Analytical Methods

Microbiological and biochemical spoilage of smoke-dried fishes sold in West African open markets

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ABSTRACT

Proximate composition and microbiological characteristics of pre-dried *Chrysichthys nigrodigitatus* and *Pseudotolithus typus* were studied over a period of 4 weeks to determine the health risks associated with delayed consumption. All analyses were conducted using standard microbiological and chemical methods. Results showed a general decline in microbiological safety and nutritive characteristics of both fish species over time, with an observed increase in microbial loads over time. *Aspergillus flavus* was also present on both species which makes consumption of the fishes hazardous to the health of consumers due to its ability to produce carcinogenic aflatoxins. In order to minimise the health risks to consumers, it is recommended that smoke-dried fishes be consumed with minimal delay and cooked properly before consumption. The findings of this study will prove important in the development of more stringent regulations regarding food safety in Nigeria.

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1. Introduction

Fish is an important dietary component of people all around the world and represents a relatively cheap and accessible source of high quality protein for poorer households. In West Africa, fish has been reported to provide 40–70% of the protein intake of the population (Béné & Heck, 2005) and is a critical source of dietary protein that is not readily available in the carbohydrate-based staple foods of the population.

Depending on consumer preference, there are several forms in which fish can be consumed; fresh, dried, frozen, fermented, brined etc. In a study by Mafimisebi (2012), it was discovered that majority of the Nigerian people reported a preference for fresh fish; however limitations such as the low keeping quality of the fish after harvest and the distances between fishing grounds and marketing outlets make this very difficult. This results in a higher reported consumption of smoke-dried fish, which has a longer shelf-life (Mafimisebi, 2012).

Methods such as smoking and/or drying, frying, salting, freezing and fermentation are the most commonly employed fish preservation methods used in West Africa. In Nigeria, particularly, where electricity supply is unreliable at best, about 70–80% of domestic fish catch is smoke-dried, as this is the most affordable and practicable method of fish preservation (Akinyemi, Adejola,

Obasa, & Ezeri, 2012). In addition to a longer shelf-life, smoke-dried fish gives a desirable taste and remains whole in soups and stews, making it more appealing to consumers than fresh fish which disintegrates when cooked (Mafimisebi, 2012).

Although the smoke-drying process may extend the shelf-life of fish by reducing the moisture content which aids microbial spoilage, the final product is often sold in open markets where the fishes are displayed uncovered atop tables in the market place. This exposes the products to contamination by unsanitary handling (by both sellers and buyers), dust and insects such as flies and beetles.

Retailers of smoke-dried fish in Nigerian markets occasionally re-smoke their leftover products in order to extend the shelf-life even further as this is reported to reduce the incidence of spoilage microorganisms (Plahar, Nerquaye-Tetteh, & Annan, 1999). These re-dried products are then mixed up with freshly-procured dried fish and displayed for sale again, with no regard for differing storage times. The heat and smoke associated with the smoke-drying process has also been reported to cause the denaturation of the protein content of fish (Morris, Barnett, & Burrows, 2004), thereby reducing its nutritional value.

The fact that old and newly-smoked fishes are mixed and sold together in the open markets in Nigeria, with no regard for differing storage periods, raises many concerns about the safety and quality of the smoke-dried products offered for sale in the open markets. The importance of these concerns cannot be over-emphasised as most dried fish are bought in larger quantities than will

immediately be consumed, leading to an even longer storage period in the homes of consumers prior to consumption.

Changes in the proximate composition of fish have been extensively reported to provide an indication of the nutritional quality and extent of spoilage in dried fish (Abolagba & Melle, 2008; Chukwu & Shaba, 2009; Dainty, 1996; Daramola, Fasakin, & Adeparusi, 2007; Fraser & Sumar, 1998; Gram & Huss, 1996; Huis in't Veld, 1996; Morris et al., 2004; Puwastien et al., 1999; Vishwanath, Lilabati, & Bijen, 1998; Yola & Timothy, 2013). A decrease in pH, for example, could be due to the bacterial fermentation of carbohydrate to lactic acid, which enables the pH-sensitive spoilage bacterium *Shewanella putrefuciens* to grow (Gram & Huss, 1996). The reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA) in a pH-dependent reaction is also universally accepted as an indicator of fish spoilage (Fraser & Sumar, 1998).

A number of studies (Adebayo-Tayo, Onilude, & Patrick, 2008; Akinyemi et al., 2012; Daramola et al., 2007; Fafioye, Efuntoye, & Osho, 2002; Fafioye, Fagbohun, & Olubanjo, 2008; Hood, Ness, Rodrick, & Blake, 1983; Kumolu-Johnson, Aladetohun, & Ndimele, 2010) have also been carried out to determine the shelf-life and microbiological characteristics of smoke-dried fish. These studies have been carried out under controlled conditions and beginning from the smoke-drying process itself. The reality, however, is that the people who buy these smoke-dried products from the open markets do not know when these fishes were smoke-dried or even how long they had been in storage before being sold to them. The present study therefore adds to existing literature on the keeping qualities of smoke-dried fish by examining smoke-dried fish purchased from open markets, thereby assuming the position of every-day consumers.

Although physical examination is the method that is readily available to an intending fish buyer, the microbiological assessment of any processed food product provides information which serves as the most important criterion for judging the success of the processing method used as well as the microbiological stability and safety of the food (Plahar et al., 1999). Proximate analysis is also an important tool that can be used to assure consumers of the nutritional quality of the smoke-dried products. The aim of this study, therefore, is to assess the proximate composition and microbiological characteristics of smoke-dried fish species sold in open markets in Nigeria; by taking a consumer's stand-point. This will aid the determination of the health risks associated with delayed consumption of smoke-dried fish as well as the development of more effective food safety regulations.

2. Material and methods

Two fish species, *Chrysichthys nigrodigitatus* (Silver catfish) and *Pseudotolithus typus* (Long-neck croaker) were chosen for this study based on consumer popularity (Ndimele, Kumolu-Johnson, & Anetekhai, 2011; Williams, 2013). Fifteen smoke-dried samples of each fish species were purchased from Choba and Rumuokoro markets in Rivers state, Nigeria. The fishes had been in storage for indeterminate periods of time prior to their procurement for the investigations.

Five samples of each species were obtained from three different sellers and then mixed together to make composite samples of fifteen. The samples were hand-picked with sterilized gloved hands and taken to the laboratory in separate sterilized polythene bags to avoid contamination from handling. On commencement of the study, the initial microbial and proximate compositions of the samples were analysed and recorded (Week 0), representing the biochemical composition and microbiological characteristics of samples at the time of purchase. Thereafter, the samples were stored under ambient temperature (27–29 °C) and humidity (65–70%) pending further weekly analyses over a period of 4 weeks.

2.1. Microbiological analysis

All growth media and glassware (petri dishes, beakers, test tubes etc.) used in this study were sterilized by autoclaving at 121 °C in an electrically-operated autoclave. Ten-fold serial dilutions of the fish samples were carried out until 10^{-6} dilution was obtained. From each dilution, 0.1 ml was spread onto the agar plates in triplicate, using a glass spreader. The plates were then incubated at 37 °C for 24 h and a mean value of counts (cfu/g) was obtained after incubation. The identification of micro-organisms was based on characteristics described in Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974) as well as biochemical tests such as motility, indole, catalase, oxidase etc. The media used in the bacteriological analysis were non-selective Nutrient agar (NA), MacConkey agar (MAC), Salmonella Shigella agar (SSA), Manitol Salt agar (MSA) and Thiosulphate Citrate Bile Salt agar (TCBS). Potato Dextrose agar (PDA) was used in the culture, isolation and identification of fungal colonies present on the fish samples.

Gram staining was used to provide a broad classification of bacteria either as gram-positive or gram-negative. A smear was made for 18-hour old isolates on clean, dry and grease-free slides. The smear was then fixed by passing through an open flame and flooded with 0.5% crystal violet for 60 s then rinsed off with water. A mordant Gram's iodine solution was applied and left for another 60 s before it was drained off and decolourized by holding the slide in a slant position and applying absolute alcohol. It was then rinsed off with water and counterstained with carbon fucchin for 30 s and rinsed off again. Finally, the stained smear was air dried and examined under a microscope.

2.2. Proximate analysis

The proximate compositions (moisture, ash, lipid, protein, carbohydrate and fibre) of the samples were determined using descriptions by the Association of Official Analytical Chemists (William, 1980). Moisture content was determined by drying the samples in an oven at 105 °C until a constant weight was obtained. The total lipid content was obtained by the incorporation of petroleum ether into the Soxhlet system. Nitrogen (N) and crude protein contents (N × 6.25) were determined using the Kjeldahl digestion method and the Clegg and Anthrone method was used to determine the carbohydrate content, using a dilution factor of 25. Finally, the fibre content was calculated by obtaining the difference between 100% and the summation of the percentage content of protein, carbohydrate, moisture, ash and lipid as follows:

$$\text{Fibre}\ (\%) = 100 - (\%\text{Protein} + \%\text{Ash} + \%\text{Moisture} + \%\text{Lipid} + \%\text{Carbohydrate}).$$

2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA) which was followed by the Least Significant Difference (LSD) post hoc test (defined at $p < 0.05$) in order to determine which parameters significantly differed between fish species.

3. Results

3.1. Microbiological analysis

The results of microbiological analysis carried out on the smoke-dried fish species, over a period of 4 weeks are presented in Tables 1 and 2.