hexadecanoic acid	0.93
9	Niralin	1.18
10	9-Octadecenal, (Z)-	0.49
11	Oleic Acid	2.38
12	Oxirane, hexadecyl-	0.44
13	Oxirane, hexedecyl-	0.35
14	Phenol, 2-methoxy-4-(1-propenyl)-	1.34
15	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	0.72
16	(Phenylthio)acetic acid, 1-adamant ylmethyl ester	5.43
17	2-Propyn-1-one, 1-(2-thienyl)-, (2, 4-dinitrophenyl)hydrazone	1.03
18	Silane, dimethyl(2-naphthoxy)heptyloxy-	5.46
19	3-Tetradecene,	18.54
20	2,6,10,14,18,22-Tetracosahexaene,	0.85
21	9-Tricosene, (Z)-	0.32
22	Tridecane, 3-methylene-	0.39
23	5H-Thiazolo[3,2-a]pyrimidine-6-car boxylic acid, 7-methyl-3-oxo-5-phe nyl-2-(1H-pyrrol-2-ylmethylene)-2, 3-dihydro-, ethyl ester	0.02

In view of the fact that there is a dearth of information on the effect of different wood smoke on the quality of smoked fish, the effects of *Lophiraalata* could not be adequately compared with other woods used as fuel for smoking. However, this result will contribute to the meagre literature on the effect of different wood types on the smoking of fish so that in future, proper wood recommendation can be made to fish processors since they choose woods based on easy accessibility, affordability and dryness and most often, they use mixture of woods for smoking fish.

Conclusion

This study showed that smoking *C. gariepinus* with *L. alata* wood produced fish samples that are good to taste, retain their minerals and proximate components and have long shelf life. It also prevents insect infestation and produced no significant high level of carcinogenic PAH. All these make *L. alata* a safe and fish smoking wood that can be recommended to fish processors.

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Conflict of Interest

The authors have no conflict of interest in the products and the execution of the research.

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