

Research Article

Monitoring the Levels of Biogenic Amines in Canned Fish Products Marketed in Ghana

Alexander Weremfo ¹, Meinster Kodjo Eduafo,² Hakim Agyei Gyimah,¹
and Samuel Abassah-Oppong¹

¹Department of Biochemistry, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana

²Histamine and Mycotoxins Laboratory, Ghana Standards Board, Accra, Ghana

Correspondence should be addressed to Alexander Weremfo; aweremfo@ucc.edu.gh

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An ion-pair HPLC method with postcolumn o-phthalaldehyde (OPA) derivatization and fluorescence detection was validated for quantitative determination of five biogenic amines (histamine, tyramine, cadaverine, putrescine, and agmatine) in canned fish products (mackerel, sardine, and tuna) marketed in Ghana. The validated method exhibited excellent selectivity and good linearity ($R^2 > 0.9990$) for all the amines. The limits of detection and quantification for studied biogenic amines were in the range of $0.32\text{--}0.78\text{ mg}\cdot\text{kg}^{-1}$ and $1.10\text{--}2.57\text{ mg}\cdot\text{kg}^{-1}$, respectively. Also, a satisfactory recovery was obtained for all the amines (82.1–101.4%), and the relative standard deviations were lower than 9.3% under repeatability conditions for the studied amines. Subsequently, the method was applied to the analysis of biogenic amines in canned fish products to estimate the safety of Ghanaian consumers. The maximum levels of histamine, tyramine, cadaverine, putrescine, and agmatine detected in the analysed canned fish products were $64.05\text{ mg}\cdot\text{kg}^{-1}$, $27.44\text{ mg}\cdot\text{kg}^{-1}$, $27.23\text{ mg}\cdot\text{kg}^{-1}$, $18.74\text{ mg}\cdot\text{kg}^{-1}$, and $52.72\text{ mg}\cdot\text{kg}^{-1}$, respectively. Thus, the levels of biogenic amines detected in the canned fish products were lower than the acceptable levels and, therefore, can be considered relatively safe for human consumption.

1. Introduction

Fish is an important source of dietary proteins, minerals, and vitamins and has become a necessity in many households globally. It provides a unique and well-balanced source of nutrients for persons of all ages. Fish also forms an integral part of regular and therapeutic diets due to their low caloric value, ease of digestibility, moderate cost, and high nutritional content. However, fish is a highly perishable food commodity, which deteriorates soon after death, if not properly preserved. They are extremely susceptible to biogenic amine formation due to the metabolism of spoilage microbes, which lead to loss of quality and spoilage [1, 2]. Hence, analysis of spoilage metabolites (biogenic amines) is significantly considered as a quality index and gives more information about eating quality and freshness of fish [1, 3].

Biogenic amines (BAs) are low molecular weight organic bases that are formed in foods by microbial decarboxylation of certain amino acids or by transamination of aldehydes and ketones by amino acid transaminases [4, 5]. Amines such as histamine, tyramine, tryptamine, putrescine, cadaverine, agmatine, spermine, and spermidine are frequently observed in foods such as fish, meat, eggs, cheese, fruits, vegetables, beer, and wine [6–8]. Biogenic amines are normally present at very low concentrations in fresh foods especially fish. High levels of BAs may be found in food as a result of the use of poor-quality raw materials, contamination, and inappropriate conditions during food processing and storage. Bacterial growth results in gradual accumulation of BAs, and high levels are indicative of microbial spoilage.

The presence of BAs in foods is of public health importance not only due to their potential toxicity but also due

to their usage as indicators for quality of food products [9]. Low levels of BAs in food products do not present a serious risk to human health as the amine oxidases in the human intestine can rapidly detoxify the amines. However, their consumption in large quantities is known to cause deleterious effects in human beings [5]. Common symptoms of BAs intoxication in human are nausea, respiratory distress, hot flushes, sweating, heart palpitations, headaches, a bright red rash, oral burning, hypertension, and hypotension. Histamine and tyramine are the main causes of numerous cases of food intoxication. However, other amines such as putrescine and cadaverine can potentiate the undesirable effects of tyramine and histamine by inhibiting metabolizing enzymes such as monoamine or diamine oxidase and histamine methyltransferase [5, 10]. Furthermore, other biogenic amines such as putrescine, cadaverine, agmatine, and spermidine may react with nitrite to form carcinogenic nitrosamines [5, 11]. In addition, BAs such as histamine, putrescine, agmatine, and cadaverine have been used as indicators of spoilage in foods such as fish and meat products [7, 12]. Nout indicated that the maximum allowable level of histamine and tyramine in foods should be in the range of 50–100 mg/kg and 100–800 mg/kg, respectively [13]. For the US Food and Drug Administration (FDA) and European Union (EU), the acceptable levels of 50 mg/kg [14] and 100 mg/kg [15], respectively, have been established for histamine in the edible portion of fish. According to Codex standard [16], which Ghana subscribes to, 100 mg/kg of histamine in fish and fish products is set as the acceptable limit.

Many of the several methods that are used to quantify biogenic amines in food are based on liquid chromatography, which is highly sensitive and allows for simultaneous quantification of most biogenic amines in food [17]. Before detection, biologically active amines require chemical derivatization due to the lack of chromophore or fluorophore in their structure [18]. The most common derivatizing agents are *o*-phthalaldehyde (OPA), dansyl chloride, and benzoyl chloride. The fluorimetric detection of OPA derivatives offers higher selectivity and sensitivity for primary amines in comparison with spectrophotometric detection of other derivatives [19].

Canned fish is widely consumed in Ghana. The main fish species used in these canned products are scombroid fish (mackerel and tuna) and nonscombroid fish (sardine), which are commonly associated with histamine fish poisoning [5, 20]. The use of unwholesome fish as raw materials for canning or poor hygienic conditions of the fish processing and storage increase the levels of biogenic amines in canned fish products [21, 22]. Furthermore, biogenic amines are nonvolatile and heat resistant and as such can survive food processing conditions such as cooking, freezing, canning, and smoking [22]. To date, there is no information regarding the levels of biogenic amines in canned fish products marketed in Ghana. Considering the toxicological implications of biogenic amines and the general interest in occurrence data for risk assessment of fishery products, the aim of the present study is to use an ion-pair HPLC method with postcolumn OPA derivatization and fluorescence

detection to quantitatively determine biogenic amines (histamine, cadaverine, tyramine, putrescine, and agmatine) in canned fish products marketed in Ghana to estimate their safety for human consumption. This method is based on the procedure previously developed for determination of biogenic amines in vegetable products [23]. Fishery products have a variety of matrices and therefore the suitability of the method for determination of the biogenic amines is investigated in three fish products, namely, canned mackerel, canned tuna, and canned sardine.

2. Materials and Methods

2.1. Chemicals. All chemicals and reagents used were of analytical or HPLC grade or equivalent. Biogenic amine standards such as histamine hydrochloride, putrescine dihydrochloride, tyramine hydrochloride, cadaverine dihydrochloride, and agmatine sulphate were obtained from Sigma (USA). Perchloric acid and boric acid were also obtained from Sigma (France). *O*-phthalaldehyde (OPA), mercaptoethanol, polyoxyethylene (Brig-35), and sodium octanesulfonate were obtained from Merck (Germany). Double distilled water was used to prepare solutions.

2.2. Sample Preparation. Fish products including canned mackerel (21 samples), canned sardine (14 samples), and canned tuna (8 samples) from different manufacturers were purchased from retail markets in Accra, Ghana, in February 2017. After opening each can, the content was homogenised using a blender and immediately subjected to the extraction procedure.

2.3. Preparation of Biogenic Amine Standards. Histamine dihydrochloride (16.5 mg), putrescine dihydrochloride (18.3 mg), tyramine hydrochloride (12.7 mg), cadaverine dihydrochloride (17.1 mg), and agmatine sulphate (17.5 mg) were dissolved in 10 mL of 0.6 M perchloric acid and used as the working solution. The final concentration of each biogenic amine (free base) was 1 mg/mL.

2.4. Extraction of Biogenic Amines. Extraction of biogenic amines from fish samples was carried out as described recently with modification [23]. In brief, 5 g of homogenised fish sample was extracted two times with 10 mL of 0.6 M perchloric acid. The mixture was vortexed for 5 min and centrifuged at 4400g for 10 min at 4°C. The supernatants collected were combined and filtered through Whatman paper no.1. The final volume was adjusted to 25 mL with 0.6 M perchloric acid.

2.5. Chromatographic Conditions. The quantification of biogenic amines was carried out using an HPLC unit that consisted of an LC-20AD pump coupled to a RF-20A fluorescence detector, SIL-20A HT auto sampler, DGU-20A5 degasser, and CBM-20A communication bus module (Shimadzu, Kyoto, Japan) and detected fluorometrically after postcolumn derivatization with OPA.

Amines were separated using a Teknokroma Tracer Excel (15 cm × 0.46 cm; 5 μm) column and were eluted with a mobile phase consisted of eluent A as a solution of 0.1 M sodium acetate and 10 mM sodium octanesulfonate adjusted to pH 4.5 with acetic acid; and eluent B was a mixture of solvent B-acetonitrile (6.6:3.4), where solvent B was a solution of 0.16 M sodium acetate and 10 mM sodium octanesulfonate solution adjusted to pH 4.5 with acetic acid [23]. The gradient elution was set for a linear gradient as shown in Table 1. The flow rate was 1.2 mL/min, and the column temperature was set as 40°C. A postcolumn derivatization reaction was performed using a reagent which was prepared as follows: 30.9 g of boric acid and 20.0 g of potassium hydroxide were dissolved in 500.0 mL of water and 1.5 mL of 30% Brij and 1.5 mL of 2-mercaptoethanol as a reducing agent were added; finally, 0.5 g of OPA dissolved in 5.0 mL of methanol was added to the above solution. The derivatization reagent was delivered at the flow rate of 0.7 mL/min. Automatic injection of 10 μL of the standard solution and samples was carried out. The fluorescence detection was performed at an excitation wavelength of 350 nm and emission wavelength of 450 nm.

2.6. Statistical Analysis. All statistical tests were performed using the Statistical Software Package for Window SPSS, Version 11.0 (SPSS, Chicago, IL, USA).

3. Results and Discussion

3.1. Method Validation. Biogenic amines (histamine, cadaverine, tyramine, putrescine, and agmatine) in canned fish samples were analysed using ion-pair HPLC with post-column derivatization and fluorimetric detection. The counterion octanesulfonate was added to the mobile phase to enhance amine interaction with the column and improve separation from matrix interferences [24, 25]. In this study, fluorescence detection was used after postcolumn derivatization with o-phthalaldehyde to enhance the detection and identification of histamine and other amines that do not have chromophores (i.e., putrescine and cadaverine) [19, 25]. A derivatization reagent, o-phthalaldehyde was used due to its high selectivity for amines when compared with other reagents such as dansyl chloride and fluorescamine [23].

A typical chromatographic profile of the five standard biogenic amines by the gradient elution system is shown in Figure 1(a). The retention time for all the amines was stable and consistently reproducible. The separation of the amines was achieved in less than 30 min run time with good peak resolution, sharpness, and symmetry. The analytical method was validated in terms of specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. Tables 2–4 give an overview of the performance of the ion-pair HPLC-fluorescence method. The specificity of the method was validated by comparing the peaks of the amine's standards in a solvent (0.6 M perchloric acid) to that spiked to the fish (matrix). As shown in Figure 1, no

TABLE 1: HPLC gradient condition for the separation of biogenic amines.

Time (min)	Mobile phase composition	
	Eluent A (%)	Eluent B (%)
0.1	85	15
13.0	50	50
20.0	27	73
22.6	27	73
22.7	5	95
29.0	5	95
30.0	85	15
35.0	85	15

Eluent A = 0.1 M sodium acetate and 10 mM sodium octanesulfonate (pH 4.5). Eluent B = eluent A and acetonitrile (6.6:3.4).

interfering peaks, either from derivatised amino acids or secondary by-products of the OPA, appeared at the retention times of the analytes (20.0–27.5 min).

The linearity of the calibration curves was determined from the mixed standard amine solutions (0–30 mg·kg⁻¹). A good linearity between the peak area and concentration ($R^2 > 0.9990$) was obtained for all the amines. Matrix effect was investigated by means of statistical comparison of the slopes of calibration curves in the solvent and in the matrix (tuna, mackerel, and sardine). The slopes were not significantly different at a 95% confidence level ($P > 0.05$) for all the amines, clearly showing the fish components had minimal effect on the analytical responses. The observed minimal effect may be probably due to the dilution and protein precipitation techniques employed, which have been shown to adequately reduce matrix effect [26]. LOD and LOQ values were obtained using the following equations: [27] $LOD = (3S_a/b)$ and $LOQ = (10S_a/b)$, in which S_a is the standard deviation of the intercept (response) and b is the slope of the calibration curve obtained from the standard amine solutions. As shown in Tables 2–4, the values of LOD ranged from 0.32 to 0.78 mg·kg⁻¹ whilst that of LOQ varied from 1.10 to 2.57 mg·kg⁻¹.

The accuracy of the method was evaluated by means of a spiking and recovery study on canned fish samples. The spiking levels of amine standards were 10 mg·kg⁻¹, 50 mg·kg⁻¹, and 100 mg·kg⁻¹ in each type of fish sample. The analysis was conducted fivefold. As shown in Tables 2–4, a satisfactory recovery (82.1–101.4%) was obtained for the amines in the three matrices (tuna, mackerel, and sardine). The method exhibited good repeatability of the peak area as the relative standard deviation (RSD) obtained for each amine (both intraday and interday) was less than 9.3%. These results indicate that the extraction procedure and the quantification by the ion-pair HPLC method with post-column derivatization and fluorimetric detection was appropriate for the determination of the five biogenic amines in canned fish.

3.2. Biogenic Amines in Canned Fish Products. Forty-three samples of canned fish products (canned sardines, canned mackerel, and canned tuna) were analysed for biogenic amines using the validated method. Figure 1(c) shows a

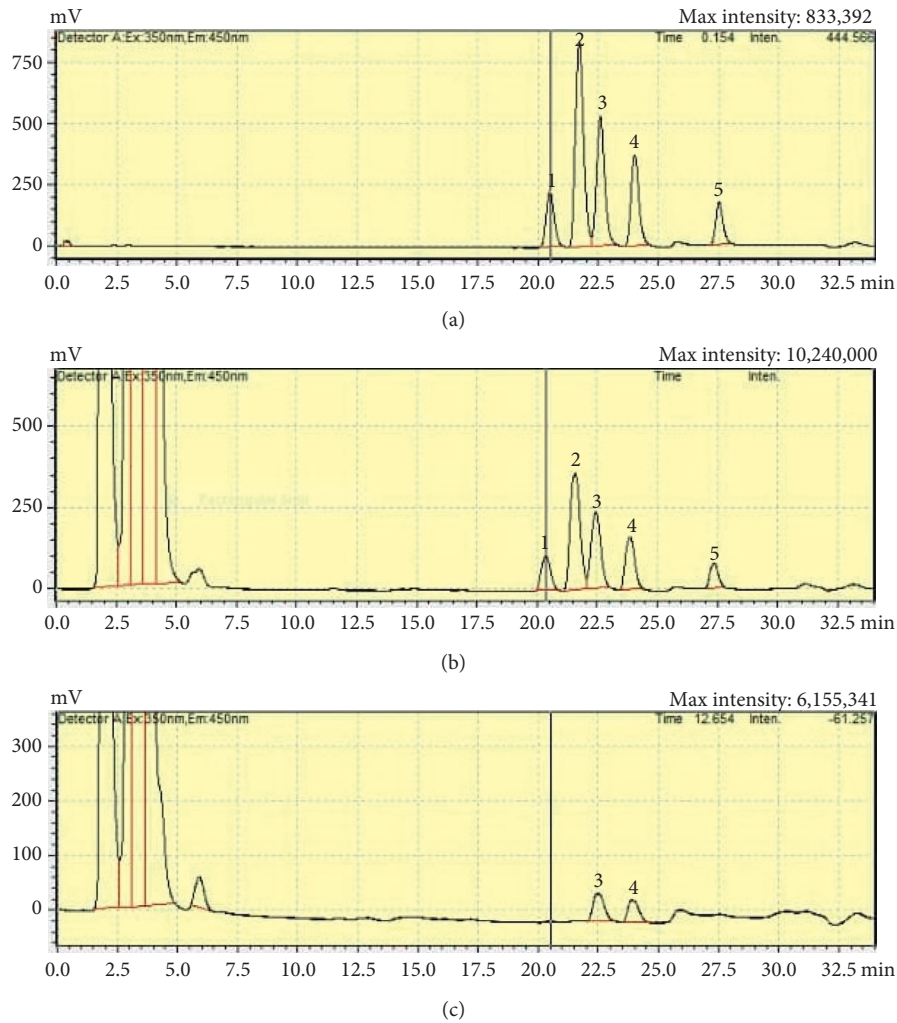


FIGURE 1: Typical HPLC chromatograms of (a) standard mixture of biogenic amines ($30 \text{ mg}\cdot\text{L}^{-1}$), (b) mixed biogenic amines ($50 \text{ mg}\cdot\text{kg}^{-1}$) in the spiked canned fish sample, and (c) biogenic amines in the extracted canned fish sample. Peak identity: tyramine (1), putrescine (2), cadaverine (3), histamine (4), and agmatine (5).

TABLE 2: Performance of the ion-pair HPLC method for biogenic amine determination in canned tuna.

Analyte	Regression equation	R^2	LOD (mg/kg)	LOQ (mg/kg)	Repeatability (%)		Recovery (%)		
					Intraday	Interday	<i>a</i>	<i>b</i>	<i>c</i>
CAD	$Y = 668196x - 566250$	0.9997	0.42	1.41	5.6	8.5	92.4	96.8	95.4
PUT	$Y = 931609x - 5580$	0.9998	0.47	1.58	7.3	6.5	95.9	101.4	97.2
HIS	$Y = 414513x - 212445$	0.9998	0.39	1.29	4.4	4.1	93.2	97.4	94.7
TYR	$Y = 217003x + 56548$	0.9991	0.78	2.53	2.8	1.2	95.7	96.3	92.5
AGM	$Y = 166697x - 67346$	0.9994	0.60	2.00	9.2	6.1	88.9	95.6	90.7

R^2 , square of regression coefficient; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; TYR, tyramine; PUT, putrescine; CAD, cadaverine; HIS, histamine; AGM, agmatine; *a*, 10 mg/kg; *b*, 50 mg/kg; *c*, 100 mg/kg.

TABLE 3: Performance of the ion-pair HPLC method for biogenic amine determination in canned mackerel.

Analyte	Regression equation	R^2	LOD (mg/kg)	LOQ (mg/kg)	Repeatability (%)		Recovery (%)		
					Intraday	Interday	<i>a</i>	<i>b</i>	<i>c</i>
CAD	$Y = 754848x - 566250$	0.9996	0.50	1.66	4.8	7.6	82.9	83.6	90.8
PUT	$Y = 1046890x - 908786$	0.9997	0.43	1.43	8.9	7.7	93.4	91.6	97.3
HIS	$Y = 393695x - 286586$	0.9999	0.32	1.10	5.5	3.5	90.5	93.6	89.4
TYR	$Y = 242031x + 96700$	0.9995	0.54	1.82	8.2	3.5	92.3	96.0	94.6
AGM	$Y = 162774x - 86120$	0.9995	0.57	1.88	6.8	7.7	93.2	90.6	91.6

TABLE 4: Performance of the ion-pair HPLC method for biogenic amine determination in canned sardine.

Analyte	Regression equation	R^2	LOD (mg/kg)	LOQ (mg/kg)	Repeatability (%)		Recovery (%)		
					Intraday	Interday	<i>a</i>	<i>b</i>	<i>c</i>
CAD	$Y = 758719x - 784386$	0.9998	0.33	1.10	5.2	3.0	85.4	82.1	87.5
PUT	$Y = 1016090x - 1050750$	0.9998	0.38	1.26	3.8	5.8	88.9	92.4	90.5
HIS	$Y = 423044x - 173483$	0.9997	0.43	1.43	4.4	3.9	85.2	90.3	88.5
TYR	$Y = 217003x + 56548$	0.9991	0.76	2.53	4.8	2.9	87.9	92.5	91.3
AGM	$Y = 163470x - 74402$	0.9997	0.47	1.57	4.5	4.3	86.2	87.4	89.8

TABLE 5: Range and mean of five biogenic amines (mg kg⁻¹) in canned fish products in Ghana.

Samples (<i>n</i>)	TRY	PUT	CAD	HIS	AGM
Tuna (8)	ND	ND–8.05 (2.63)	ND–14.48 (2.90)	ND–26.12 (5.22)	ND
Sardine (14)	ND–27.44 (4.76)	ND–18.74 (3.10)	ND–5.90 (0.42)	ND	ND–52.72 (5.90)
Mackerel (21)	ND–17.72 (4.07)	ND–5.85 (1.31)	ND–27.23 (2.80)	ND–64.05 (5.65)	ND–28.69 (3.65)
Total (40)	ND–27.44 (2.80)	ND–18.74 (2.10)	ND–27.23 (1.98)	ND–64.05 (3.62)	ND–52.72 (3.97)

n, number of samples analysed; ND, not detected; TYR, tyramine; PUT, putrescine; CAD, cadaverine; HIS, histamine; AGM, agmatine.

chromatogram of biogenic amines in a canned fish sample. The biogenic amines in the canned fish samples were identified based on the retention time by comparison with standard solutions. The levels of biogenic amines in the canned fish samples are summarised in Table 5. All the biogenic amines were not detected in all the canned fish products. Tyramine was detected in 42.5% of the canned fish samples, followed by putrescine (37.5%), agmatine (17.5%), histamine (15%), and cadaverine (12.5%). In general, the canned fish products analysed contained low levels of biogenic amines. The maximum concentrations of histamine (64.05 mg·kg⁻¹) and tyramine (27.44 mg·kg⁻¹), which are the amines with toxicological effects, were detected in mackerel and sardine, respectively. Though histamine was not detected in canned sardines, its detected level ranged from ND–64.05 mg·kg⁻¹ in canned mackerel and ND–26.12 mg·kg⁻¹ in canned tuna products. None of the samples analysed contained histamine levels higher than 100 mg·kg⁻¹, which is the limit established by the European Union [15]. However, one of the canned mackerel had the histamine level of 64.05 mg·kg⁻¹ which is above 50 mg·kg⁻¹ limit established by the FDA [14]. Tyramine was detected in canned mackerel (ND–17.72 mg·kg⁻¹) and canned sardines (ND–27.44 mg·kg⁻¹) but not in canned tuna samples. The level of tyramine in all the samples analysed was less than the recommended level (100 mg/kg) [28].

Although putrescine, cadaverine, and agmatine have no documented adverse health effects, they can potentiate the toxic effects of tyramine and histamine and may also react with nitrite to form carcinogenic nitrosamine [5, 28]. In addition, they have been proposed as spoilage indices in fish and other products [29, 30]. The maximum levels of cadaverine (27.23 mg·kg⁻¹), putrescine (18.74 mg·kg⁻¹), and agmatine (52.72 mg·kg⁻¹) were detected in mackerel and sardine, respectively. Though cadaverine and putrescine were detected in canned sardine, tuna, and mackerel, agmatine was detected only in canned sardines and mackerels. In general, the canned fish products analysed in this study contained low levels of amines. The possible cause of the small variations of biogenic amines in the fish products may be attributed to several factors including raw materials, processing conditions, growth kinetics of microorganisms,

and their proteolytic and decarboxylase activities which affect the production of biogenic amines in food [31]. Similar results have been reported [5, 6, 8, 32], where relatively low levels of these biogenic amines were found in canned fish. These findings indicate that the fish used for these products were fresh, and the products were produced under good manufacturing practices.

4. Conclusion

An HPLC method with fluorescence detection after post-column derivatization with OPA was validated for the quantitative determination of five biogenic amines (HIS, TYR, CAD, PUT, and AGM) in canned fish products. The method showed high accuracy, sensitivity, and selectivity irrespective of the characteristics of the fish matrix. Biogenic amines detected in the canned fish products ranged ND–64.05 mg·kg⁻¹ for HIS, ND–27.44 mg·kg⁻¹ for TYR, ND–27.23 mg·kg⁻¹ for CAD, ND–18.74 mg·kg⁻¹ for PUT, and ND–52.72 mg·kg⁻¹ for AGM. In general, the levels of histamine and tyramine detected in the canned fish products were within the acceptable ranges and, therefore, can be considered relatively safe for human consumption. However, one of the canned mackerel had relatively high level of histamine (64.05 mg·kg⁻¹); hence, the product must be monitored carefully to ensure its safety for human consumption.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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