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(Research Article)

Determination of biogenic amines in dry fish samples (*Scomber scombrus)*

Oriji O. G [1] and Okpara-Akpotu J. O [2,*]

[1] *Department of Pure and Industrial Chemistry, University of Port Harcourt, River State, Nigeria.*
[2] *Department of Science Laboratory Technology, Delta State University of Science and Technology, Ozoro, Nigeria.*

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Abstract

The research focuses on the comprehensive analysis and quantification of biogenic amines in dry fish samples, an essential aspect of food safety and quality. To achieve accurate and reliable results, a robust analytical method was adopted using high-performance liquid chromatography (HPLC) equipped with DAD UV/ Vis detector. The extraction of biogenic amines from dry fish samples was optimized to ensure maximum recovery, and suitable derivatization techniques were employed to enhance the detectability of these compounds. Nine (9) biogenic amines; Histamine (HIS), Cadaverine (CAD), Tyramine (TYM), Ethylamine (ETH), Methylamine (MET), Putrescine (PUT), Typtamine (TYP), Agmatine (AGM) and Isoamlamine (ISO) were analyzed in the fish samples. The correctness and repeatability of the method were assessed by performing replicate analyses (n = 3) for two samples of dried fish from Mile 2 and Choba sites respectively. The detection levels of biogenic amines in the dried fish samples ranged from 1.mg/kg to 1.70 mg/kg for agmatine; from 0.03 mg/kg to 0.04 mg/kg for cadaverine; from 5.52 mg/kg to 5.60 mg/kg for Ethylamine; from 0.42 to 0.48mg/kg for histamine; from 7.15 mg/kg to 7.16 mg/kg for Methylamine; from 5.13 mg/kg to 5.29 mg/kg for Isoamalamine; from 0.84 mg/kg to 0.90 mg/kg for Putrescine; from 2.70 mg/kg to 2.90 mg/kg for tryptamine and from 4.26 mg/kg to 4.44 mg/kg for tyramine across all sample locations respectively. The maximum histamine, Cadaverine and Agmatine detection levels were significantly less than other bioamines detected. Nine biogenic amines were detected where Cadaverine tends to be the lowest and high Methylamine concentration was recorded in both samples, though less than those of the regulation and recommendation set by the EU regulations and national standards for fish products.

Keywords: Bioamines; Food safety; Public health; Scombroid poisoning; *Scomber scombrus*

1. Introduction

Biogenic amines (BAs) are organic nitrogenous substances that are primarily created by the decarboxylation of the corresponding amino acids. Several biological occurrences are caused by BAs. However, if the concentration of BAs reaches a certain point, it might lead to mild to severe health issues among individuals (Sivamaruthi *et al*., 2021). BAs have been reported as present in various foods where they have been linked to health damage. Two well-known BA poisonings are "scombroid poisoning" from the consumption of fish from the Scombridae family which contains histamine and "cheese reaction" from cheese consumption related to tyramine. The first reported case of BA poisoning occurred in 1967 in the Netherlands and involved Gouda cheese (Linares *et al*., 2016)

Fish is regarded as a significant source of high biological value protein with low cholesterol, rich in trace elements, and vitamins. Fish is also regarded as being necessary for a healthy existence due to their amount of n-3 and n-6 polyunsaturated fatty acids (PUFA), particularly docosahexaenoic and eicosapentaenoic fatty acids (Brito *et al*., 2019; Lira *et al*., 2019, 2020). Fish is a very perishable item that must be handled and preserved carefully if it is to have a long shelf life, maintain a desired quality, and retain its nutritional content (FAO, 2010). A simple dietary option with a longer

* Corresponding author: Okpara-Akpotu J. O

shelf life than fresh fish, dry fish is a crucial source of protein and minerals in Nigeria. In contrast, the storage and processing of dried fish can result in the formation of bioamines such histamine, putrescine, and cadaverine, which could be harmful to consumers' health. Many civilizations have a strong tradition of eating dried fish as a long-lasting, nutrient-rich substitute for fresh seafood. The existence of bioamines, however, is a vital component that requires focus with regards to dry fish.

Fishes are vital providers of animal protein and other essential nutrients for the maintenance of healthy bodies, and they play a significant part in human diets (Ravichandran *et al*., 2012). Fish must be preserved because it is a food that loses quality rapidly after obtained (Musa *et al*., 2010; Khan and Khan, 2001). Fish is preserved in many ways around the globe, including drying, salting, and smoking, to increase its shelf life. For the majority of traditional fishing groups in tropical nations like Bangladesh, fish drying is an essential method of fish preservation (Balachandran, 2001). If not properly controlled, bioamines found in a variety of meals, including fish, meat, and their products, can have negative effects. more specifically Histamine.

According to Ekici and Omer (2019), the intestinal detoxification process' effectiveness and the presence of additional BAs determine the toxicity rate of BAs, which is exceedingly difficult to determine. In order to assure compliance with food safety requirements and protect the public health, specific bioamine levels in dry fish products must be determined. The findings of this research contribute significantly to the existing knowledge on bioamines in dry fish samples (*Scomber scombrus*) collected from two retail marketplaces in Port Harcourt, Nigeria, offering valuable insights to food regulatory authorities, food industry professionals, and consumers.

2. Material and methods

2.1. Collection of samples

Dry fish samples were purchased at local retail markets from two different sites in Port-Harcourt, Nigeria (Okwelle water side, (Mile 2 Diobu), Port Harcourt and Choba market, near Choba river, (Obio/Akpor), Dried fish samples were brought by tightly packing in polyethylene bags and stored at room temperature (24-29 ^{o}C) until further analysis. The samples were transported to the laboratory using portable coolers (4 ± 1 °C) and immediately homogenized (100 g) and stored at −20 °C. All the samples were analyzed within a week after the purchase of the products or before end of shelf-life period.

2.2. Biogenic amine analysis

2.2.1. Standards and reagents:

The standard stock solution (1000 mg/L) for each of the eight biogenic amines was prepared in ultrapure water using tryptamine hydrochloride, phenylethylamine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride. The working solution (100 mg/L) containing the mixture of eight biogenic amines was prepared by diluting the stock solution with 0.4 M perchloric acid. 1,7-Diaminoheptane was used as an internal standard. All chemicals used in study were of HPLC grade and purchased from Sigma Aldrich (Seoul, Korea).

2.2.2. Bioamines Extraction and Derivatization of Sample Extracts

The nine (9) biogenic amines (Histamine, Ethylamine, Cadaverine, Putrescine, Methylamine, Typtamine, Tryamine, Agmatine, Isoamlamine were detected and quantified using high-performance liquid chromatography equipped with a diode array detector (WR G7115 A, Agilent Technologies, Santa Clara, CA, USA) at 254 nm, as described in (Eerola *et al*.,1993)**.** The extraction of BA's was carried by homogenizing each fish sample (5g) with 20 mL of 0.4 M perchloric acid followed by centrifugation (3000 rpm, 10 min, 4 °C). A second extraction was performed again with 20 mL 0.4M perchloric acid and both extracts were pooled together and the total volume was made up to ;50mL with 0.4M perchloric acid. The extract was further filtered through Whatman paper No.1 (0.2µm). Before derivatization, the extract/standard solutions (1ml) were initially alkalinized by adding 200µl 2M NaOH and 300µl saturated sodium bicarbonate. To this, 2 mL of dansyl chloride (1% *w*/*v* in acetone) was added and allowed to react in a dark room at 40 °C, 45 min with intermittent stirring. For removal of the residual dansyl chloride; 100µl of 25% ammonium hydroxide was added to the mixture and kept in room temperature for 30 min. The sample volume was made up to 5 ml with acetonitrile and centrifuged at 3000 rpm for 10 min at 4 °C. The final supernatant was filtered using 0.2µm membrane filter (Sartorius, Goettingen, Germany) and stored at −25°C until HPLC analysis.

2.2.3. HPLC-DAD Analysis

HPLC analysis was performed using a liquid chromatography (Agilent 1260 infinity II Series HPLC, Santa Clara, CA, USA) equipped with a 1260 DAD UV/Vis detector (WR G7115A) according to (Eerola *et al*.,1993). The chromatographic separation was done using Nova-Pak C18 column (particle size 4μm, 3.9 × 150 mm; Waters, Milford, MA, USA) at 40 °C, with an injection volume of 20μL. The mobile phase comprised of 0.1 M ammonium acetate (Solvent A) and 100% acetonitrile (Solvent B) processed in a gradient mode at the flow rate of 1 mL/min. For the gradient, the initial was set at 50% acetonitrile and raised to 90% after 19min. Revert to initial settings was achieved in one minute and equilibrated for 10 min until the next run. The compounds were quantified using internal calibration curves plotted for each BA and expressed as mg/kg (wet weight).

2.2.4. Statistical analysis

All experiments were carried out in triplicate. Data were subjected to a one-way analysis of variance (ANOVA). Duncan multiple range test was used to separate means of Biogenic Amines at p<0.05 level of significance. All statistical analyses were performed using SPSS Version 23.0 for Windows (SPSS I nc., Chicago, I L, USA).

3. Results and discussion

The level of Biogenic amines in dry fish samples in both sites were presented in Table 1. Significant (p<0.05) differences between biogenic amine contents in fish samples were identified. Biogenic amine levels were low or moderate in both dry fish samples. The concentration of Bioamines of fish product samples showed less than 10 mg/kg of each biogenic amine. Statistically, there is significant differences between all Biogenic amines tested in this study. Cadaverine and putrescine are important in foods, especially fish and fish products because they have been shown to potentiate the toxicity of histamine.

In figure 1 below, it was generally observed that at the concentration points of 0.42 ± 0.46, 5.60± 0.61, 0.03± 0.00, 0.84 ± 0.05, 7.15 ± 0.16, 2.90 ± 0.01, 4.26 ± 0.26, 1.66 ± 0.03 (mg/kg) and 5.13± 0.00 mg/kg respectively, nine biogenic amines were detected where Cadaverine tends to be the lowest with a concentration point of 0.03± 0.00 mg/kg and Methylamine tends to be the highest with a concentration point of 7.15 ± 0.16 mg/kg. In figure 2, it was generally observed that at the concentration points of 0.48± 0.02, 5.52 ± 0.04, 0.04 ± 0.01, 0.90± 0.00, 7.16± 0.20, 2,.70± 0.02, 4.44± 0.04, 1.70± 0.03, and 5.29± 0.03 (mg/), several biogenic Amines were detected, where cadaverine tends to be the lowest with a concentration point of 0.04 ± 0.01 mg/kg and methylamine tends to be the highest with a concentration point of 7.16± 0.20mg/kg. The results demonstrate that samples in this study were below the legal limit for Bioamines levels.

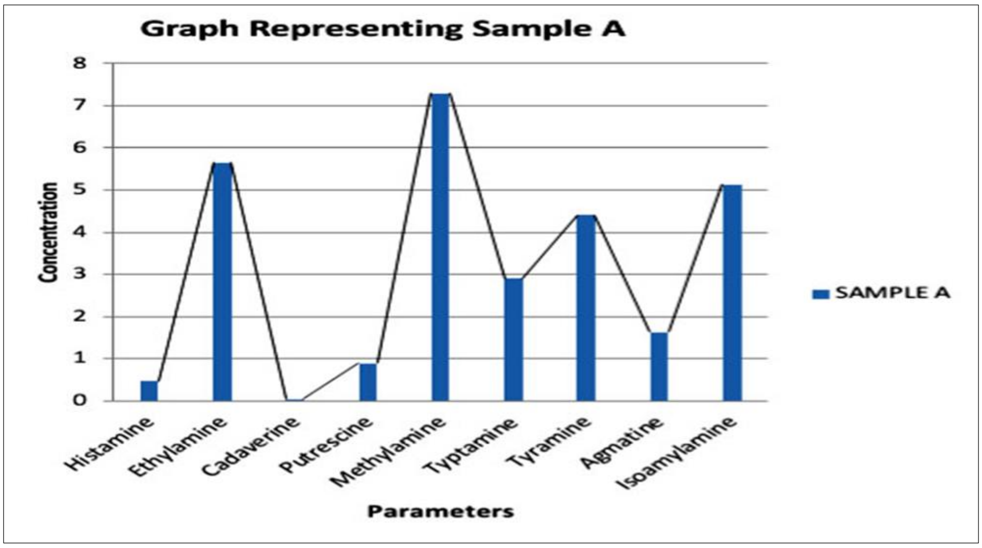

Figure 1 Graphical Representation for the Biogenic amines levels in Sample A (Mile 2)

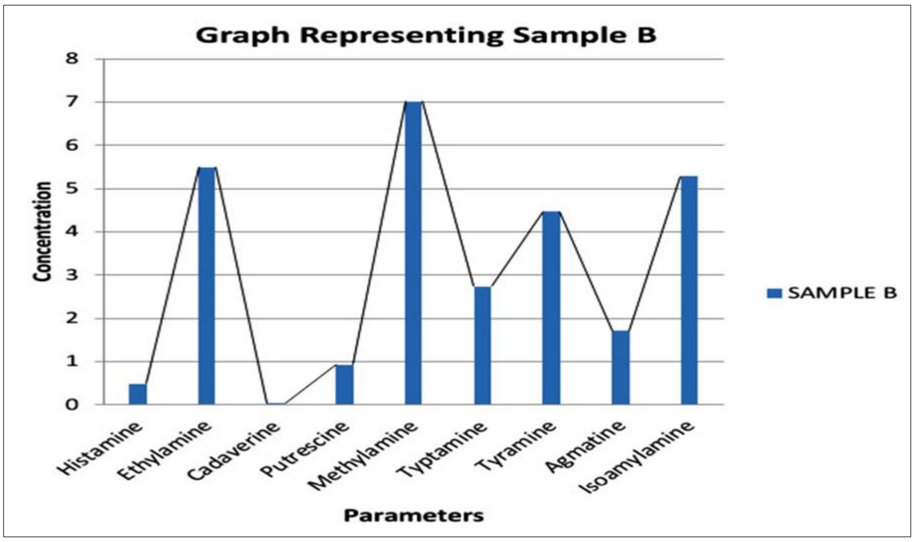

Figure 2 Graphical Representation for the Biogenic amine's levels in Sample B (Choba Market)

Table 1 Biogenic Amine levels in *Scomber scombrus* (mg/kg, mean ± SD)

Bioamines	Sample A (Mile 2)	Sample B (Choba market)
Histamine (HIS)	0.42 ± 0.46[h]	0.48 ± 0.02[h]
Ethylamine (ETH)	5.60 ± 0.61[b]	5.52 ± 0.04[b]
Cadaverine (CAD)	0.03 ± 0.00[i]	0.04 ± 0.01[i]
Putrescine (PUT)	0.84 ± 0.05[g]	0.90 ± 0.00[g]
Methylamine (MET)	7.15 ± 0.16[a]	7.16 ± 0.20[a]
Typtamine (TYP)	2.90 ± 0.01[e]	2.70 ± 0.02[e]
Tryamine (TRY)	4.26 ± 0.26[d]	4.44 ± 0.04[d]
Agmatine (AGM)	1.66 ± 0.03[f]	1.70 ± 0.03[f]
Isoamlamine (ISO)	5.13 ± 0.00[c]	5.29 ± 0.03[c]

Different lowercase letters within each sample column indicate significant differences between biogenic amines in fish samples ($p<0.05$).

3.1. Contents of BAs in the examined fish samples

Histamine concentration for both dried fish samples (Choba and mile 2) showed average mean of 0.42 ± 0.46 and 0.48 ± 0.02 mg/kg respectively and were below the maximum requirement of 200 mg/kg. The recorded concentration Histamine in the current work were comparable to that recorded in (Thadhani and Peiris 2001) Histamine levels in live seafood are generally low, as shown by several authors who measured histamine concentrations in freshly caught fish: values below 1 mg/kg were found in scombroid species, such as the skipjack (*Katsuwonus pelamis*) (Mazorra - Manzano *et al*.,2000) whereas, in non-scombroid fish, such as in hake, (*Merluccius merluccius*), no histamine was found at all, While, lower HIS levels were recorded in Tilapia in a study conducted in Poland (Kulawik *et al*., 2013). In literature, there are nearly comparable outcomes for histamine levels in fish products.

The result in Table 1 showed relatively high levels of Ethylamine (5.60 ± 0.61 and 5.52 ± 0.04 mg/kg), Isoamalamine (5.13± 0.00 and 5.29 ± 0.03 mg/kg) and Tryamine (4.26± 0.26 and 4.44 ±0.04 mg/kg) across both sample sites respectively and were below the allowable limits. Specifically, Methylamine with mean values for fish samples (7.15 ± 0.16 and 7.16 ± 0.20 mg/kg) showed elevated levels compared to the other Bioamines. It is obvious from the outcomes exhibited in Figure 1 and 2 that the highest levels of Bioamines present in both samples were detected in methylamine. The obtained results show that Cadaverine and Putrescine had the lowest levels with mean values of (0.03 ± 0.00 and

0.04 ± 0.01 mg/kg) and (0.84 ± 0.05 and 0.90 ± 0.00 mg/kg) respectively. The prevalence of bioamine contamination in fish products may be related to the levels of additives used in processing.

4. Conclusion

Food safety is crucial and needs to be prioritized on a global scale. The demand for food is rising rapidly along with the global population. The safety of food products may be compromised by this demand, especially in underdeveloped nations where food safety regulations may not be fully adhered to. According to the study's findings, checking fish products for biogenic amines can serve as an indicator of both quality and safety. There is a shortage of information on levels that may be used as guidelines in the various products that are offered on the market, despite their importance for both public health and meat shelf-life. Currently, only HIS is regulated for BA, at least within the European Union, and only for fish and fish products. Despite the fact that the biogenic amine levels in the dry fish samples were similar, they were all below what the EU considers to be an acceptable level of contamination in fish. In this investigation the dry fish samples were discovered to have substantially lower levels of BA than the recommended values and can thus be regarded as safe for human consumption. To protect public health, it is important to always verify the product's quality and safety.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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