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Microbial Flora and Nutrient Content of Market Bought Smoked African Cat Fish Clarias gariepinus from Jos, Nigeria

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Abstract
Clarias gariepinus, one of the many fishes sold in Nigerian market, is the most preferred smoked fish in Jos where large quantities are smoked and stored for sale. This study assessed the nutritional value and health of smoked *C. gariepinus* sold in Jos markets.Live and smoked *C. gariepinus* were purchased from the four major markets in Jos metropolis. Microorganisms isolated from the smoked fish were identified. The live fish were smoked in the laboratory and inoculated with the isolated microorganisms. Nutrient content of the fishes were monitored weekly for four weeks, un-inoculated laboratory smoked fish served as controls.*Bacillus brevis, Aspergillus fumigates* and *Mucor species* were isolated from purchased smoked fish. The nutrient value of these fish were significantly lower ($p<0.05$) than the laboratory smoked fish. There was however a gradual decline in the nutrient content of the infected laboratory smoked fish. The carbohydrate content decreased to zero while the moisture content increased. *Mucor* had the most significant effect on protein (62.06 ±13.39) and carbohydrate (1.11±0.95) levels in infected fish. pH dropped below7.0 by the end of four weeks in *Mucor* infected fish and fat content was lowest (14.19±3.82) in *A. fumigates* infected fish. There was a significant difference ($p>0.05$) between the nutrient values in the control and infected fish. The microbial content and lower nutrient values of infected fish emphasize the need to ascertain the health and nutrient content of market sold fish. This will ensure that consumers receive optimum nourishment and avoid the likely health implications of consuming infected fish.
Keywords: Fish nutrient, smoked *Clarias gariepinus*, microflora, Jos markets

1. Introduction

Fish makes up about 60% of world protein supply and developing countries derive more than 30% of their annual protein from fish (FAO 1994). In Nigeria, there is an increasing demand for fish because it is a cheaper source of animal protein, it is also a delicacy with demands cutting across socio-economic, religious, educational or age groups (Adebayo-Tayo *et al.,* 2008). Fish is eaten fresh, processed or preserved and fish protein makes up 40-80% of the optimal protein consumed (Adebayo-Tayo *et al* 2008). Fish nutrients show appreciable depletion in storage, it is therefore best consumed fresh to ensure maximum optimization of the nutrients. Eyo (1989) reported a 50% annual loss of the fish caught in Nigeria to post harvest spoilage irrespective of the preservation methods employed. The high ambient temperatures and humid tropical conditions speed up spoilage processes in harvested fresh water fishes (Saliu 2008). The speed of spoilage is related to the initial bacterial load: the higher the count, the sooner spoilage occurs (Adam and Tobaias 1999). Kvenberg,(1991) and Rodrick,(1991) classified bacteria pathogen of fish into two: the indigenous bacteria, those living naturally in fish and its habitat and non-indigenous bacteria, which are contaminant of fish or its habitat. Many bacteria that are potential spoilers abound in the surface slime, gill and intestine of live fish but the natural defenses prevent invasion while the fish is alive. Multiplication and invasion occurs soon after death of fish. (Agbolagba and Uwagbai, 2011).

To reduce the loss associated with such spoilage, preservation methods such as smoking, salting, sun drying, freezing and cold storage are employed. Smoking of fish from smoldering wood dates back to early civilization (Eyo,2001 and Olokor *et al.,* (2007) and about 66% of preserved fish in Nigeria are smoked. Smoking is the traditional method of fish preservation in many developing countries (Tawari and Abowei, 2011). Smoking is desirable partly due to the ease of the procedure, and consumer preferences. Wood smoke gives fish a desirable taste, toughens and dehydrates fish muscle providing a longer shelf life, lowering the pH making it less susceptible to spoilage (Sengor *et al*., 2004, Olokor *et al.,* 2007, Abolagba and Igbinevbo, 2010). In spite of the qualities of smoking, smoked fish still has the problem of insect infestations and microbial contamination. A wide range of beetles and microbes have been reported as infesting smoked and fresh fish in Nigeria (Nduh 1984,Ufodike and Obureke. 1989, Olokor *et al.,* 2007, Abolagba and Igbinevbo, 2010, Shinkafi *et al.,* 2010, Eze *et al.,* 2011). Abolagba and Iyeru (1998) reported that improper smoking and unhygienic handling of smoked fish results in high microbial infestation and that storage temperature close to 37^{o}C are ideal for the growth of pathogenic bacteria. They also stated that high humidity and high to moderate temperature support mould growth in stored food. Moulds produce mycotoxins some of which are carcinogenic while fungal groups may cause mycoses and allergies in man.

C. gariepinus is the most predominant smoked fish sold in Nigeria and it is also the most abundant fish caught from artisanal fishing. Mohammad (1981) reported a high commercial preference for *C. gariepinus* over

Tilapia or *Cyprinus* species because of the ease of smoking and consumer preference for the less bony body. In this study, an attempt was made to indentify the microbial flora in smoked *C. gariepinus* sold at the markets in Jos metropolis, Nigeria and also to determine the nutrient quality of this fish during storage. The aim was to determine the safety of fish consumed and ascertain if the fish provided the expected nutritional value to the consumer.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Jos, Plateau State, Nigeria. Jos is located on the plateau where temperatures generally range from 4 to 28^{o}C. Jos metropolis has four major and several satellite markets. Smoked fish including *C. gariepinus* is sold in all the markets. The fish used in the study were identified as *C. gariepinus* in the Hydrobiology and Fisheries Unit, Department of Zoology, University of Jos.

2.2 Sample Collection

Smoked (30) and live (30) fish used in the study were purchased from the four major markets in Jos. At point of purchase, live fish were placed in water tanks and the smoked fish in sterile plastic bags. All samples were transported to the Department of Zoology, University of Jos for analyses.

2.3 Sample Preparation

Market bought smoked *C. gariepinus* was prepared by crushing fish muscle in a sterile mortar and 10g of this was homogenized in 10ml distilled water to prepare a stock sample. Homogenization was to obtain uniform distribution of cells. Stock homogenate (1ml) was serially diluted (10 fold) and 0.1ml of $10^{-10}$was used for plate inoculations.

The live *C. gariepinus* were acclimatize in the Department of Zoology Research ponds for 72 hours and subsequently harvested, sacrificed and de-gutted. Smoking immediately followed on smoking grills for 4 days at an average temperature of 95^{o}C until dry, adopting method locally employed for smoking of market sold fish. Laboratory smoked fish were sterilized by immersion in acetone for 2 minutes and allowed to air dry.

2.3.1 Inoculation of Culture Plates and Isolation of Microorganisms

Microbial organisms from bought smoked fish were isolated into pure culture and identified.

Bacteria: Freshly prepared sterile nutrient Agar plates were inoculated with 0.1ml of fish homogenate, spread with sterile glass rod and incubated at 37^{o}C for 24 hours. Representative colonies were sub-cultured onto fresh sterile nutrient Agar plates to ensure production of pure cultures.

Fungi: Sterile plates of Potato Extract Agar (PEA) were inoculated with 0.1ml homogenate fish sample. Plates were incubated at 25^{o}C for 5 days after which distinct representative fungi were sub cultured on fresh plates for 5 days at 25^{o}C.

Pure isolates were identified using features such as morphology, motility test, gram staining biochemical reactions and fermentation of sugar (Bergey1974, Cheesbrough 2002).

2.3.2 Inoculation of Laboratory Smoked Fish

Sterilized laboratory smoked fish were divided into three groups consisting of two experimental groups (1 and 2) and a control group (3) and each group had 10 fish. Bacteria cells scrapped off the surface of the pure culture on agar plates were suspended in 1% peptone water. The suspension (0.5ml) was inoculated into each fish in group 1. Also pure fungi on PEA were washed into a sterile petri dish with 25ml distilled water and 0.5ml of this was inoculated into each of 10 fish in group 2. Fish in group 3(control) were not inoculated with isolates. All fish samples were placed in sterile cabinets to avoid contamination with aerial microorganisms.

2.4 Nutrient Assays

The nutrient value of experimental and control fish was determined over a period of 4 weeks by analyzing two or three fish from each group weekly. The major nutritional indices of fish: protein, fat(oil), moisture and carbohydrate were analyzed based on methods of the Association of Official Analytical Chemist (AOAC, 2000). The pH of the samples was also recorded weekly since pH gives an indication of the type of flora and extent of microbial activity in the fish.

2.5 Identification of Microorganisms

Features such as spore and hyphae morphology were observed and compared with standard atlas (Ochei and Kohatkar 2000). Colour, texture, colonial morphology and pigmentation of each sample was recorded. Biochemical tests described by Bergey *et al*.,(1974),was employed in the identification of the bacterium isolated.

3. Results and Discussion

The microorganisms isolated from the market bought fish were two fungi species *Aspergillus fumigatus, Mucor spp* and one bacterium *Bacillus brevis.* The morphological characteristics used in identifying the fungi showed that *Aspergillus fumigates* had many celled conidia represented in chains while *Mucor spp* had a round spore head. Biochemical tests used in the identification of *Bacillus brevis* showed reaction to lactose, sucrose and catalase(Table 1).

Table 1: Biochemical tests for identification of *Bacillus brevis* isolates.

Tests	Results
Gram reaction	-
Motility	+
MR	+
Glucose	-
Lactose	+
Mannitol	-
Sucrose	+
Catalase test	+
H_2S	-

Several workers over time had identified a diverse range of microorganisms from fresh as well as preserved fish including smoked fish, (Nduh 1984, Ufodike and Obureke 1989, Eyo 2001, Abolagba and Igbinevbo 2010). The microorganisms isolated and identified from smoked fish purchased from Jos markets gave an index of the health and nutritional value of the fish. The fungi isolated from these fish were similar to some identified in earlier studies on smoked fish and stockfish (Junaid et al.,2010). All three isolated microorganisms in this study were organisms usually found in soil and decaying organic matter suggesting that the fish may have been improperly smoked or contaminated while handling the after smoking. Akinneye *et al.,* (2007) reported that handling and preservation practices after fish capture affect the degree of spoilage of the fish. Usually in Jos markets, smoked fish were displayed exposed in stacks on tables while unsold fish were packed in open baskets or boxes and displayed on subsequent days. Agbolagba and Iyeru (1988) reported that exposure of fish to dust, microbial and other environmental contaminates results in spoilage.

Microbial population isolated from smoked fish in the study were fewer than from previous reports in fresh or frozen fish (Sengor *et al.,* 2004, Shinkafi and Ukwaje 2010, Eze *et al.,* 2011), probably because the anti-bacterial effect of smoke may have reduced infestation by microorganisms (Olokor *et al.,* 2007). Some microorganisms such as *Bacillus* species are said to be normal microbial flora of fish, which are not harmful but could become pathogenic under some enabling environments (Agbolagba and Igbinevbo 2010, Emikpe *et al.,* 2011). Though *B. brevis,* the bacterium isolated in the smoked fish is rarely associated with infectious diseases, it is associated with food poisoning, which manifests in a myriad of pathogenic disorders of the gastro intestinal tract (GIT) and the Central Nervous System (CNS). *A. fumigatus*, a fungi also isolated in the study has severe health implications for immuno-deficient individuals and could also cause chronic infections or allergies in immuno-competent hosts (Hohl and Feldmesser 2007). *Mucor* has also been reported to cause such pathologies as infections of the lungs, otitis and psoriasis. Therefore all three microorganisms isolated from fish bought in the markets could cause severe health problems and consumption of food containing these organisms could compromise the health of the consumers. Although the intensity of the disease caused depends on the densities of the organism, the large quantities of fish consumed by Nigerians may increase the probability of such diseases. Even where fungus is dead, they may have produced mycotoxins which could poison the food (Junaid et al.,2010). Mycotoxins are reported to resist decomposition even by temperature treatments such as cooking, and freezing resulting in ingestion of such toxins by consumers of such infected fish (Adebayo-Tayo *et al*., 2008, Junaid *et al*., 2010,).

The nutrient indices of market smoked fish was 69.37% protein, 19.15% fat, 3.47% carbohydrate, 10.24% moisture and pH 7.0 compared to 77.36% protein,18.24% fat, 2.32% carbohydrate, 10.09% moisture and pH 6 of the laboratory smoked fish. The nutritional indices of inoculated laboratory smoked fish, were significantly higher ($P>0.05$) than values of market smoked fish. However, after four weeks, the nutritional values of the infected laboratory smoked fish were shown to have declined significantly compared to the uninfected control. Microbes degrade fish muscle and spoilage is enhanced by the storage method and duration. The nutritional quality of the fish in this study, declined with the presence of microorganisms and length of storage.

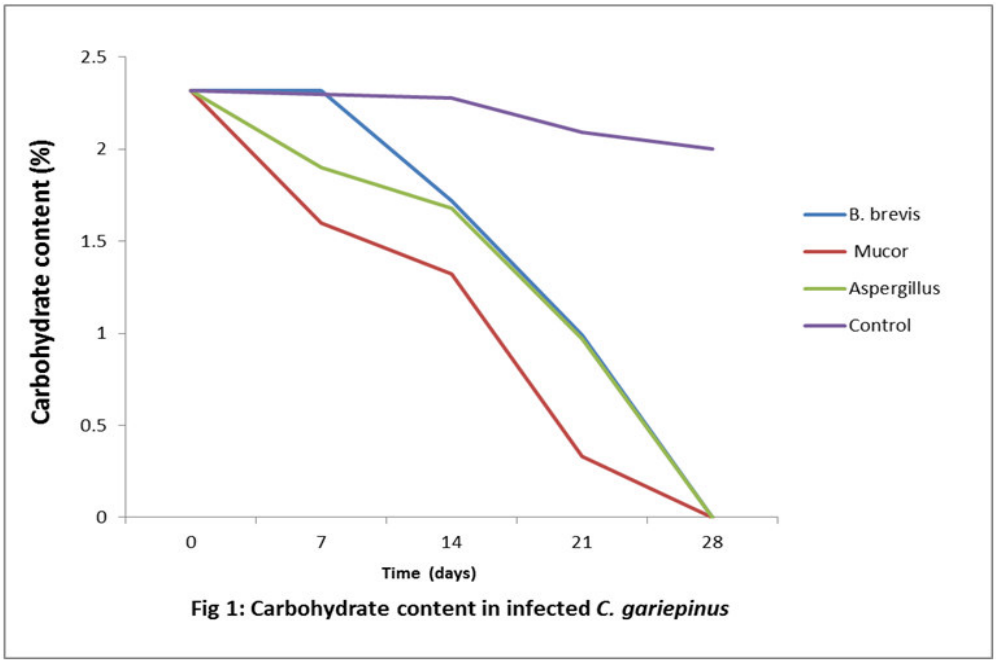

Fig 1: Carbohydrate content in infected *C. gariepinus*

The microbial infestation had a significant effect on carbohydrate content. Carbohydrate was completely depleted in the fish by 28 days post infection (Fig 1). Fish is known to have low carbohydrate content and microorganisms require a carbon source for energy metabolism, therefore utilizing and quickly depleting the carbohydrate in the fish. This could also account for the reduction in the fat content as fat probably becomes the alternative energy source for the organisms.

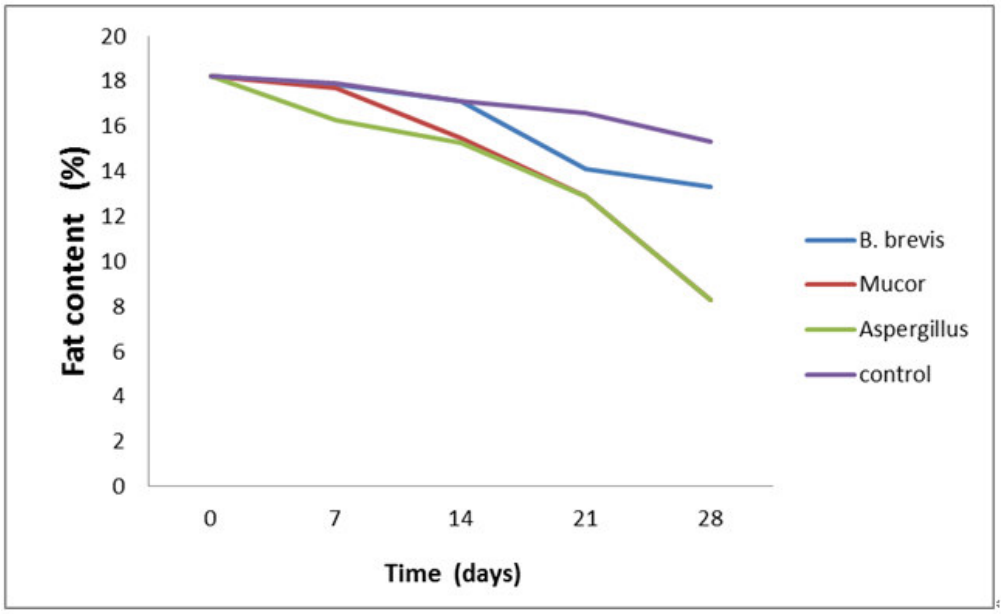

Fig. 2: Fat content of fish infected with microorganisms

However, *B. brevis* showed no significant effect on the fat content of infected fish. Though there was a significant decrease ($P<0.05$) in the fat content of fish infected by *A. fumigatus* and *Mucor* in comparison to control fish (Fig. 2).

Fish experimentally inoculated with isolated microorganisms showed appreciable changes in the protein content in contrast to controls. The protein content decreased significantly ($P<0.05$) with length of storage period (Fig.3). The LSD 6.55 showed microbial effect on protein content by organism in the order *Mucor>B.brevis>A.fumigatus*.

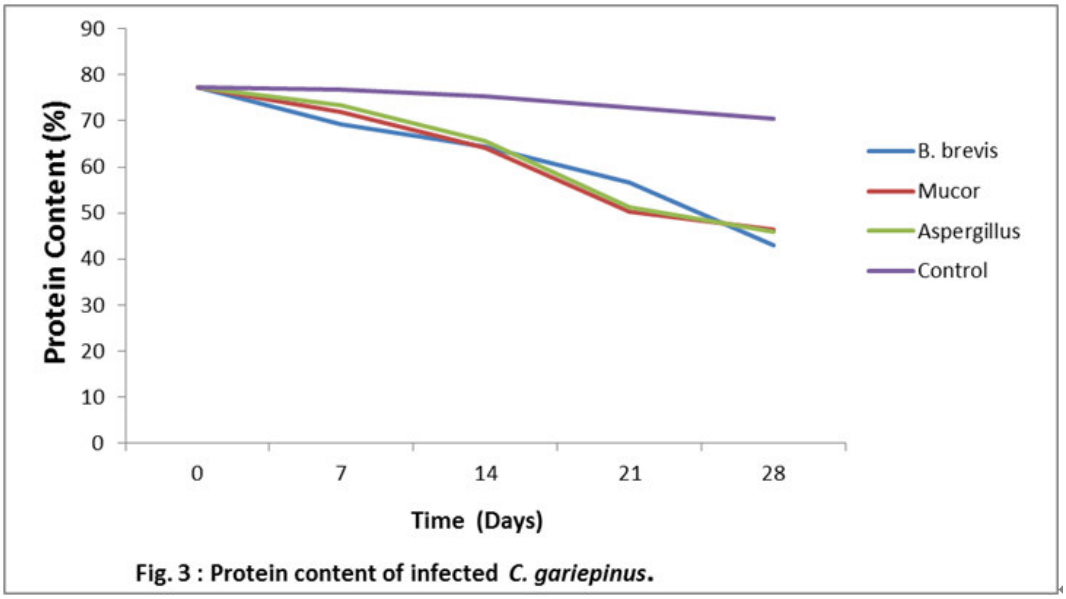

Fig. 3 : Protein content of infected *C. gariepinus*.

The moisture content of experimental fish decreased in the first 2 weeks post infection and began to increase thereafter (Fig. 4).

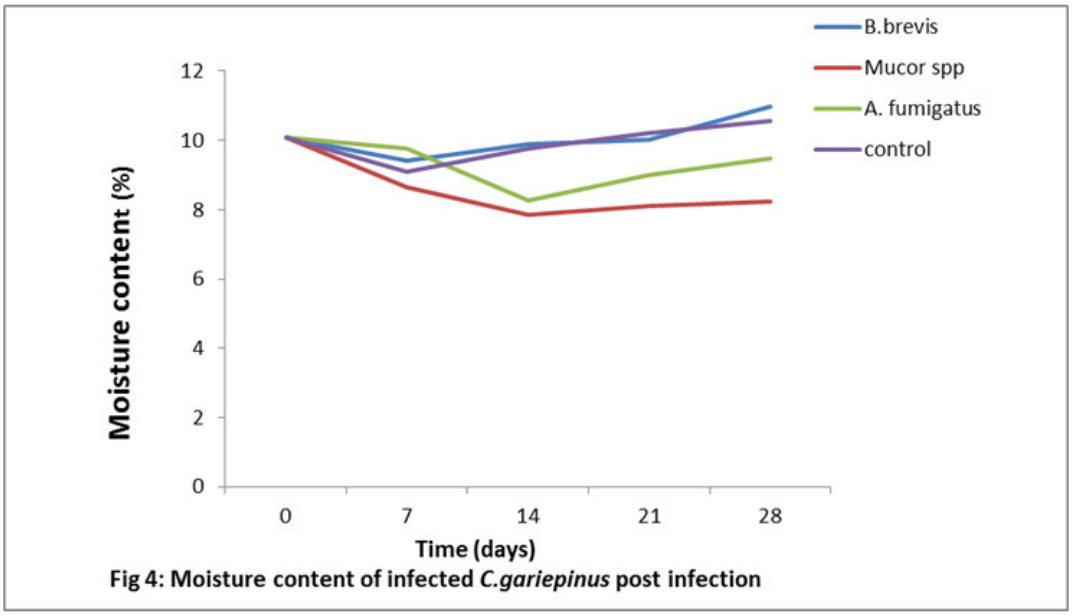

Fig 4: Moisture content of infected *C.gariepinus* post infection

Microbial deteriogens are known to require moisture for enzymatic hydrolysis of food components (Ogbonna 1987). The high moisture content may be necessary to make the food soluble for absorption by the microorganisms.

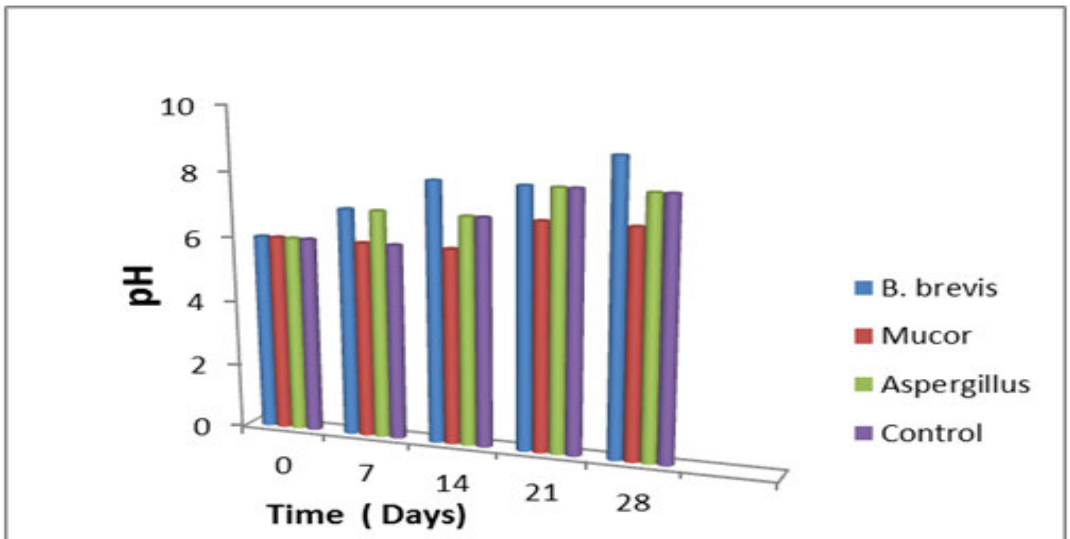

Fig 5: pH of *C. gariepinus* infected with various microorganism.

There was also a recorded gradual increase in pH of fish during the study (Fig.5). The breakdown products of fish nutrient increase the alkalinity of the fish. This results in increased pH.
At LSD 0.54, all microorganisms were responsible for decrease in nutrient values in the following order of rapidity *Mucor* > *A. fumigates* > *B. brevis.*

4. Conclusion

It can therefore be concluded from the study that the microorganisms identified on market smoked fish may render the fish unsuitable for consumption since these microorganisms and or toxins produced could be harmful to the health of the consumers. In addition, the level of fish nutrients depletion suggests that the consumers receive very little nutritional benefits from such fish. It is of public health importance that adequate measures are taken to ensure that market sold fish are processed and handled in the most hygienic manner to ensure the safety of this important food source for its teeming consumers.

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