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Research Article

Microbiological Analysis and Organoleptic Assessment of Smoked *Sarotherodon melanotheron* from Retail Market in Lagos, Nigeria

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ABSTRACT

The microbiological analysis and organoleptic assessment of exposed and cellophane wrapped smoked *Sarotherodon melanotheron* were investigated for 26 days. The changes in the sensory analysis of smoked *S. melanotheron* was observed to have decreased in quality as the day increases in both the exposed and cellophane wrapped samples. The microorganisms isolated and identified include the following species of fungi: *Penicillium spp.* (53.13%, 27.78%), S*accharomyces spp.* (15.63%, 16.67%), *Trichoderma spp.* (6.25%, 16.67%), *Fusarium spp.* (18.75%, 16.67%), *Aspergillus fumigatus* (3.13%, 11.11%) and *Mucor spp.* (3.13%, 11.11%). The species of bacteria: *Bacillus spp.* (12.5%, 31.82%), M*icrococcus spp.* (20.0%, 15.91%), *Staphylococcus aureus* (35.0%, 27.27%), S*taphylococcus saprophyticus* (30.0%, 22.73%) and *Pseudomonas aeruginosa* (2.5%, 2.27%) were found in the exposed and cellophane wrapped samples respectively. It could be concluded that the cellophane wrapped smoked fish were infested with more microbes compared to the exposed smoked fish samples. This may be as a result of heat that was absorbed by the cellophane wrapped samples which increase the smoked fish moisture that allowed the proliferation of the microbes. Therefore, there is a need to educate and advocate for good handling of smoked fish and proper hygiene practices.

Keywords: Cellophane, Deterioration, Microbial, Sanitation, Smoked fish
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INTRODUCTION

Fish is a highly nutritious protein source of food and it is particularly valued for its high quality compared to those of meat and egg (Ojutiku *et al.*, 2009). Its harvesting, handling, processing and distribution provide livelihood for millions of people. It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis, (Abolagba & Mella, 2008). Fish is also widely acceptable because of its high palatability, low cholesterol and tender flesh. To satisfy the consumer demand, it is necessary to produce good quality and safe smoked seafood products that stand the taste of time.

Fish is highly perishable because it provides favourable medium for the growth of microorganisms after death (Aliya *et al.*, 2012; Oparaku & Mgbenka, 2012). Microorganisms such as bacteria, moulds and yeast are known to be responsible for putrefaction and development of poor marketing appearance and toxic substances in fish sold to consumers. However, the role of bacterial flora has not been given full attention. The activities of microbial organisms can be reduced through fish processing. Hence, it has become increasingly important to ensure that the fish once caught is fully and efficiently utilised to avoid deterioration and wastage of resources. To prolong the shelf life of fish, it is preserved by many processes including sun drying, solar drying, canning and smoking among others. According to Eyo (2001) all preservative methods are geared towards making the condition in the fish uncomfortable for bacteria and reduction of chemical reactions in the fish.

In Nigeria, fish is eaten fresh, preserved or processes (smoked) and form a much cherished delicacy that cuts across socioeconomic, age, religious and educational barriers in Nigeria (Adedayo-Tayo *et al.*, 2008). Most of the fish are caught by the artisanal sector which is dominated by the fishery folks that do not have access to means of preserving their products apart from smoking. Dried fish is a major component

of harvested fisheries in many countries including Nigeria. About 25 to 30% of the world fish catch is consumed in the dried, salted smoked form or combination of these processes (Aliya *et al.,* 2012).

Smoking involves heat application to remove water and it inhibits bacterial and enzymatic actions of fish (Komolu-Johnson & Ndimele, 2001). It is a traditional method of processing fish around the globe, thereby extending the shelf-life of the smoked fish. The shelf-life of smoked fish product is usually extended primarily due to the reduced water activity. To ensure short time storage of dry fish that is safe from moulds and bacteria infestation, the moisture content must be less than 30% (Eyo, 2001). Clucas and Ward (1996), Horner (1997), Eyo (2001), Sengor *et al*. (2004), Olokor *et al.* (2007), and Abolagba and Melle (2008), noted that apart from giving the product a desirable taste and odour, smoking provides a longer shelf life through its anti-bacterial and oxidative effect, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting as antagonist to spoilage.

Smoked fish and shell fish products can be a source of microbial infection associated with *Listeria monocytogenes*, *Salmonella spp* and *Clostridium botulinum* (Heintz & Johnson, 1998). In addition, human infections may be caused by bacteria endogeneous to fish and bacterial pathogens, which may be transferred from fish to human include, *Clostridium botulinum* (botulism), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa*(wound infections), *Salmonella spp* (Food poisoning) and *Vibrio parahaemolyticus* (food poisoning) (Austin & Austin,1989).

Escherichia coli is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria as *Staphylococcus* spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish (Aberoumand, 2010). Scientists have shown that the contamination of food of fish origin with pathogenic *E. coli* probably occur during handling of fish and production process. The microorganisms associated with smoked fish pose a great threat to the populace as the transfer of the microorganisms attack the immune system of the consumer, usually man, thereby, giving room for the invasion of disease.

Ogbondemdimu *et al.* (1996) stated that modern agricultural practices are quite new in Nigeria. Therefore, basic information on the bacterial populations and types associated with cultured fish species are not available for the development of preventive measures to safe guard against infections agents which could cause disease and eventual, financial losses. Although, smoking of fish and the associated effect have been of interest to several researchers, yet no reference concerning the microbial load analysis of *Sarotherodon melanotheron* has been found in the literature. Thus, this study was designed to investigate the microbiological analysis and organoleptic assessment of smoked *S. melanotheron* collected from Lagos Lagoon.

MATERIALS AND METHODS

Collection of Samples

Forty freshly harvested tilapia fish (*Sarotherodon melanotheron*) were purchased from fish market at Ilaje in Bariga, Lagos, and then eviscerated, de-scaled and thoroughly washed. To determine the moisture content before smoking, the fish was weighed in a moisture dish of known weight using an electronic balance (Camry ISO 9001) and its weight recorded and then placed in the smoking kiln for six hours. The sample was removed from the kiln, and then left to cool, after which its dry weight was recorded. The moisture content percentage was determined using equation (1) described by Eyo (2001). After which the average moisture content was determined using equation (2).

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet sample} - \text{Weight of dried sample}}{\text{Weight of wet sample}} \times 100$$

…… equation (1)

$$\text{Average moisture content (\%)} = \frac{\text{Sum total of the body weight}}{\text{Total number of fish}}$$

…… equation (2)

Preparation of Serial Dilution

One gram of the fish sample for microbial evaluation was weighed into 7 ml of sterile water in the test tube and taken as the original stock. One millimeter of the original stock was transferred into 9 ml of sterile distilled water and mixed thoroughly to give 10^2 dilution of the original sample. Samples were labeled 10^{-1}, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8. The several dilutions were carried out using a sterilized micropipette from one test tube to the last test tube.

Media Preparation

Nutrient Agar, Potato Dextrose Agar, MacConkey Agar and Mannitol Salt Agar were prepared according to the manufacturers' instructions and sterilised using autoclave f or 15 minutes at 121^{o}C. It was removed and allowed to cool before it was poured into plates. After which one millimeter of the serial diluted samples of 10^{-8}, 10^{-4}, 10^{-3} and 10^{-3} dilution was inoculated on the surface of the well dried Nutrient Agar Potato Dextrose Agar, MacConkey Agar and Mannitol Salt Agar plates and gently swirled to completely spread. The inoculated plates were incubated at 37 ^{o}C for of 24 hours, while the Potato Dextrose Agar was incubated at room temperature (26.5 ^{o}C) for 5 days.

Bacterial and Fungal Colony Count

Bacterial and fungal colonies were counted using colony counter (Scan 300). The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was done for 1ml of original sample.

Biochemical Test

Each bacteria colony was identified by their Gram stain and biochemically characterised by their catalase, oxidase and sugar fermentation test as described by Cheesbrough (2000).

Organoleptic Test

The quality of the smoked fish was evaluated immediately after smoking on and these included appearance, flavour, texture, odour and overall acceptability using a six-point hedonic scale of unacceptable (1.0 -1.9), fair (2.0 - 2.9), medium (3.0 - 3.9), Good (4.0 - 4.9), very good (5.0 - 6.0) conducted by a 5 man panelist as described by Eyo (2001).

Statistical Analysis

The mean and standard deviation (Mean ± STD) for the obtained results were calculated using SPSS software (version 20.0).

RESULTS

Sensory Characteristics of Smoked *S. melanotheron*

Appearance

The mean appearance of the exposed smoked fish observed lowest value of 1.60 at day 26 and highest quality value of 4.80 at day 1, while the lowest value recorded for cellophane smoked fish was 1.20 and highest value of 5.00 at day 1. The analysis of variance (ANOVA) clearly indicated that there was a significant difference ($p<0.05$) in the appearance of the smoked fish from day 1 to 26 (Table1 and 2).

Flavour

The mean value of flavour of the exposed smoked fish observed lowest value of 1.40 at day 26 and highest quality value of 4.40 at day 1, while the lowest value recorded for cellophane smoked fish was 1.40 and highest value of 4..60 at day 1. The analysis of variance (ANOVA) clearly indicated that there was a significant difference ($p<0.05$) in the flavour of the smoked fish from day 1 to 26 (Table 1 and 2).

Texture

The mean value of texture of the exposed smoked fish observed lowest value of 1.80 at day 26 and highest quality value of 5.00 at day 1, while the lowest value recorded for cellophane smoked fish was 1.40 and highest value of 5.00 at day 1. The analysis of variance (ANOVA) clearly indicated that there was a significant difference ($p<0.05$) in the texture of the smoked fish from day 1 to 26 (Table 1 and 2).

Odour

The mean value of odour assessed in the exposed smoked fish observed lowest value of 1.20 at day 26 and highest quality value of 4.80 at day 1, while the lowest value recorded for cellophane smoked fish was 1.40 and highest value of 4.80 at day 1. The analysis of variance (ANOVA) clearly indicated that there was a significant difference ($p<0.05$) in the odour of the smoked fish from day 1 to 26 (Table 1 and 2).

Acceptability

The mean value of acceptability of the exposed smoked fish observed lowest value of 1.60 at day 26 and highest quality value of 4.80 at day 1, while the lowest value recorded for cellophane smoked fish was 1.20 and highest value of 5.00 at day 1. The analysis of variance (ANOVA) clearly indicated that there was a significant difference ($p<0.05$) in the acceptability of the smoked fish from day 1 to 26 (Table 1 and 2).

Comparison of the Organoleptic Characteristics of Exposed and Cellophane Wrapped Fish.

Appearance

It was observed that the cellophane packed fish had better appearance in day 1 and 11th day compared to the exposed fish from day 16 to day 26. The exposed fish had better appearance compared to the cellophane packed fish (Figure 1).

Table 1: Mean sensory scores of exposed smoked *Sarotherodon melanotheron*

Parameter	Days					
	1	6	11	16	21	26
Appearance	4.80±0.44^{b}	4.60±0.54^{b}	3.40±1.34^{ab}	3.00±1.41^{ab}	2.20±1.30^{a}	1.60±0.89^{a}
Flavour	4.40±1.34^{b}	4.20±1.30^{b}	3.80±1.30^{b}	3.20±1.64^{ab}	2.20±1.30^{ab}	1.40±0.54^{a}
Texture	5.00±0.00^{b}	4.60±0.54^{b}	3.60±1.51^{ab}	3.20±1.64^{ab}	2.60±1.34^{a}	1.80±0.83^{a}
Odour	4.80±0.44^{c}	4.60±0.54^{c}	3.40±1.34^{bc}	3.40±1.34^{bc}	2.60±0.89^{ab}	1.20±0.54^{a}
Acceptability	4.80±0.44^{b}	4.06±0.54^{b}	3.80±1.30^{b}	3.20±1.64^{ab}	3.20±1.30^{ab}	1.60±0.89^{a}

NB: These values are the 6-point Hedonic scale of 5 men panel response to each attribute. The Hedonic scales are: 1 = extremely poor; 2 = very poor; 3 = poor; 4 = fair; 5 = good; 6 = very good.
Values in the same row with same superscripts are not significantly different at 0.05 level of probability

Table 2: Mean sensory scores of cellophane smoked *Sarotherodon melanotheron*

Parameter	Days					
	1	6	11	16	21	26
Appearance	5.00±0.00^{d}	4.40±0.54^{cd}	3.60±0.89^{c}	2.40±0.89^{b}	1.80±0.83^{ab}	1.20±0.44^{a}
Flavor	4.60±0.54^{c}	4.20±0.44^{c}	3.40±1.34^{bc}	2.60±1.51^{ab}	1.80±0.83^{a}	1.40±0.54^{a}
Texture	5.00±0.00^{c}	4.60±0.54^{c}	3.60±1.14^{bc}	2.80±1.30^{ab}	2.40±1.14^{ab}	1.40±0.54^{a}
Odour	4.80±0.44^{c}	4.20±0.44^{c}	3.00±1.00^{b}	2.80±1.30^{b}	2.20±0.83^{ab}	1.40±0.54^{a}
Acceptability	5.00±0.00^{c}	4.40±0.54^{de}	3.40±0.89^{cd}	2.60±1.51^{bc}	1.80±0.83^{ab}	1.20±0.44^{a}

NB: These values are the6-point Hedonic scale of 5 men panel response to each attribute. The Hedonic scales are: 1 = extremely poor; 2 = Very poor; 3 = Poor; 4 = Fair; 5 = Good; 6 = Very good.
Values in the same row with same superscripts are not significantly different at 0.05 level of probability.

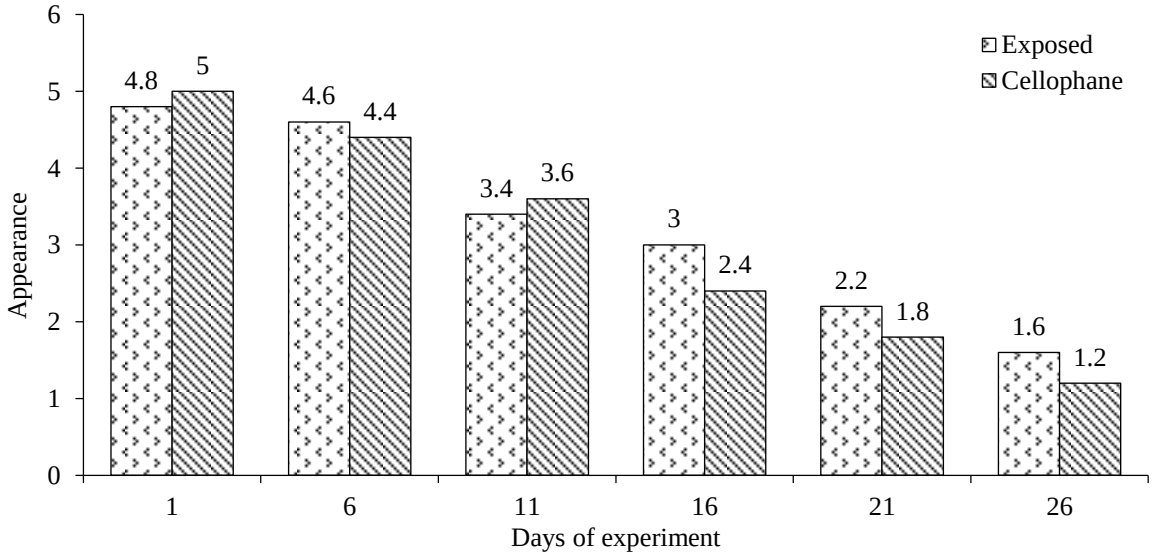

Figure 1: Comparison of the appearance in the exposed and cellophane packed samples

Flavour

It was observed that the exposed fish had better flavour in day 1 to day 21 compared to the cellophane packed fish from day 11 to day 26. The exposed fish had better flavour than the cellophane packed fish (Figure 2).

Texture

It was observed that the exposed fish had better texture in day 16 to day 26 compared to the cellophane sample from day to 16 today 26. The exposed sample had better texture than the cellophane packed fish (Figure 3)

Odour

It was observed that the exposed fish had better odour from 11^{th} day to 26^{th} day compared to the cellophane packed fish from the 11^{th} day to 26^{th} day. The exposed sample had better odour than the cellophane packed fish (Figure 4).

Acceptability

It was observed that the exposed fish was well accepted from the 6^{th} day to the 26^{th} day when compared to the cellophane packed fish from day 1. The exposed fish was well accepted compared to the cellophane sample (Figure 5).

Biochemical Characteristics of Microbes Isolated from *Sarotherodon melanotheron*

The biochemical characteristics of the bacterial isolated from smoked *Sarotherodon melanotheron* showed that the presence of catalase, Gram stain, oxidase, glucose, fructose, sucrose and lactose with positive as represented in Table 3.

Fungi Isolated from Smoked *S. melanotheron*

In the exposed fish *Penicillum* spp had the highest occurrence (53-13%) *Aspergillus fumigatus* and *Mucour* spp had the lowest percentage occurrence (3.13%). In the cellophone packed fish *Penicillium* spp had the highest percentage occurrence (27.78%) while *Aspergillus fumigates* and *Mucour* spp had the least frequency (11.11%). (Table 4)

Bacteria Isolated from Smoked *S. melanotheron*

In the exposed fish *Staphylococcus aureus* had the highest frequency occurrence (35.0%) while *Pseudomonas aeruginosa* had the least frequency (2.5%). The cellophane packed fish *Bacillus* spp had the highest occurrence (31.82) while *Pseudomonas aeruginosa* had the least (2.27%) frequency (Table 5).

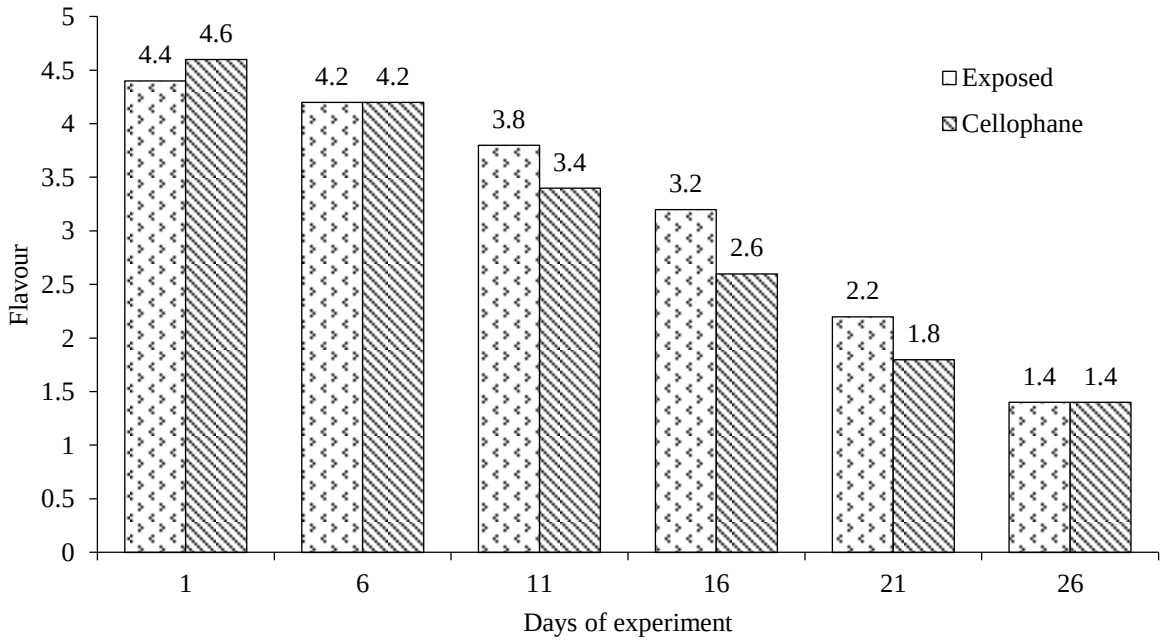

Figure 2: Comparison of the flavour in the exposed and cellophane packed samples

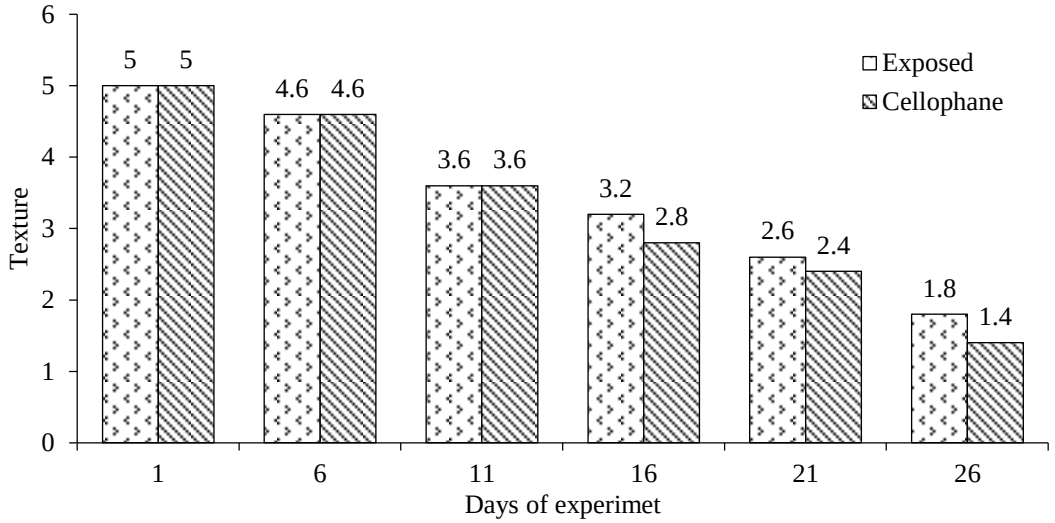

Figure 3: Comparison of the texture in the exposed and cellophane packed samples

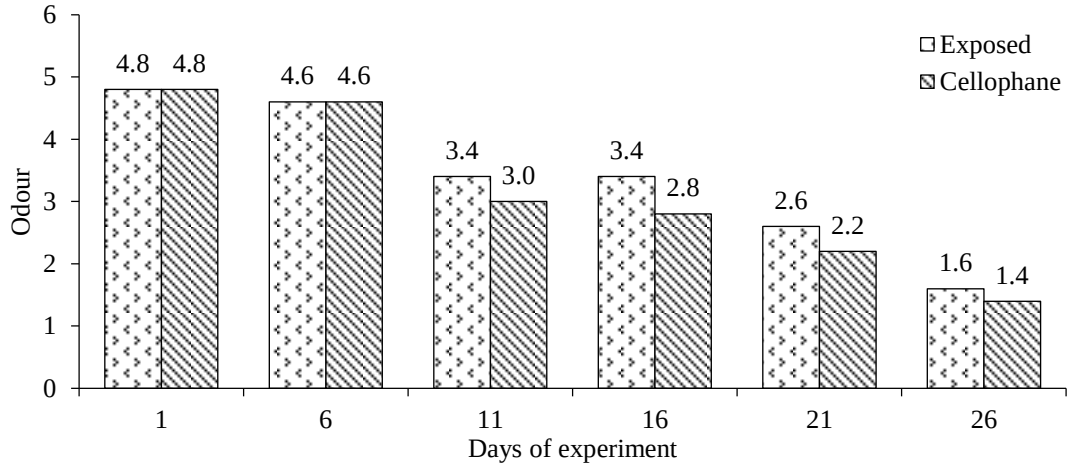

Figure 4: Comparison of the odour in the exposed and cellophane packed samples

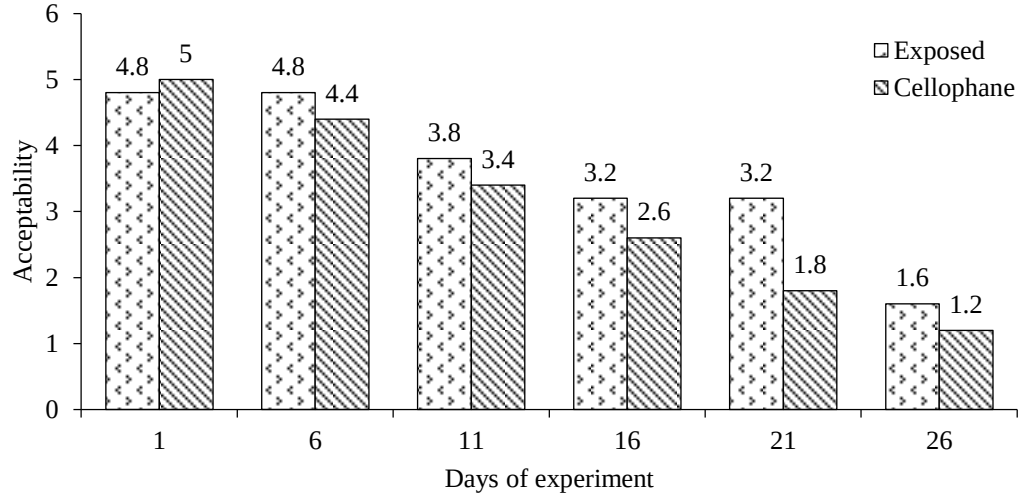

Figure 5: Comparison of the acceptability in the exposed and cellophane packed samples

Table 3: Biochemical characteristics of the bacterial isolates from smoked *Sarotherodon melanotheron*

General morphology	Gram stain	Catalase	Oxidase	Glucose	Fructose	Sucrose	Lactose	Suspected organism
Golden yellow slightly raised with smooth edges	+	+	-	A	AG	AG	AG	*Staphylococcusaureus*
Creamy deep yellow slightly raised with smooth edges	+	+	-	A	AG	AG	AG	*Micrococcus spp*
Creamy white raised with rough edges	+	+	-	AG	AG	A	A	*Bacillus spp*
Creamy slightly raised with smooth edges	+	+	+	AG	AG	AG	AG	*Staphylococcus saprophyticus*
Blue green slightly raised with smooth edges	-	+	+	A	-	-	-	*Pseudomonas aeruginosa*

Key: + (Positive); - (Negative); A (Acid); AG (Acid and gas)

Table 4: Frequency and percentage occurrence of fungi isolates of smoke *Sarotherodon melanotheron*

Fungi	Exposed		Cellophane	
	Frequency	% Occurrence	Frequency	% Occurrence
Penicillium spp	17	53.13	5	27.78
Saccharomyces spp	5	15.63	3	16.67
Trichoderma spp	2	6.25	3	16.67
Fusarium spp	6	18.75	3	16.67
Aspergillus fumigatus	1	3.13	2	11.11
Mucour spp	1	3.13	2	11.11
Total	32	100.00	18	100.00

Table 5: Frequency and percentage occurrence of bacterial isolates of smoked *Sarotherodon melanotheron*

Bacteria	Exposed		Cellophane	
	Frequency	% Occurrence	Frequency	% Occurrence
Bacillus spp	5	12.5	14	31.82
Micrococcus spp	8	20.0	7	15.91
Staphyllococcus aureus	14	35.0	12	27.27
Staphyllococcus saprophyticus	12	30.0	10	22.73
Pseudomonas aeruginosa	1	2.5	1	2.27
Total	40	100.00	44	100.00

DISCUSSION

In this study, there were marked variations between the means of viable bacteria counts. Result showed high coliform contamination in exposed smoked fish in comparison with cellophane storage. This is in line with the report of Nyarko *et al.* (2011) who reported the presence of coliform bacteria, yeast and moulds in smoked *Sardinella aurita* at smoking sites and market centres.

The organoleptic assessment observed a decline in the quality of the smoked fish in both exposed and cellophane with time as the day increased. The quality on the 6-point hedonic scale indicated that the exposed and cellophane ranged from fair to extremely poor quality of the smoked *Sarotherodon melanotheron*

from day 1 to day 26. The organoleptic assessment indicated that there was a significant difference ($p<0.05$) in the exposed and cellophane smoked fish.

This study indicated that the presence of bacteria and fungi on both exposed and cellophane packed smoked fish. This is in agreement with Maga, (1988), who considered smoking process, a mild preservative treatment, which kills bacteria and prevents microbial proliferation due to combined effects off heating, drying, pH and anti-microbial smoke components. Hence, as a mild treatment, smoking does not achieve complete elimination of microbial load of a fresh fish which has been proven to be naturally high due to the high microbial load of their habitat (water) (Frazier & Westhoff, 1995).

The exposed smoked fish samples observed higher fungi counts when compared with the cellophane packed samples. This may be due to the exposure and improper hygiene in handling during fish preparation resulting in high fungi contamination as the samples was observed to be kept in a rack, exposing it to contact of insects and dust particles. It is obvious that the exposed smoked *S. melanotheron* possess the possibilities of accumulating more contaminants than the cellophane packed one. This supports the observation of Eklund *et al.* (1993), which stated that any handling of fish and the associated sanitary practice from the point of harvesting can potentially contribute to the micro- flora on the final product.

Also, the isolation of *Staphylococcus aureus and Bacillus spp.* is an indication of poor handling or cross contamination of smoked fish products, since the two organisms have been indicted in food poisoning (Gupte, 2006). *Penicillium* spp., *Fusarium* spp., and *Aspergillus* spp. as identified in this work have all been incriminated in food spoilage.

Studies have also identified similar organisms from other fish species. For instance, Adelaja *et al.* (2013) isolated *Staphylococcus* sp. and *A. fumigatus* in smoked *Chrysichthys nigrodigitatus* at selected fish markets in Southwest Nigeria. Okareh and Erhahon (2015) reported that *Staphylococcus* spp have pathogenic strains which could cause food poisoning due to the heat stable Staphylococcal enterotoxin which is resistant to gastrointestinal enzymes. *S. aureus,* a normal flora of human skin and mucous membrane, is one of the most common causes of boils, impetigo and folliculitis and in some cases, bactereamia and infections of the bones and wounds (Herman *et al.,* 2011). The *A. fumigatus* in the exposed smoked fish was more than cellophane wrapped ones. This could be as a result of moisture generated from the cellophane in relation to the fish surface. The presence of *A. fumigatus* in the studied fish samples is of great health concern because of their mycotoxigenic potentials. Essien *et al.* (2005) reported that *A. flavus* and *A. fumigatus* produced aflatoxins, which destroyed the liver and kidney in man resulting to death. The presence of these organisms in the fish could be as a result of handling processes during smoking and cross contamination during storage, or during sales of smoked fish.

However, several techniques exist in the prevention of the growth of pathogenic micro-organisms during distribution and storage of processed fish. Huss *et al*, (2000), observed that the hazards related to contamination, recontamination or survival of biological hazards during processing could be controlled by applying good manufacturing practice and good hygiene practice. Smoking at adequately high temperatures is capable of controlling microbial contamination in fish, although, the heat supplied might not be sufficient enough to kill all the microbial contaminants (Eyo, 2001). A combination of smoking and treatments with antimicrobial agents and antioxidants have been found to retard microbial spoilage, extend shelf life, and enhance safety of smoked catfish (Adelaja *et al*., 2013).

The microbiological and organoleptic assessment of smoked *Sarotherodon melanotheron* fish samples are of public health importance. Proper storage of smoked fish is necessary because poor storage methods and unhygienic handling of the items are known to predispose dried fish to microbial contamination as this study unveiled. The cellophane smoked fish were infested with more microbes compared to the exposed smoked fish samples. This may be as a result of heat that was absorbed by the cellophane wrapped samples which increase the smoked fish moisture that allowed the proliferation of the microbes. The identified organisms are entirely preventable by practicing good sanitation and proper food handling techniques.

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