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STUDIES ON THE TRADITIONAL STORAGE OF SMOKED ANCHOVIES IN GHANA

PROGRESS REPORT #1C

MICROBIOLOGICAL AND MYCOTOXICOLOGICAL QUALITY OF FRESHLY SMOKED ANCHOVIES (*Anchoa guineensis*) FOR STORAGE AT TEMA MANHEAN

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MICROBIOLOGICAL AND MICOTOXICOLOGICAL QUALITY OF FRESHLY SMOKED ANCHOVIES (Anchoa guineensis) FOR STORAGE AT TEMA MANHEAN

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ABSTRACT

Whole and edible portions of freshly smoked anchovies (Anchoa guineensis) were milled and analysed for total viable counts, mould and yeast counts, coliforms, pathogenic microorganisms as well as aflatoxin content. The edible portions of the fish samples had less bacterial load than the whole fish, but the mould count was approximately the same for both samples. In general, however, the microbial loads for the smoked fish were low, ranging between 760 and 850 bacterial organisms per gram of fish and between 320 and 450 moulds per gram. Microorganisms isolated were Rhizopus, Aspergillus spp., Micrococci, Bacillus sp. and Yeasts. Predominant Aspergillus spp. identified include Aspergillus niger. Coliforms as well as faecal coli and pathogenic microorganisms were absent from from from both whole fish and the edible portions of the smoked anchovies; a good indication of hygienic processing conditions. Both fish samples were also negative for aflatoxins B_1, B_2, G_1 and G_2

TABLE OF CONTENT

INTRODUCTION

The most significant pelagic species of fish landed by Ghanaian canoe fisheries are the sardinellas (Sardinella aurita and Sardinella eba) and the anchovies (Anchoa guineensis). Remarkable increases in anchovy landings in recent years are indicative of its increasing economic and nutritional significance in Ghana and neighbouring West African countries.

Among the various traditional processing methods employed in Ghana to preserve fish, smoking and sun-drying are the most widely used techniques for anchovies. However, smoking is conducted without proper hygienic conditions, the result being that products become susceptible to bacteria and fungi spoilage as well as insect infestation. Although no statistics are available on storage losses of dried smoked anchovies in Ghana, reports have indicated post-processing losses of unprotected dry fish as high as 20 - 70 % (Kagan, 1970; James, 1976; Osuji, 1976; Waterman, 1976; Plahar, et al., 1990). Microbial spoilage in storage depends largely on the initial microbial quality of the freshly smoked fish. In samples of fish freshly smoked to about 70°C, Okafor and Nzeako (1985) isolated Micrococcus was the main spoilage organism, while Lactobacillus, Acinetobacter and Micrococcus were isolated in samples that were smoke treated at 40°C.

The processing method therefore can have a profound effect on the types and numbers of microorganisms. Drying of fish before smoking aids in "pellicle" formation (a glossy firm surface) which

gives the desired appearance and allows for the development and absorption of the delicate smoke flavour and preservatives for a resultant good and relatively stable product. On the other hand, soft wet fish when smoked is more susceptible to microbial spoilage.

The need to protect smoked anchovies from excessive microbial infection can also be considered in the light of increased awareness of the hazards of mycotoxins in stored foods. Mycotoxins can be produced by certain strains of a number of species of fungi when grown under favourable conditions on a wide variety of different substrates. The most important and toxic mycotoxins are the aflatoxins which are products of the mould Aspergillus flavus and Aspergillus parasiticus. Aflatoxins have been detected in several commodities including smoked, dried and salted fish from South East Asia. In a survey in the Philippines, 93% of 15 samples of smoked fish were found to contain aflatoxins. A similar survey also showed 83% of 24 samples of dried fish to be positive for aflatoxins (FAO, 1979).

With the fast growing smoked anchovy industry in Ghana and its socio-economic and nutritional significance, it was considered necessary to study the traditional storage techniques for possible improvements. A project under the Ghana-Netherlands Artisanal Fish Processing Programme was initiated to evaluate a traditional anchovy storage method used at Tema Manhean. This report provides information on the microbiological and mycotoxicological quality of the freshly smoked anchovies prepared for storage.

2. MATERIALS AND METHODS

2.1. Sampling and sample Preparation

Five samples of freshly smoked anchovies were ramdomly taken from each of forty large baskets filled with smoked anchovies prepared for storage. The samples were bulked together and mixed thoroughly. The bulk was divided into two batches and one batch was milled whole in a laboratory hammer mill. The other batch was treated to obtain the edible portion by removing the scales, the head and the tail. This was also ground as before and the milled samples were kept in separate sterile polyethylene bags for microbiological and mycotoxicological analysis.

2.2. Total viable counts (Pour plate technique)

The sterile bag containing whole fish powder was opened near a bunsen burner flame and 10g of the sample was aseptically removed into a sterile sample bottle. A 90 ml portion of quarter strength Ringers solution was added and mixed thoroughly by shaking several times. The suspension was allowed to stand for 5 min. to soak well. The mixture was again shaken vigorously and 1 ml portion was pipetted and used to prepare 10^{-1} to 10^{-6} serial dilutions. One milliliter of each serial dilution was then pipetted into sterile plates in duplicate. Each plate was overlaid with about 20 ml of Plate Count Agar cooled to 45°C. Thorough mixing was ensured by clockwise and anti-clockwise rotation of the plates. The plates were

allowed to stand to solidify after which they were incubated at 30°C for 72 h. The edible portion of the smoked anchovy was treated in the same way to obtain the total viable counts (Harrigan and McCance, 1966).

2.3. Mould and Yeast Counts

For the enumeration of yeast and mould, a low acid medium was used. This medium was prepared by sterilizing 250 ml of Potato Dextrose Agar (PDA) and adding 7.5 ml of sterilized acid (i.e. 1.5 ml acid to 50 ml of PDA). Employing the Pour Plate technique, 1.0 ml of the 10^{-1} dilution of smoked fish suspension was pipetted into duplicate sterile petri dishes. This was overlaid with acidified PDA and carefully rotated in a clockwise and anti-clockwise direction for thorough mixing. The plates were then incubated at 30oC for 24 h.

2.4. Enumeration of Enterobacteriaceae (Coliforms)

MacConkey broth with glass vials in test tubes were prepared and sterilized. One milliliter of 10^{-1} and 10^{-2} dilutions of fish suspension were pipetted into 10 ml duplicate broths. These were incubated for 72 h at 37°C. Incubated samples were then identified for acid and gas production. For direct plating out, streaks were made on MacConkey agar plates using the stock fish solution prepared from each of the samples. The plates were then incubated at 37°C for 48 h.

2.5. Pathogenic Organisms

2.5.1. Staphylococcus sp.

A 5 g sample of smoked fish powder was aseptically weighed and placed in cooked meat medium with 10% salt added. It was mixed thoroughly and incubated for 12 - 18 h at 37°C. The sample was then subcultured onto Mannitol salt agar and incubated for 72 h at 37°C for pure culture isolation and identification.

2.5.2. Salmonella sp.

Twenty-five gram sample of smoked fish powder was weighed and placed in 100 ml Selenite enrichment broth and mixed well by shaking. The broth was then incubated for 12 - 18 h at 37°C. This was subsequently subcultured onto Bismuth Sulphite agar and the plates incubated for 72 h at 37°C.

2.6. Culture Identification

Smears of growth from the plates were made on clean slides with sterile loop. These were Gram stained and viewed under the microscope to identify the morphology and Gram reaction.

2.7 Hydrogen Ion Concentration (pH)

pH of the samples were determined with a Metrohm 620 pH meter (Swiss-made). Approximately 10 g of fish powder was weighed into 200 ml beakers and 90 ml of carbon dioxide free distilled water was added and thoroughly mixed. The mixture was left to stand for 5 min.

before pH measurements were taken. The pH meter was calibrated prior to sample measurements using a standard buffer solution of pH 7.0.

2.8. Extraction and Estimation of Aflatoxins

The method of extraction was based on that of Romer (1975). Ground samples were extracted with 250 ml acetone : water (85 : 15 v/v). The extract was filtered through Whatman #1 filter paper. Clean-up of filtrate was carried out using cupric carbonate and ferric gel (170 ml sodium hydroxide + 30 ml ferric chloride). After a second filtration the first 250 ml of filtrate were collected and aflatoxins extracted into chloroform (2 x 10 ml). The chloroform layer was run off into 100 ml potassium hydroxide wash solution in a separating funnel which was gently swirled for 15 seconds and the layers allowed to separate. The chloroform layer was run through a bed of anhydrous sodium sulphate, then evaporated to dryness. The residue was picked up in 200 ul chloroform.

Thin layer chromatography was carried out on silica gel 60 aluminium-backed TLC plates (Merck No 5553, BDH Ltd., Dorset, U.K.). Bi-directional development first in diethyl ether to remove interferences followed by chloroform : acetone (9 : 1 v/v) was carried out.

Visual comparison of the intensity of the fluorescence under ultraviolet light using a Chromato-vue Ultra violet light cabinet fitted with a UVL 56 Blakray lamp (Ultra Violet Products Ltd., Cambridge, U.K.) of sample aliquots and aflatoxin standards (Sigma Chemical Co. Ltd., U.S.A.) was carried out. All chemicals and

reagents used were of the AnalaR grade (British Drug House, BDH Chemicals Ltd., Poole, U.K.).

3. RESULTS AND DISCUSSION

3.1. Microbiological Quality of Smoked Anchovies

Table 1 shows the results of microbiological analysis of whole and edible portions of smoked anchovies (Anchoa guineensis) sampled before storage at Tema Manhean. Microbial examination of any processed food product provides information which serves as the most important criterion for judging the success of the process used, the effectiveness of the production controls as well as the microbiological stability and safety of the food. In this study, both the whole and edible portions of the freshly smoked anchovies had very low and acceptable bacterial and fungi loads.

The whole fish samples had slightly higher bacterial loads (85 x 10^1 per gram) than the edible portions (76 x 10^1 per gram). This difference may be attributed to the fact that the skin and head portions of the fish that were removed and discarded from the edible portion had excess bacteria as compared to the flesh. On the other hand, the absence of significantly higher mould counts in the whole fish samples as compared to the edible portions may be indicative of the fact that much of the mould growth was on the inner core of the fish and not on the skin. Removal of the skin did not therefore reduce the mould counts on the edible portion to any significant extent. In addition, the smoke treatment might have destroyed most of the surface skin contaminating moulds during processing.

Microorganisms isolated from both whole and edible samples

Table 1. Microbiological quality of whole and edible portions of freshly smoked anchovies (Anchoa guineensis)

Test	Whole fish	Edible portion
Physical appearance	Dark-brown meal	Light-brown meal
Viable organisms		
Aerobic bacterial count per gram	85×10^1	76×10^1
Mould count per gram	32×10^1	46×10^1
pH	6.5	6.4
Culture	Rhizopus, Aspergillus sp. Micrococci Bacillus sp. Yeast	Micrococci Bacillus sp. Aspergillus sp. Rhizopus Yeast
Coliforms (in 0.1 g)	Absent	Absent
Faecal coli	Absent	Absent
Pathogens	Absent	Absent

were Rhizopus, Aspergillus sp., Micrococci, Bacillus sp. and Yeasts. The most predominant Aspergillus spp. identified include Aspergillus niger. Plahar et al.(1990) isolated similar organisms in freshly smoked herring (Sardinella eba). Coliforms as well as faecal coli and pathogenic microorganisms were absent from both whole fish and edible portions of the smoked anchovies. The absence of Escherichia coli (coliforms) in the freshly smoked anchovy samples shows that there was no faecal contamination of the fish. Coliforms, other than E. coli are a good indicator of unsatisfactory processing or sanitation. The absence therefore of other coliform organisms shows that proper and hygienic procedures were used during the drying and smoking of the anchovies.

3.2. Aflatoxin content of freshly smoked anchovies

Both fish samples were negative for aflatoxins B_1, B_2, G_1 and G_2. Aflatoxins are toxic mycotoxins produced by the moulds Aspergillus flavus and Aspergillus parasiticus under favourable conditions of temperature and moisture, especially during storage. They have been detected in various processed fish samples (FAO, 1979), but nothing has been reported of aflatoxins in freshly smoked fish. Aflatoxin contamination of foods is mainly a storage problem and this usually occurs when foods are stored under conditions that are conducive to fungal growth.

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