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acta SATECH 3(2): 64 - 68 (2010)

Mycological evaluation of smoked fish (*Ethmalosa fimbriata*) from retail outlets in Ago-Iwoye, Ogun state, Nigeria

+* Christianah I. Ayolabi and **Fagade, Ezekiel O.

**Department of Botany and Microbiology University of Lagos, Akoka, Lagos, Nigeria*

***Department of Botany and Microbiology University of Ibadan, Nigeria*

+ Corresponding author: <ciayolabi@yahoo.co.uk>

ABSTRACT

The mycological and some biochemical quality of smoked fish (Ethmalosa fimbriata) obtained from Oja-Oba market, Ago-Iwoye, Ogun State of Nigeria was studied. Mycological examination of fish samples was carried out on freshly prepared Czapekdox agar and potato dextrose agar. Samples were collected bimonthly for six months. Results indicate the prevalence of fungal genera Aspergillus, Rhizopus and Penicillium in the samples tested. The genus Aspergillus consists of A. niger, A. flavus and A. ochraceous. The optimum growth temperature was found to be 28^{o}C for all the fungal isolates except A. niger and Rhizopus sp which grew optimally at 35^{o}C. All the isolates were found to degrade the proteins of the fish with A. ochraceous having the highest rate of degradation followed by A. niger and A. flavus. The results indicate the need to improve the post-harvest storage condition of these samples to ensure high quality product on preservation.

Keywords: *Fish, fungi, protein, degradation*

INTRODUCTION

Fish farming is a common occupation of people living in the coastal areas and along major river banks in Nigeria. Fish has a high source of protein. The flesh is consumed when prepared as one form of delicacy or the other while various brands of oils are also extracted from the flesh and the fluids of the fish (Eyo, 1993). The flesh and body fluid of newly caught healthy fish is generally considered sterile (Shewan, 1991), however it was reported the presence of bacteria in muscles of fish (Bisset, 1948). More studies revealed that the microbial flora of caught fish and other aquatic specimens is largely a reflection of the microbial quality of the water where they are harvested (Pelzar et al., 1986). In most cases, fresh fish flora are mainly bacteria, fungi are absolutely absent in fresh fish except a few yeast which have been shown to play no role in spoilage (Wood, 1940). The natural microflora of fish that play a predominant role in spoilage include the genera *Pseudomonas, Vibrio, Micrococcus Achromobacter, Corynebacterium, Flavobacterium* amongst others (Horsely, 1973).

Fish is a highly perishable product due to its high susceptibility to autolysis, oxidation and hydrolysis of fats and microbial spoilage (Frazier and Westhoff, 1978). Care is therefore required in handling as well as its preservation for food. The post harvest methods of preserving fish include refrigeration (4^{0}C) and freezing which is effective when such fish is conditioned to a temperature of -10^{o}C. Other methods frequently employed include sun drying and smoke drying all associated with increased germicidal action with increasing temperature (Frazier and Westhoff, 1978).

Since most Nigerians utilize fish in their smoked form, it is inevitable and imperative that the quality of smoked fish product be determined to asses the extent of fungi deterioration which has not been studied in this locality. This paper attempts to determine the mycological quality of smoked fish from retail outlets in Ago-Iwoye, Ogun State of Nigeria. Also it seeks to determine the deteriorative effects of the fungal isolates on the protein content of smoked fish.

Materials and methods Collection of sample

Fresh and non-mouldy smoked fish samples of *Ethmalosa fimbriata* were purchased from Oja-Oba market, Ago-Iwoye bimonthly for up to six months (3 sample collections). They were tied in clean polythene bag previously sterilized by 70% (v/v) ethanol and carefully transported to the laboratory for microbiological analysis adopted from the methods of Booths (1971).

Isolation of fungal species from fish samples

Preparation of medium: Czapek dox agar which consisted of sodium nitrate (2.0 g), KCl (0.5 g), $FeSO_4.7H_2O$ (0.01 g), K_2SO_4 (0.35 g), sucrose (30.0 g) and Agar-agar (20.0 g) in 1 liter of distilled water was used for fungal isolation. Potato-dextrose agar was prepared by peeling and weighing 200.0 g of Irish potato. The weighed potato was cut into smaller pieces and boiled with moist heat and mashed after which it was filtered using muslin cloth. To the mashed potato filterate, was added 20.0 g of glucose and 25.0 g agar-agar were added and mixed thoroughly after which the medium was made to 1 liter with distilled water. Sterilization of the prepared media were done in the autoclave at 121 ^oC for 15 mins. After which they were allowed to cool to 45 ^oC before 0.01 g/l of chloramphenicol was added to act as a bactericidal agent. The media were shaken vigorously and asceptically dispensed into oven-sterilized Petri dishes and allowed to solidify under laminar air-flow.

Isolation of fungi from fish samples

Isolation was carried out by first sterilizing the surface of the smoked fish with 70% (v/v) ethanol and later cutting portions of the smoked fish with sterile scalpel. The cut pieces were asceptically placed on the prepared isolation media and the inoculated plates were incubated at 28 ^oC for up to 5 days. The microbes that grew on the plates were sub-cultured into fresh agar plates using the same medium to obtain pure microbial isolates.

Stock of sporulating cultures were made in potato-dextrose agar (PDA) slant in McCarthney bottles after microscopic examination and stored in the refrigerator (4^0C) prior to use.

Microscopic examination of the isolates

The fungal isolates were mounted in lactophenol cotton blue stain solution on slides with cover slips and microscopically examined for spores and vegetative bodies according to the method described by Barnett and Hunter (Barnet and Hunter, 1972).

Pathogenicity test

Fresh samples of smoked *E. fimbriata* were wrapped in aluminium foil, sterilized at 121^oC for 15 mins and allowed to cool. Discs from actively growing portion of each isolate were asceptically transferred to holes made using sterile cork borer in the flesh of the fish and sealed with the flesh earlier removed. This was later placed in transparent polythene bags which were sprinkled with sterile distilled water. The polythene bags were then tied and put in a dessicator containing water at the cotton. The desiccators were then covered and sealed with wax and allowed to stand for 7 – 14 days during which observation were made. The inoculated areas of the fish were plated onto fresh PDA and Czapekdox agar plates. Comparisons were then made between the isolates obtained and the inoculum plated initially.

Effects of temperature on fungal isolates in fish broth

Fish infusion broth was prepared by boiling 100.0 g grounded, smoked fish samples with 1 litre [l] of distilled water. On cooling, the liquid content of the broth was squeezed out through muslin cloth and supplemented with 2.5 g yeast extract and made up to 1litre with distilled water. The broth was dispensed in 30.0 ml – portion into 250.0 ml conical flasks and autoclaved at 121^oC for 15 mins. The flasks were allowed to cool and inoculated with young mycelia agar disc (5mm diameter) of 3 days old culture of each isolates. The flasks were then incubated at different temperatures of 20, 27, 30, 35 and 40 ^oC for 7 days after which the mycelia produced were suction-filtered, dried and weighed until a constant weight was obtained for each isolate.

Protein determination

Protein was assayed according to the modified method of Lowry *et al.* (1951), using Bovine Serum Albumin as the standard.

Results and Discussion

Figures 1 and 2 summarises the results obtained in this study. Five fungal species made of three genera: *Aspergillus, Penicillium* and *Rhizopus* were isolated. The genus *Aspergillus* consisted of *A. niger*, *A. Ochraceous* and *A. flavus*. Within 72 hours of incubation, *A. niger, A. flavus* and *Penicillium* sp developed in most of the fish samples examined and their frequencies of occurrence are in the order of *A. flavus* > *A. niger* > *Penicillium* and are 30, 27 and 26 respectively. In 7-days of incubation, more isolates such as *Rhizopus sp, A. ochraceous* developed with *A. ochraceous* covering all the skin of the fish samples. The diameter of cover over the inoculated area show that *A. ochraceous* > *A. flavus A. niger* > *Rhizopus sp.* This result is consistent with the fact that *A. ochraceous* proliferated in the tissue of the fish flesh than any of the isolates. *Penicillium sp* did not grow appreciably on the fish flesh when pathogenicity test was carried out. This indicates that it lacks the enzyme required to breakdown the fish tissue. The isolate might probably be a contaminant acquired during bargaining in the market or acquired during the course of isolation.

It is important to state that majority of the fungal isolates obtained in this study are of veterinary and medical importance. *A. flavus* and *A. ochraceous* are probably the most notorious of the common isolates because of their high potentials in producing aflatoxin and ochratoxin respectively. Aflatoxin has been reported to cause acute hepatitis (aflatoxicosis) while ochratoxin is responsible for mitosis in animal kidney (Bababunmi et al, 1978; Adebajo, 1992).

The occurrence of *Aspergillus spp, Rhizopus sp* and *Penicillium* could be due to the fact that during storage, the fish product reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to contamination during processing, handling and display on the market stalls.

Figure 1 shows the weight of each isolate with variation in temperature. *A. ochraceous* and *A. flavus* grew best at 28 ^{o}C while *A. niger* and *Rhizopus sp* grew optimally at 35 ^{o}C in the fish broth meaning that they are all mesophilic organisms.

Figure 2 is a summary of the effects of the isolates on the protein content of the fish. All the isolates degraded the proteins of the fish with *A. ochraceous* having the highest rate of degradation followed by *A. niger, Rhizopus sp* and *A. flavus* in that order. The results suggest that the isolates produce proteases which degrade the fish protein. This is in agreement with earlier report of Kuku (1980). The implication of the isolates degrading fish protein is that the available protein for human consumption will be drastically reduced. It is therefore suggested that better preservation method (agro waste drying) should be employed and good storage condition fashioned out for the fish product. Preservation could probably be better if the fish product is kept in a hostile environment such as the show glass equipped with heating element or in a regulated oven so as to reduce drastically the moisture content of the fish which is the basic requirement for microbial growth and hence, inhibit the growth of the microorganisms causing fish spoilage.

An ancient or local control method of putting the fish product in a wire cage ("Ayanran") and hang same over the fire for preservation on daily basis could also be employed as the smoke of the fire has some germicidal effect or chemical preservatives such as phenolic compounds (Frazier and Westhoff, 1978) which inhibit the growth of microbes.

Acknowledgements

We would like to thank Prof. J. A. Olagunju of the Department of Biochemistry, Lagos State University College of Medicine for providing us with the reagents used for protein analysis.

FIG. 1. EFFECT OF TEMPERATURE ON MYCELIAL GROWTH OF FUNGI ISOLATES IN FISH BROTH

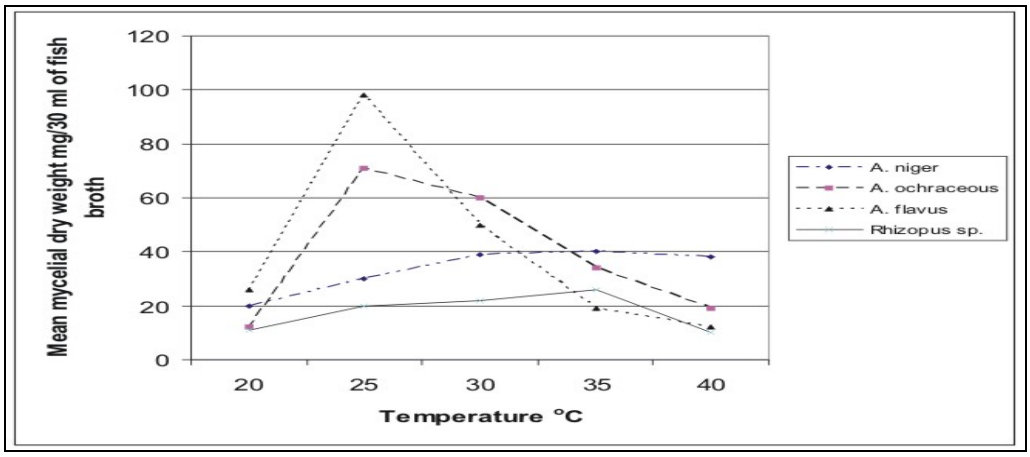
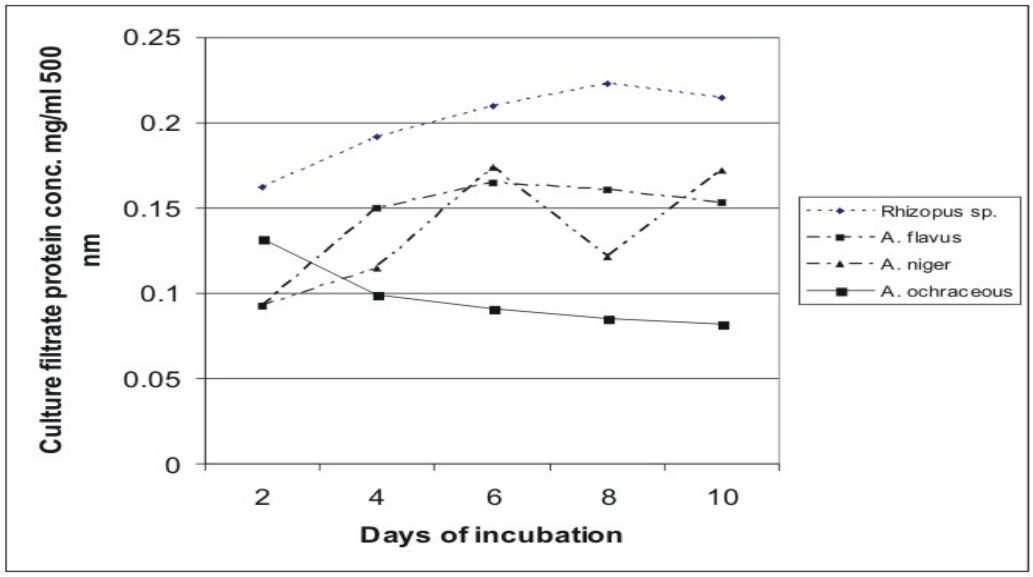


FIG. 2. EFFECT OF ISOLATES ON THE PROTEIN CONTENT OF THE FISH



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