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Full Length Research Paper

Evaluation of the microbial load on smoked fish from some markets in Ogun state, Nigeria

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A study determining the microbial load on smoked fish from various markets in Ogun State, Nigeria, showed that Ota Market had the highest bacterial count of 4.2 × 10^4, followed by Oju-Ore with 3.8 × 10^4 and Igbesa and Lusada markets with 1.1 × 10^4 and 1.0 × 10^4 respectively. Fungal count followed a similar pattern but low in Lusada and absent from Igbesa samples. *Staphylococc*i predominated due to their ubiquitous nature but *Bacilli* of different types were the possible pathogens as well as a result of the handling environment. The smoked fish were found to be of low acidity; Igbesa samples being the most acidic. Incomplete combustion possibly affected the level of coloration due to accumulation of Polycyclic Aromatic Hydrocarbons (PAHs) in intensively heated products.

Key words: Smoked fish, microorganisms, acidity (pH), polycyclic aromatic hydrocarbons (PAHs), phenolics.

INTRODUCTION

In Nigeria, fish and fish products constitute more than 60% of the total protein intake in adults, especially in the rural areas, and they supply a good balance of protein, vitamins and minerals. Fish has a caloric content of relatively 10%, which enhances its value, as well as its role in the nation's nutrition (Akinneye et al., 2007).

The problem of fish in Nigeria however, is its high rate of perishability especially since the relative ease of preservation is not readily attainable, hence the difficulty in handling. Generally, the different preservation methods are: drying, smoking, freezing, chilling and brining but the common method in Nigeria is smoking (Akinola et al., 2006).

Smoking (the commonly adopted method of preserving fish in Nigeria), as an international source of foreign exchange is gradually losing ground (Al – Jafali and Opara, 2006); this is because exportation of smoked fish to developed countries is becoming increasingly stringent due to the emergence of food safety and agricultural health standard, along with the fact that buyers keep changing their requirements. The quality of processed food usually depends on the processing and handling techniques employed (Al-Jafaili and Opara, 2006). This is particularly true of smoked fish in view of the presence of endogenous organisms. Food poisoning organisms can multiply profusely in foods without initially altering the appearance, taste or odour.

A large number of people suffer from gastro-intestinal upsets annually, as a result of eating contaminated food which leads to a considerable loss of man-hour with accompanying consequences. Sometimes death occurs.

In many instances, outbreaks result from lack of understanding of food hygiene in the preparation, cooking and care of food. In order to avert such outbreaks, safety evaluation is necessary. Determination of pathogenic loads, by microbial tests for ascertaining risk level as well as potential sources in the smoked fish could be part of the palliatives.

This study therefore, samples the microbial loads on the smoked fish sold in Lusada, Igbesa, Ota and Oju-Ore open markets in Ogun State, Nigeria, and determines the acidity level of the fish in order to be able to meaningfully evaluate the contamination potential of the locally

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Table 1. Microbial load and acidity of smoked fish sample obtained from different markets in Ogun State, Nigeria.

Sample	Total Microbial Count(cfu/g)	Bacteria		Fungi	pH
		Bacillus Count (cfu/g)	Staphylococcus Count(cfu/g)	Yeast Count (cfu/g)	
A	3.8×10^4	1.1×10^4	0.1×10^4	2.6×10^4	6.43 ± 0.01
B	1.0×10^4	0.6×10^4	0.2×10^4	0.2×10^4	6.57 ± 0.05
C	4.2×10^4	1.2×10^4	2.0×10^4	1.0×10^4	6.31 ± 0.05
D	1.1×10^4	0.3×10^4	0.8×10^4	-	5.25 ± 0.01

- = No count; A = Oju-Ore, smoked fish; B = Lusada, smoked fish; C = Ota, smoked fish; D = Igbesa, smoked fish.

Table 2. Microorganisms isolated from smoked fish at different markets in Ogun State, Nigeria.

Organisms	MARKETS			
	Oju-Ore	Lusada	Ota	Igbesa
Bacillus spp.	+	+	+	+
Staphylococcus spp.	+	+	+	+
Fungi	+	+	+	-

+ = Present; - = Absent.

preserved openly sold fish in the markets.

MATERIALS AND METHODS

Several smoked fish were obtained from different markets within Ogun State of Nigeria. The markets were; Lusada, Igbesa, Ota and Oju-Ore. The fish were collected asceptically and analyzed the same day.

The samples were homogenized in a sterile mortar and pestle then 1 gram was weighed with a sterile aluminium foil, after which they were serially diluted into sterile test tubes containing 9mls of sterile water.

In each situation, 1 ml of the sample in the test tube was plated in sterile Petri dish into which the appropriate media (of malt agar or nutrient agar or mannitol saltagar) had been previously prepared into sterile McCartney bottles for stock cultures – for storing bacterial or fungal cultures. The petri dish was rocked for even distribution before inverse incubation (in the case of bacteria plates). Gram stain was done according to standard methods (Cowan and Steel, 1965). Bacteria and fungal counts were also done according to standard methods. Various biochemical tests-coagulase, oxidase, citrate, starch hydrolysis and carbohydrate utilization - were all carried out according to standard methods.

The pH (hydrogen ion concentration) of the suspension of 10 g of the smoked fish samples from different sources, already homogenized in a sterile mortar and pestle, added to 100 ml of sterile water, was done.

Spore staining techniques were used to determine endospores. Smears of 48 h old cultures of the isolates were heat fixed on different glass slides. They were flooded with malachite green stain and heated over a beaker of boiling water for 10 min. Most stain was continuously added to the slides to prevent drying. They were consequently washed and counterstained with safranin for 20 s, washed again and then air-dried after which it was observed under oil immersion. While the vegetative portion of the organisms stained pink (to red), the spores stained green. Motility test was carried out.

RESULTS

Microbial analysis

The Microbial load of smoked fish samples were as shown on Table 1, where the fungi was high at Ota (C), followed by Oju-Ore (A). It was low in Lusada (B). Bacteria were highest at Ota (C), followed by Oju-Ore (A) with staphylococci being predominant.

Table 1 also shows the average acidity levels (pH) of the fish samples from the various markets in Ogun State of Nigeria, with those from Igbesa (D) market being most acidic followed by Ota (C), Oju-Ore (A) and Lusada (B) respectively.

The types of microorganisms isolated from smoked fish were as shown on Table 2, where *Bacillus* spp. and *Staphylococcus* spp. were predominant in the samples from all the markets, and fungi were found in all except from Igbesa samples. The various types of *Bacilli* were confirmed biochemically as shown on Table 3.

DISCUSSION

The level of growth of microorganisms on the smoked fish depends on the amount of water which has been expelled from them (Oyewole et al., 2006).This implies that smoked fish sold in Ota market still had more water retained than those from other markets with Oju-Ore, Igbesa and, Lusada following in that order hence sequential possible higher rate of deterioration. However, Igbesa should be seen in the light that fungal count was

Table 3. Biochemical tests of Bacterial isolates from smoked fish obtained in some markets at Ogun State, Nigeria.

Test Organisms	Gram	Shape	Motility	Catalase	Oxidase	Spore stain	Citrate Utilization	Starch hydrolysis	Maltose	Glucose	Sucrose	Probable and identity
A1	+	R	-	+	-	+	-	+	-	-	-	*Bacillus alvei*
A2	+	R	-	-	-	+	+	+	+	-	G	*Bacillus* spp.
B1	+	R	-	+	-	+	-	+	-	+	G	*Bacillus brevis*
B2	+	R	+	+	-	+	+	+	A	-	-	*Bacillus laterosporous*
C1	+	R	+	-	-	+	+	+	-	+	-	*Bacillus* spp.
D1	+	R	+	-	-	+	-	-	-	-	G	*Bacillus sphaericus*
D2	+	R	-	+	-	+	-	+	-	-	-	*Bacillus alvei*

R = Rod; - = Negative; + = Positive; A = Acid formation; G = Gas formation.

not included; meaning that the smoked fish there, were more properly processed, especially because of the relatively low count of bacteria.

The pH from the fish samples shows that smoked fish is a low-acid food, and also explains why fish is a perishable food knowing very well that bacteria can strive in low acid food, despite the fact that smoked fish especially with lower Aw inhibit growth of most bacteria.

A palliative to contamination by microorganisms is an organized Food Inspectorate for monitoring the preservation processes.

The type of material used for smoking usually, also, would affect the texture, aroma, and other organoleptic qualities like taste etc. (Bolaji, 2005).

This was not however, considered in this study. Rice bran and sawdust have low flame temperature; hence the quantity of heat and the maximum attainable thermal efficiency is usually very low compared with what is obtainable with charcoal and fire-wood. Very importantly, it is the heat from the fire that dries the fish; reducing the moisture content to a level that will prevent growth of microorganisms (Oyewole et at., 2006). Rice bran and sawdust may not generate enough heat to expel the moisture from the fish.

Moreover, smoke contains tar-based phenolic compounds that inhibit microbial growth (Oyewole et al., 2006). Smoke phenolics also have antioxidant properties that can delay the development of rancidity in oily fish. The level of the phenolics can therefore also be a factor in the quality of the smoked fish from the various markets of this study. During smoking, Polycyclic Aromatic Hydrocarbons (PAHs) are formed as a result of incomplete combustion of organic materials. High levels of PAHs are associated with the dark colorations in intensively heated products. Modern smoking ovens allow for control of these conditions by controlling the amount of smoke present in the oven (Karl and Leinemann, 1996).

In a study on smoked foods in Nigeria (Ogbadu and Ogbadu, 1989), benzo(a) pyrene (an indicator for PAHs in food analysis) levels in all samples (n = 9) ranged from 8.9 to 34.8 µg/kg, which by far exceeded the threshold of 1 µg/kg for products in general, set by The Dutch Inspectorate for Health in 2003.

Conclusions

Microorganisms counted on the smoked fish varied from market to market in Ogun State of Nigeria, with Bacilli being the major pathogens and the ubiquitous staphylococci predominating.

The smoked fish were of low acid level and the relative growth of microorganism on them was because of the favourable surface environment of the fish. An organized monitoring Agency could help improve the method and mode of smoking.

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