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IMPACT OF SMOKING TECHNIQUES AND STORAGE CONDITIONS ON MICROBIAL SAFETY AND STABILITY OF CATFISH (*Clarias gariepinus*)

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ABSTRACT

Microbial spoilage is one of the causes of quality deterioration of smoked catfish during storage. The impact of smoking techniques and storage conditions on the quality of smoked catfish were determined. Smoked dried catfish were produced using traditional smoking kiln (44-gallon drum kiln) and an improved smoking kiln from Nigerian Institute for Oceanography and Marine Research (NIOMR). The smoked dried catfish were packed in polythene bags and stored at ambient (30 ±3 °C), refrigerated (4 ±1 °C) or frozen at (-18±2°C) for 12 weeks. The moisture content and acidity (pH) of the smoked fish products fluctuated during the storage period. Smoking reduced the total viable count (TVC) of the microorganisms significantly ($P<0.05$) from 5.71 to between 3.18 and 3.93 log CFU/g for traditionally smoked and improved kiln smoked fish samples respectively. The microbial total viable count (TVC) of the stored smoked fish products increased as the storage period increased, the frozen stored smoked fish products had the highest TVC followed by the refrigerated stored smoked fish products and the ambient stored smoked fish products had the least TVC but there was no significant difference ($P>0.05$) among the storage conditions studied. The stored smoked fish products were acceptable up to 8th week storage period based on microbial load. Gram negative organisms, *Pseudomonas* sp. and *Aeromonas* sp. were the bacteria identified while *Penicillium* sp. and *Aspergillus niger* were the fungi identified in the smoked fish products during the storage period. Microbial safety and shelf life of stored smoked catfish depends on the smoking technique adopted, storage condition and duration of storage.

Keywords: Catfish, Smoked fish, Ambient temperature, Refrigerated, Microbial loads

INTRODUCTION

The African catfish, *Clarias gariepinus*, is the most important tropical catfish species for aquaculture. It is easily cultured in Nigeria and of great economic interest. Nigeria produce over 250,000 tonnes of catfish annually, which makes Nigeria largest producer of catfish in Africa (FDF, 2014). Catfish is generally consumed fresh, a good percentage is also consumed smoked in Nigeria.

Smoking is one of the oldest methods used to process and preserve fish (Swastawati *et al*., 2000; Simko, 2002; Stołyhwo and Sikorski, 2005). Smoking can halt the formation of toxins and reduce the growth of bacteria due to lower water activity as a result of smoking in addition to salting and drying which create a physical surface barrier (Rervik, 2000). The salt content, duration and temperature of smoking, density of smoke and component of smoke affect the presence and composition of spoilage and pathogenic microflora of the smoked product (Kolodziejska *et al*., 2002).

Depending on the way smoke gets into products, smoking can be categorized accordingly: the traditional technique – where the smoke is formed directly by burning chips or sawdust from firm wood in the oven, wood log, and locally available materials such as coconut husk (Stołyhwo and Sikorski, 2005; Visciano *et al*., 2008); or new technique - by using an improved traditional smoking technique, mechanised smoking technique where the smoke generator is separated from the smoke chamber and there is no direct firing of the smoked products. The improved and mechanised smoking techniques use charcoal and smoke derivatives such as liquid smoke and powder smoke as the source of smoke flavour.

The commonly used smoking technique in Lagos, Nigeria is the 44-gallon drum kiln (Adeyeye *et al*., 2015) while the Nigerian Institute for Oceanography and Marine Research (NIOMR) smoking kilns have been adopted for use in over 30 states out of the 36 states in Nigeria and neighbouring countries such as Benin republic, the Gambia and Cote d voire. NIOMR improved

smoking kilns have the support of international agencies such as Food and Agriculture Organization (FAO) of the United Nations, West Africa Agricultural Productivity Programs (WAAPP) and United States Agency for International Development (USAID). NIOMR smoking kilns have been designed to address the disadvantages of the traditional smoking kilns/ techniques (NIOMR, 2016).

The processing of a fish species inevitably entails a storage period for the finished product prior to marketing and consumption. Since fish are composed of perishable nutrients, storage period should be kept to a minimum with adequate storage conditions provided so as to prevent deteriorative changes occurring through oxidative damage and microbial infestation. The most important environmental factors governing the storage or shelf life of fish are ambient temperature and humidity. These factors dictate the rate at which chemical and microbial changes take place (Daramola *et al.,* 2007).

The impact of the commonly used traditional smoking kiln (44-gallon drum kiln) and improved smoking kiln by NIOMR at different storage times and conditions (such as ambient, refrigerated and frozen conditions) on the microbial safety and stability of smoked catfish (*Clarias gariepinus*) has not been reported.

MATERIALS AND METHODS

Sample Collection, Smoking, Packaging and Storage

Fifty kilograms (50 kg) of live catfish (each individual fish weighing 350 g to 450 g) were purchased from Biotechnology Department, Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos, Nigeria. It was randomly divided into two halves and smoked by adopting two smoking techniques. One half was smoked using 44-gallon drum kiln that uses wood, at a temperature of 55 – 145 $^{\circ}$C for 15 h and the second half was smoked using NIOMR kiln with charcoal at a temperature of 55- 180 $^{\circ}$C for 11 h. The smoked catfish from each techniques were cooled, packed in polythene bags, divided into three parts and stored for 12 weeks at three different temperature conditions: ambient (30 ± 3 $^{\circ}$C), refrigerated (4 ± 1 $^{\circ}$C) or frozen (-18 ± 2 $^{\circ}$C). Samples were drawn every two weeks for analysis.

Moisture Determination

Moisture content was determined by difference in weight of the homogenised samples before and after drying for 24 h in electronic oven at a temperature of 104 ± 2 $^{\circ}$C (AOAC 1994).

Hydrogen ion (pH) Determination

The pH of fresh and dried samples was measured by the method of Bragadottir *et al.,* (2007). 5 g of fresh samples was measured directly while 5 g of dried samples was mixed with 20 ml of deionised water, stirred for 5 mins prior to measurement with combined electrode SE 104- Mettler Toledo, Knick Berlin Germany connected to a portable pH meter.

Microbial Analysis

Isolation Technique: Fresh and smoked catfish samples were analysed for the presence of pathogens. A swab of the skin and gut of the fresh catfish samples were taken with sterile swab stick and 1 g representative sample was obtained aseptically from the loin muscle of the smoked catfish samples. The samples were ground and serial dilutions (10^{-1} -10^{-4}) of the homogenized samples were made using sterile distilled water and plated out on seven (7) different culture media; nutrient agar (NA) was used to determine the total viable count (TVC) of bacteria, potato dextrose agar (PDA) and malt extract agar (MEA) were used to detect the presence of fungi and moulds, *Salmonella-Shigella* Agar (SSA) was used to detect the presence of Salmonella and Shigella, manitol salt agar was used to detect *Staphylococcus* colonies, lactose broth and eosine methylene blue (EMB) agar were used to detect the presence of coliforms, while thiosulphate citrate bile salt (TCBS) agar was used to detect the presence of *Vibrio*. The plates were incubated at room temperature (30 $^{\circ}$C) for fungal isolates and 35 $^{\circ}$C for bacteria isolates (Harrigan, 1998; Joanne *et al*., 2008).

Identification of Isolates. Pure bacterial isolates were identified through their microscopic and biochemical characteristics, while the pure fungi isolates were identified using their cultural/microscopic characteristics (Holt, 1994; Joanne *et al*., 2008).

RESULTS

The effect of two smoking techniques and storage conditions on the microbial quality of smoked catfish were examined during 12 weeks storage period under ambient, refrigerated and frozen storage conditions. The moisture content of the fresh sample was significantly reduced after smoking and there was also a significant reduction in the moisture content of improved kiln smoked catfish (IKSC) to that of traditionally smoked catfish (TSC) as presented in table 1. There was also a significant difference in the hydrogen ion (pH) of the fresh, TSC and the IKSC, the pH fluctuated during the storage period as shown in table 2.

The total viable count (TVC) of fresh fish sample was 5.71 log CFU/g and smoking was able to reduce the TVC to 3.18 log CFU/g and 3.93 log CFU/g for traditionally smoked samples and improved kiln smoked samples respectively as shown in table 3. The samples smoked traditionally with wood showed greater reduction in the TVC of 3.18 log CFU/g than samples smoked with improved kiln that use charcoal with TVC of 3.93 log CFU/g as shown in table 3. The total viable count increased during storage for the fish products smoked by the two techniques in all the storage conditions up to 8th week storage period as shown in table 3. It was also observed that the TVC of the smoked samples from the two techniques stored in the freezer had higher or equal to TVC of smoked samples stored in the refrigerator and ambient condition up to week 8 storage period. The refrigerated samples had TVC higher or equal to the TVC of samples stored at ambient condition up to 8th week storage period. The order of TVC for both smoking techniques were frozen $\geqslant$ refrigerated $\geqslant$ ambient up to 8th week storage period. It was also observed that at week 10 and 12 storage periods, smoked fish products from the two smoking techniques stored at ambient and refrigerated conditions respectively had TVC too numerous to count (TNTC) at dilution seven (table 3). Also, it was observed that after week 8 storage period, the smoked fish products stored in the frozen condition had reduction in TVC up to the end of the 12th week storage period.

There was no yeast and mould growth in the freshly smoked (week 0) fish products. The yeast and mould growth identified in the study were *Penicillium sp* and *Aspergillus niger*, their load ranged between 1.00 and 2.55 log CFU/g during the storage period and by the 10th and 12th week of storage, the ambient and refrigerated stored samples from the two smoking techniques had TNTC respectively as presented in table 3. The effects of smoking techniques and storage conditions on yeast and mould growth had no clear significance as presented in table 3.

Table 1: Changes in Moisture Content (%) of Smoked Catfish during Storage at Different Temperatures

Smoked fish	Storage method	Storage Period (weeks) 0 Mean ± SD	2 Mean ± SD	4 Mean ± SD	6 Mean ± SD	8 Mean ± SD	10 Mean ± SD	12 Mean ± SD
	Fresh	78.12±0.22^{c}						
TSC	Un-stored	15.84±0.32^{b}						
	Frozen		18.00±0.00	17.00±1.73^{c}	13.00±0.00^{a}	12.67±0.58^{a}	13.00±0.00^{a}	13.67±1.52^{b}
	Refrigerated		16.00±0.00	17.67±0.58^{c}	12.33±0.58^{a}	13.67±0.58^{abc}	14.00±1.00^{b}	
	Ambient		13.00±0.00	13.00±1.00^{a}	12.33±1.15^{a}	13.00±1.00^{ab}		
IKSC	Un-stored	12.70±0.31^{a}						
	Frozen		13.00±0.00	12.33±0.57^{a}	12.67±0.58^{a}	14.00±1.00^{abc}	13.50±0.50^{ab}	11.00±1.00^{a}
	Refrigerated		12.00±0.00	12.67±0.57^{a}	13.67±0.58^{a}	14.33±0.58^{bcd}	13.50±0.50^{ab}	
	Ambient		10.00±0.00	11.67±0.57^{a}	12.33±0.58^{a}	14.00±0.00^{abc}		

SD = Standard deviation, TSC = Traditionally smoked catfish, IKSC = Improved kiln smoked catfish.
$^{a\text{-}bcd}$ Different letters within column of storage methods and each storage period indicate a significant difference at $P<0.05$.

Table 2: Changes in Hydrogen Ion (pH) of Smoked Catfish during Storage at Different Temperatures

Smoked	Storage	Storage Period (weeks) 0	2	4	6	8	10	12
fish	method	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
	Fresh	6.82±0.00[c]						
TSC	Un-stored	6.33±0.02[b]						
	Frozen		6.42±0.02[f]	6.54±0.01[d]	6.36±0.01[e]	6.32±0.01[e]	6.48±0.02[d]	6.49±0.01[b]
	Refrigerated		6.29±0.03[d]	6.31±0.01[b]	6.29±0.01[d]	6.21±0.01[c]	6.44±0.01[b]	
	Ambient		6.25±0.01[c]	6.32±0.01[b]	6.24±0.01[c]	6.22±0.02[c]		
IKSC	Un-stored	6.29±0.01[a]						
	Frozen		6.34±0.03[e]	6.37±0.00[c]	6.34±0.03[e]	6.23±0.01[c]	6.39±0.00[a]	6.45±0.01[a]
	Refrigerated		6.26±0.02[c]	6.53±0.03[d]	6.29±0.01[d]	6.27±0.01[d]	6.39±0.01[a]	
	Ambient		6.13±0.00[a]	6.31±0.01[b]	6.25±0.01[c]	6.22±0.00[c]		

SD = Standard deviation, TSC = Traditionally smoked catfish, IKSC = Improved kiln smoked catfish.
[a-f] Different letters within column of storage methods and each storage period indicate a significant difference at $P<0.05$

Table 3: Total Viable Count and Yeast and Mould Count (logCFU/g) of Smoked Catfish Products during Storage at Different Conditions

Microorganisms	Storage period (weeks)	Smoking kilns /Storage conditions					
		Traditionally Smoked Catfish (TSC)			Improved Smoked Catfish (IKSC)		
		Ambient	Refrigerated	Frozen	Ambient	Refrigerated	Frozen
Total Viable Count	0	3.18	3.18	3.18	3.93	3.93	3.93
	2	4.40	4.78	4.90	4.80	5.14	5.18
	4	4.00	3.48	4.18	4.00	4.18	4.40
	6	4.30	4.30	5.30	5.02	5.30	5.41
	8	7.08	7.08	7.08	7.06	7.08	7.08
	10	TNTC	6.60	6.60	TNTC	6.60	6.78
	12	TNTC	TNTC	6.18	TNTC	TNTC	6.30
Yeast & Mould	0	ND	ND	ND	ND	ND	ND
	2	1.30	1.60	1.30	1.00	1.00	1.00
	4	1.9	1.90	1.70	1.40	1.40	1.48
	6	1.48	2.11	1.48	1.70	1.30	1.30
	8	1.48	2.18	1.70	2.04	1.30	1.60
	10	TNTC	2.55	2.19	TNTC	2.48	2.00
	12	TNTC	TNTC	2.40	TNTC	TNTC	2.20

TNTC = Too numerous to count, ND = Not detected
The maximum recommended bacterial count for good quality products is 5.7 log CFU/g, (ICMSF 2005).
The maximum recommended bacterial count for marginally acceptable quality products is 7 log CFU/g (ICMSF 2005)

DISCUSSION

There are so many ways pathogenic organisms can enter a food process and handling at every stage of production determines the quality of the food product. It can be introduced into foods during processing from the environment, air, water, unsanitary utensils, equipment, unclean hands and sewage. It can also be introduced through cross contamination between raw and cooked product (FDA, 2001). Moisture content is a determinant of the quality of dried food products and Clucas (1982) reported that dried fish of 15% moisture content or less could inhibit microbial growth. It was observed that the moisture content of the smoked catfish in this study fluctuated between 10.00 and 18.00% during the storage period. The moisture content of the smoked catfish fluctuated during storage as a result of differences in relative humidity and temperature of storage conditions.

The pH value is an indicator of the degree of freshness or spoilage of food. The pH of the fresh catfish in this study was quite neutral, dropped after smoking and fluctuated during storage. Nester *et al.,* (2007) reported that a high pH favours microbial growth and that most bacteria will grow best at neutral pH 7 although they can still tolerate ranges from pH 5 (acidic) to pH 8 (basic). The pH of the smoked catfish from both smoking techniques and the three storage conditions throughout the storage period fall in the range at which most spoilage bacteria will thrive.

The study found out that there was significant reduction in the TVC of the fresh samples to that of smoked fish products from the two smoking techniques. Antonia da Silva *et al*., (2008) and Salaudeen *et al*., (2010) also observed reduction in TVC of smoked catfish as a result of hot smoking. Udochukwu *et al.,* (2016) reported decrease in microbial loads of smoked fish as compared to fresh fish of the same species from open markets as a result of smoking effects. The temperature of smoking in the two smoking techniques used in this study was high enough to kill all the viable microorganisms in the freshly smoked products but presence of microorganisms in the freshly smoked (week 0) fish products might be due to post processing contamination during cooling and packaging (FDA, 2001). Greater reduction in TVC of the TSC to that of IKSC despite the higher moisture content of TSC might be due to the higher concentration of phenolic compounds and other smoke derivatives present in the wood smoke compared to that of charcoal which has been burnt off during the production of charcoal. Kester *et al*., (2013) also reported that phenolic fraction of wood smoke has inhibiting ability on bacteria. The highest TVC recorded in frozen stored samples, followed by the refrigerated stored samples and the least TVC recorded in the ambient stored samples could be attributed to multiplication of the microorganisms as a result of changes in environment and temperatures during the laboratory analysis.

The study recorded presence of Gram negative bacteria *Pseudomonas* sp. and *Aeromonas* sp. in the smoked catfish products from both smoking kilns during storage. *Pseudomonas* and *Aeromonas* are considered one of the most important fish pathogens (El-Sayyad *et al*., 2010). González-Rodríguez *et al*., (2002) identified *Pseudomonas* in vacuum packed cold smoked salmon and trout. It was observed that TVC increased during storage up to the 8th week storage period for smoked fish products from the two smoking techniques. Yanar (2007) also reported increase in TVC of hot smoked catfish during refrigerated storage with time. The increase in TVC might be due to fluctuation in moisture content and pH of the smoked catfish during storage as a result of changes in relative humidity and temperature. Increase in microbial load over time was also reported by Ikutegbe and Sikoki (2014). TVC is an important factor for quality determination of food products; the maximum recommended bacterial count for good quality products is 5.7 log CFU/g, and the maximum recommended bacterial count for marginally acceptable quality products is 7 log CFU/g (ICMSF 2005).

Penicillium sp. and *Aspergillus niger* have been identified as important food spoilage moulds. The presence of *Penicillium* sp. and *Aspergillus niger* could be as a result of handling during packaging and fluctuation in the moisture content during storage. Martin (1994) reported *Penicillium sp* and *Aspergillus niger* as the most common fungi associated with smoked fish. Christianah *et al.,* (2010) also attributed occurrence of *Penicillium sp*

and *Aspergillus niger* to poor processing, handling and reabsorption of moisture during storage.

CONCLUSION

The study showed a general decline in microbiological safety of smoked fish products from both smoking techniques and the three storage conditions with an observed increase in microbial load over time. The microbial safety and shelf life of stored smoked catfish depends on the smoking technique, storage condition and duration of storage. Therefore, to prevent and minimise health risks to consumers, smoked dried catfish should be hygienically handled, properly stored for short storage period.

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