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EVALUATION OF HISTAMINE-PRODUCING BACTERIA IN COLD-SMOKED MACKEREL (*Scomber scombrus*) RETAILED IN OTA, OGUN STATE, NIGERIA

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ABSTRACT

Background: The occurrence of histamine-producing bacteria (HPB) in poorly preserved mackerel fish smoked and retailed in the majority of Nigerian markets is of public health concern and therefore necessitated this investigation.
Objectives: The aim of this study was to evaluate histamine-producing bacteria in cold-smoked mackerel (*Scomber scombrus*) retailed in Ota, Ogun State, Nigeria.
Methods: Cold-smoked mackerel fish samples (36 pieces) were purchased from four selected markets in Ota: Oju-Ore (OJ), Iyana-Iyesi (IY) Iju (IJ) and Oja Oba (OO). Each of the fish samples cut into Head (H), Trunk (Tr) and Tail (Tl) were analyzed for microbial loads; total plate count (TPC; log $CFUg^{-1}$) and histamine-producing bacteria (HPB; log $CFUg^{-1}$) using pour plate method of isolation. Data were generated in triplicates and analyzed using ANOVA and Duncan's multiple range at α 0.05.
Results: The microbial loads showed that all the fish samples were of unsatisfactory microbial quality having mean counts of $\geq 10^7$, with the highest TPC values of 8.59±0.17, 8.44±0.47 and 8.43±0.21 log $CFUg^{-1}$ respectively recorded in TL, TR and H parts. Iyana-Iyesi market recorded the least (7.80±0.51 (TL) to 7.89±0.35 (H) log CFU/g) while Iju market had the highest value range of 8.43±0.21 (H) to 8.59±0.17 (TL). *Pseudomonas* spp. (45%, 299 colonies), *Proteus* spp. (24%, 130 colonies), *Escherichia coli* (20%, 157 colonies) and *Morganella morganii* (11%, 95 colonies) were the isolated HPB.
Conclusion: This indicates that the safety of smoked mackerel fish retailed in Ota is compromised and may therefore be unsafe for human consumption. This information can be useful for critical monitoring to improve their quality and safety.

Keywords: Cold-smoked, Erratic electricity supply, Histamine-producing bacteria, Mackerel fish, Poor cold storage

INTRODUCTION

Owing to its velvet skin, mackerel, also known locally as "monkere," "eja ice" (iced fish), or "eja alaran" in Nigeria, is a popular food fish that is consumed all over the world for its high protein content and low saturated fat content (Bae *et al*., 2011). This fish is at its best when frozen (Shamsan *et al*., 2019), with a higher concentration of healthy fats-polyunsaturated fatty acids, in particular that are essential for preventing non-communicable diseases like cancer and diabetes (Das *et al.,* 2024). Regular mackerel consumption has also been shown to improve cognitive function, aid in weight loss, lower visceral fat, and the risk of cardiovascular disease (Tørris *et al.,* 2018). PUFAs also increase immunity, regulate blood pressure, and lessen rheumatoid arthritis symptoms (Šimat *et al.,* 2020).

Due to its high susceptibility to microbial spoilage, fat autolysis, oxidation, and hydrolysis, fish is a highly perishable product (Getu *et al.,* 2015). Defrosted fish typically soften and are more susceptible to damage, which can result in severe bruises. Handling can also contaminate the flesh (Adelaja *et al.,* 2013). Abuse of temperature during the freezing process of fish results in increased

bacterial multiplication and histidine decarboxylase activity, with exposure to temperatures above 15°C (Abuhlega and Ali, 2022), favoring the activity of the enzyme. Huge economic losses have reportedly resulted from the alleged frozen storage of fish and other products being rendered ineffective by the unpredictable electricity power supply that plagues many parts of Nigeria. The bulk of Nigerian fish retailing outlets specialize in defrosted soft and subpar fish products, including mackerel.

When fish reach the end of their shelf life, a biogenic amine called histamine is produced, and its level is more appropriately regarded as a spoilage index than a quality index (Visciano *et al.,* 2014).

The flesh of poorly maintained mackerel has been linked to the presence of enteric bacteria, including *Escherichia coli*, *Proteus* spp., *Morganella morganii*, *Pseudomonas* species, *Clostridium* spp, *Klebsiella* spp, and *Proteus vulgaris* (Tembhurne *et al.,* 2013). As these bacteria proliferate, the amino acid histidine that occurs in most proteins is modified, resulting in the production of high concentrations of the food toxin histamine and other bioactive amines. (Schirone*et al.,* 2017). The histamine is released by the body tissues in allergic reactions, causing irritation. It also stimulates gastric secretions, dialates blood vessels and contracts smooth muscles. (Kovacova-Hanuskova *et al.,* 2015).

The most common sources of histamine have reportedly been identified as *Proteus* spp. and *Morganella morganii*. Hassan *et al*. (2022) found that four chosen fish species on display in three fish markets in Tripoli, Libya, had a high prevalence of HPB isolates from the families Enterobacteriaceae and Vibrionaceae. Enterobacteriaceae were found to be the most common histamine-producing isolates in smoked herring, mackerel, fillet of carp, Morgan, and pilchards that were acquired from Giza retail stores in Egypt, according to a research done by Refai *et al*. (2020). According to Oktariani *et al*. (2022), fish exposed to high temperatures after harvesting multiply HPB, which in turn causes a significant increase in histamine production. Scombrotoxic fish poisoning (SFP) is associated with the consumption of contaminated fish of the Scombridae family, to which mackerel belongs (Zapata *et al*., 2020). This intoxication causes cardiovascular, gastrointestinal, and neurological symptoms, such as skin rashes, urticaria, oedema, local inflammation, nausea, vomiting, diarrhoea, cramping, hypotension, headache, palpitation, and oral burning (Anusha *et al*., 2021).

Smoke-drying of fish is one of the common traditional processing and affordable methods of fish preservation in Nigeria and some West African countries. It is a practice directed at reducing postharvest losses of fish and fishery products but unfortunately, smoke-dried fish could be contaminated with different contaminants including bacteria and other pathogens, which could be of public health interest (Abiala *et al*., 2020). Ozoh and Orji, (2022) submitted that smoking eliminates water from fish thereby inhibiting spoilage bacterial and enzymatic activities on fish. Madejska *et al*. (2022) however postulated that histamine develops before fish is smoked, though histamine-producing bacteria are eliminated during smoking.

While Gbolagunte *et al*. (2012), Kester *et al*. (2012) and Daramola *et al*. (2020) conducted some microbiological quality assessment studies of smoked fish products retailed in some selected markets in Ota, Ogun State, none of these studies looked into the occurrence of HPB in smoked fish products, especially mackerel (*Scomber scombrus*). Even though there is a great demand for this fish among the general public, eating it can pose a risk to public health.

Thus, the purpose of this study is to determine whether smoked mackerel fish sold in Ota, Ogun State, is safe for human consumption by looking into the presence of histamine-producing bacteria in the fish.

MATERIALS AND METHODS

Study site

A preliminary survey was carried out to identify the retailing locations of smoked mackerel fish in Ota, Ogun State. The selected markets were Oju-Ore (OJ), Iyana-Iyesi (IY) Iju (IJ) and Oja Oba (OO), Ado-Odo Local Government Area of Ogun State,

located between latitude 6.38^0N to 6.41^0N and longitude 3.8^0E to 3.12^0E in a South-western geopolitical zone of Nigeria.

Materials and Methods

Samples collection

Thirty sixty (36) pieces of mackerel fish were bought from each of the four markets, labeled appropriately, and transported in sterile Ziploc bags for examination by microbiologists. The fish samples were subjected to microbiological analysis at Bells University of Technology's microbiology lab in Ota, Ogun State, Nigeria.

Microbiological analysis of smoked mackerel fish samples

The head, trunk, and tail of fish samples were aseptically cut from each of the four market locations and then individually pounded into pieces in a clean mortar. Each tissue sample weighed about 10g, and it was aseptically homogenized in 90 mL of sterile normal saline. To ascertain the bacteriological loads, a ten-fold serial dilution was prepared up to 10^{-8}. Total aerobic bacterial count (TABC), total coliform count (TCC), total staphylococcus count (TSC), and total enterobacterial count (TEBC) were the parameters determined using pour plate technique. These were determined using nutrient agar (NA), MacConkey agar (MCA), mannitol salt agar (MSA), and eosine methylene blue agar (EMBA), respectively. Three identical inocula (1 mL) were added to labeled, sterile Petri dishes in triplicate from the preferred dilution. Each of the inoculated Petri dishes received 15–20 mL of sterile, melted agar medium that had been cooled to roughly 45^0C. The inoculum was then carefully and evenly mixed with the medium before being left to gel. Additionally, a few control plates were made to verify the sterility of the medium, glassware, and diluents. Every Petri dish that had been poured was inverted and incubated at 37^0C for an entire day. Using a colony counter, Petri dishes containing 30-300 colonies per dilution were counted and recorded after incubation. The total counts per gram of the sample were expressed using colony-forming units ($CFUg^{-1}$). Every suspected HPB underwent a standard protocol-compliant biochemical examination for further identification.

Biochemical tests of the suspected HPB isolates

The following tests were performed on pure culture colonies of the suspected bacterial isolates that were 18 to 24 hours old following standard protocols to further identify them:

Gram staining reaction:

The isolate was thinly applied to a clean glass slide, allowed to air-dry, and then heat-fixed. The fixed smear was stained for 30 to 60 seconds with crystal violet stain and then rinsed with tap water. Rinse with tap water after 30 to 60 seconds of Lugol's iodine application. The film was decolored with 70% ethanol until the slide no longer displayed any crystal violet stain. The slide was counterstained with safranin for sixty seconds, and then it was rinsed again with tap water. The prepared slides were examined under a microscope using an oil immersion objective lens (X40 and X100) after being allowed to dry. Isolates that retained the purple hue of crystal violet stain were indicative of gram-positive bacteria, while those that looked pinkish or reddish were indicative of gram-negative bacteria.

Catalase test

The 18–24 hour-old culture of the organisms was suspended and placed on a sterile slide. A few drops of hydrogen peroxide were added using a wire loop. The observation of gas bubbles caused by the release of free oxygen indicated the presence of the catalase enzyme and, therefore, a positive reaction; the absence of bubbles suggested a negative reaction.

Oxidase test

Using a sterile inoculating needle, a colony of the test organism was selected and applied to the oxidase strip. Within ten seconds, a blue-purple color development was noticed. A positive oxidase test would have a blue-purple color; one that was negative would not have any blue-purple color.

Citrate test

Each isolate's 18–24 hour old culture was aseptically inoculated onto Simmons citrate agar, which had been prepared in test tubes. After that, it was incubated for 24 hours at 37^0C. Positive citrate usage was indicated by a color shift from green to deep blue; negative citrate utilization was indicated by no color change.

Sulphide Indole Motility test

Test-tube-prepared semi-solid Sulphide-Indole medium was aseptically stabbed straight and incubated for 24 hours at 37°C. The organisms spreading beyond the line of stab indicated motility.

Indole Test

McCartney bottles containing distilled water were sterilized for 15 minutes at 121^0C. The test organism was added and incubated for 24 hours at 37^0C after cooling. After this incubation time, the culture was given a few drops of Kovac's reagent, which was then left to react for two minutes. The formation of a red ring around the rim of the medium signified a positive outcome, whereas the absence of such ring formation suggested a negative outcome.

Triple Iron Sugar/Kliger Iron agar test

This four-in-one test looks for the production of gas (CO_2), the release of H_2S (hydrogen sulphide), and acid as a result of the fermentation of lactose (slope) and glucose (butt). Using an inoculating needle, each pure isolate was added to the Kligler iron medium, causing the slope to be streaked in a zigzag pattern. The mixture was then incubated at 37°C for a full day. Cracks and bubbles in the medium indicated gas production in the fermentation of glucose; a yellow slope and a yellow butt indicated the fermentation of lactose; a red-pink slope and butt indicated no fermentation of glucose or lactose; blackening along the stab line or throughout the medium indicated the production of hydrogen sulfide. A yellow butt (acid production) and red-pink slope indicated the fermentation of glucose only. The data from these biochemical tests were recorded for analysis.

Data Analysis: ANOVA was utilized to analyze the data derived from the H, Tr and Tl subsets of the fish samples. Duncan's Multiple Range test was next used to look for significant differences between the means of the values using Duncan's Multiple Range test. Windows Inc., Chicago, U.S.A.'s SPSS (Version 17.0) was used for both statistical analyses.

RESULTS AND DISCUSSION

The highest TPC values of 7.89±0.35 log $CFUg^{-1}$ (H), 8.14±0.52 log $CFUg^{-1}$ (TL), 8.44±0.47 log $CFUg^{-1}$ (TL) and 8.59±0.17 log $CFUg^{-1}$ (TL) respectively recorded in the smoked mackerel fish samples purchased from Iyana-Iyesi, Oja-Oba, Oju Ore and Iju markets (Figure 1) differed significantly ($P<0.05$). These values exceeded the ICMSF (2018) maximum recommended bacterial count for a marginally acceptable quality product (M) which is 7 log 10 CFU/g) for fish and fishery products. This finding shows a higher microbial load of 6.26 log CFU/g recorded by Akpabio *et al*. (2018), in their study on isolation and identification of bacteria in smoked fish retailed in Umuahia Metropolis. This implies that the artisanal fish merchants had to have smoked and low-quality mackerel fish for sale. It is possible that the fish lot was improperly stored because of the unstable electricity supply, which encourages the growth of scrombotoxigenic bacteria in the samples of mackerel fish. The contamination of the fish samples during storage, transportation, and sales may have also been caused by the unhygienic handling techniques used by the fish retailers, posing a health risk to consumers.

Displayed in Tables 1 and 2 are the morphological and biochemical characteristics of the isolated HPB from the cold smoked mackerel fish (*Scomber scombrus*). *Pseudomonas* spp., *Proteus* spp, *Escherichia coli* and *Morganella morganii* were morphologically suspected and biochemically identified. The occurrence of these bacteria isolated from smoked mackerel fish samples purchased from selected markets in Ota agreed with the findings of Ayeloja *et al*. (2018) and Anihouvi *et al*. (2019) in their studies on microbial loads of smoked fish respectively traded in Ibadan, Oyo State and Benin, Edo State.

Pseudomonas spp. (45%) was the most prevalent HPB isolate in Figure 2, with a total of 299 colonies. *Proteus* spp. (24%, 130), *Escherichia coli* (20%, 157) and *Morganella morganii* (11%, 95) were the next most prevalent isolates. These HPB must have arisen because tropical temperatures are ideal for the development of decarboxylase-containing microorganisms (Kalhotka *et al*., 2012). According to Oktariani *et al*. (2022), histidine, which is abundant in mackerel fish by nature, can be readily transformed by HPB into toxic histamine in unfavorable storage circumstances.

The majority of the bacteria found in all of the smoked mackerel fish samples that had their HPB levels examined were Pseudomonas spp. This demonstrates the widespread

nature of *Pseudomonas* species as a cause of nosocomial infections and food poisoning (El-Aziz, 2015). They can survive in extremely challenging ecological niches (Oku and Amakoromo, 2013). It has been suggested by Virupakshaiah and Hemalata, (2016) that *Pseudomonas* spp. are linked to fish and seafood contamination. When such products are consumed, the users run the risk of contracting serious infections such as pneumonia, septicemia, meningitis, endocarditis, and malignant external otitis (Raposo *et al*., 2016). The revelation of *Proteus* spp. as a contaminant of the smoked mackerel fish of this study also calls for health concerns. Though they are part of normal human intestinal flora, *Proteus mirabilis* are considered to be the causes of some community-acquired infections. The occurrence of *E. coli* in this study of smoked mackerel samples is indicative of unhygienic handling along the fish processing and distribution chains as established by Bedane *et al*. (2022). The ingestion of such smoked mackerel fish contaminated with *E. coli* tends to cause bacterial gastroenteritis, kidney damage as well as uncomplicated community-acquired urinary tract infections as reported by Adelaja *et al*. (2013). *Morganella morganii,* the least dominant isolate of this study, has been demonstrated by Liu *et al.* (2016) as an unusual opportunistic pathogen capable of causing an array of infections, ranging from urinary tract infections to systemic bacteremia.

With the potential to produce histamine, every bacterial contaminant found in the smoked mackerel fish samples sold in Ota, Ogun State, has implications for public health and food safety. It is not impossible that the cooking procedure these retailed fish samples may undergo is insufficient to bring their elevated microbial loads down to levels that are safe for consumption. Therefore, the fish samples may be dangerous and harmful to human health, particularly if improperly cooked before consumption.

CONCLUSION

The bacterial loads on the smoked mackerel fish retailed in Ota markets exceeded the maximum recommended limits for marginally acceptable fish and fishery products. The occurrence of histamine- producing bacteria was extremely high in the smoked mackerel fish samples retailed in Ota, indicating the likelihood of high levels of histamine in the products. Some of the smoke fish retailers in Ota markets are apparently smoking spoiling mackerel fish in order to reduce the impending monetary loss they may likely incur in their sales.

All local markets should be regulated by the State chapter of the National body in charge of food control. Further research on the levels of histamine (scombrotoxin) in smoked and frozen mackerel fish should be carried out.

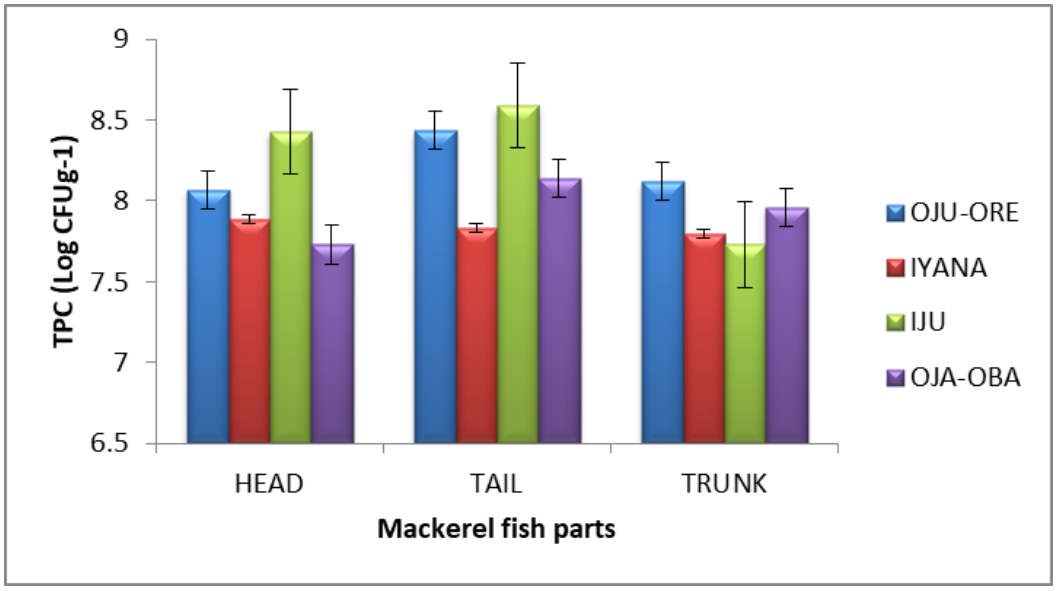

Figure 1: Total bacterial densities of the Head, Tail and Trunk of smoked mackerel fish samples retailed in the selected markets

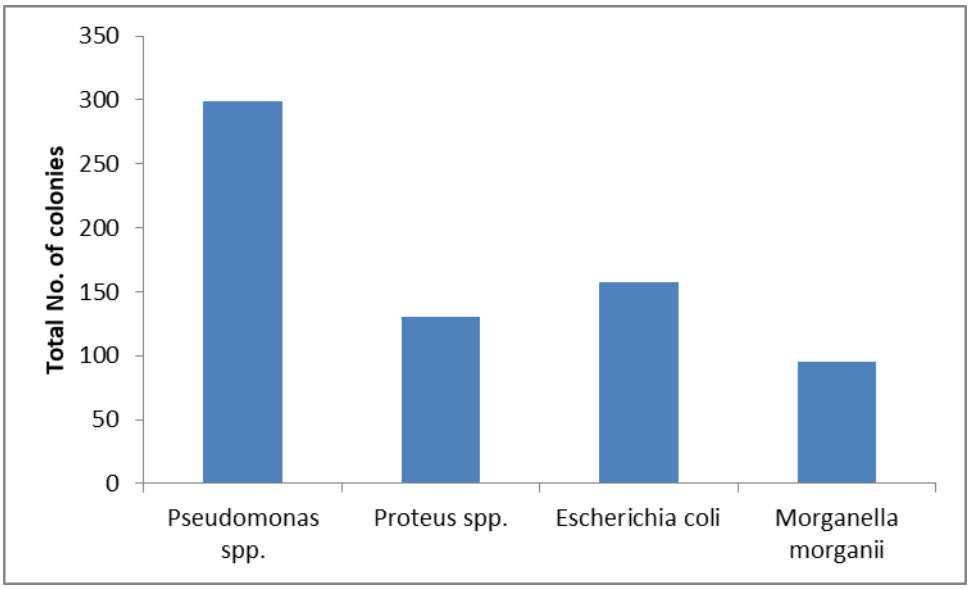

Figure 2: Percentage occurrence of bacteria isolated from smoked mackerel fish samples

Table 1: Morphological characterization of the isolated bacteria

Cellular Shape	Colony Pigmentation	Colony Appearance	Colony Surface	Suspected Organisms
Rod	Cream	Swarmy	Smooth	*Proteus spp.*
Rod	Green sheen	Shiny	Smooth	*Escherichia coli*
Rod	Off white	Opaque	Rough	*Morganella morganii*
Rod	Greenish yellow	Opaque	Flat	*Pseudomonas spp.*

Table 2: Biochemical characteristics of the bacterial isolates

Gram reactions	Ca	Co	Mo	G	SFM	SFG	IND	CIT	Ox	H_2S	Ur	Probable isolates
-	+	-	+	+	-	+	+	-	-		+	*Morganella morganii*
-	+	-	+	+	+	+	+	-	-	+	-	*Escherichia coli*
-	+	-	+	-	+	-	-	+	+	+	-	*Pseudomonas spp.*
-	+	-	+	+	+	+	-	+	-	+	+	*Proteus spp.*

Ca – Catalase, Co – Coagulase, Mo – Motility, G - Gas Production, Ind – Indole, CIT – Citrate, H_2S – Hydrogen Sulphide**,** Ur – Urease SFG - Sugar Fermentation Glucose, SFM - Sugar Fermentation Maltose, Ox – Oxidase

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