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Original Research

Occurrence and Control of Biogenic Amines in Fresh Fish and Products of Fish

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Abstract

In this study, the detection of biogenic amines in fresh and products of fish is focused and biological trials have been used to reduce their levels. 120 random samples of fresh fish (*Lates niloticus*), sardine salted, herring smoked, and tuna canned (for every thirty samples) were collected from different markets in Shibine, Menoufia, Egypt. The biogenic amine residues have examined in all samples that were collected (histamine, putrescine, cadaverine and tyramine). The average levels of biogenic amines in the samples under investigation of fresh fish, canned tuna, herring smoked, and sardine salted are 10.74±0.54, 15.08±0.76, 17.93±0.81 and 26.12±0.89 mg % for histamine, 7.61±0.49, 11.13±0.57, 14.35±0.64 and 19.59±0.81 mg% for putrescine, 5.96±0.43, 10.22±0.49, 12.41±0.58 and 16.85±0.62 mg% for cadaverine each, accordingly. Finally, the tyramine mean value was 2.05±0.10 mg % in fresh fish, 6.37±0.19 mg % in tuna canned, 7.9±0.24mg % for herring smoked and10.44±0.27 mg % for sardine salted. Fish fillets were experimentally inoculated with *B. polymyxa* culture ($2x10^7$), the effect was ideal, the level of histamine reduced to 31.4 mg/kg after eight hours, 19.5 mg/kg after 12 h and 12.8 mg/kg 24 hours later with reduction percentages of 37.2%, 61.0%, and 74.4%, respectively and cadaverine level reduced to 22.1 mg/kg after 8 hours, 15.4 mg/kg after 12 h and 9.7 mg/kg 24 h later with reductions percentages were 26.3%, 48.7%, and 67.7%, respectively.

KEYWORDS
Fish, Fish products, Biogenic amines, *B. polymyxa*

INTRODUCTION

Fish and fish products should be a component of a person's diet since fish meat has a high nutritional value (high level of polyunsaturated fatty acids, omega-3, fat-soluble vitamins, and B complex as well as iron and zinc). There are various ways to determine the shelf life of fish meat, but one of the more reliable ways is to measure the quantities of biogenic amines (El-Sayed, 2014).

Due to their significance in food safety, particularly concerning the toxic effects of histamine and it is applied as a spoilage marker in several products, BAs have drawn attention and research. Depending on the environmental circumstances in the fish's native habitat, different types of bacteria can be found in fish. and it can be either Gram-positive or Gram-negative bacteria. Although in various ways, both positive and negative bacteria, can produce decarboxylase (Abuhlega and Ali, 2022).

Moreover, histamine is one of the most significant poisons. As it builds up in some fish, it can cause negative effects in people similar to typical food allergies (Arulkumar *et al*., 2021).

The amount of free histidine in fish with red muscles ranges from 1 gram/kg in herring to 15 grams/kg in tuna. Under some circumstances, the enzymes' activity can cause the free histidine to transform into histamine (Hassan *et al*., 2017).

The formation of BAs is influenced by the raw material, processing technique, transportation, and storage circumstances. Moreover, it is impacted by the surface natural flora of the raw material, as well as fish or microbial enzymes (Ruiz-Capillas and Jiménez-Colmenero, 2005).

Eating fresh raw or tuna processed has been linked to several cases of histamine poisoning around the world (Yemmen and Gargouri, 2022).

Due to their ability to increase histamine toxicity, cadaverine and putrescine are crucial in the development of food toxicity. Those on monoamine oxidase inhibitor medications may get headaches after consuming dietary tyramine at a dose of 60 mg/kg although a hypertensive crisis will occur at 100–250 mg/kg. Some BAs have also been linked to the production of carcinogenic nitrosamines (Kočar *et al*., 2021).

It is essential to keep in mind that BAs cannot degrade when preserved using traditional methods like smoking, freezing, drying, or cooking (Houicher *et al*., 2021).

Histamine, putrescine and cadaverine are the most biogenic amines that are usually found in products of fish, and they are regarded as markers of fish meal quality and freshness. They can be caused by inadequate production procedures and improper storage during processing, and the large levels denote food spoiling by microbial infection. At low temperatures (8°C), the histamine creation in fish is delayed, however, even after four days of storage, it might reach dangerous levels. Moreover, histamine is thermally stable and won't be destroyed by high temperatures like those that occur during frying and smoking (Giva-

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noudi *et al*., 2023).

Starter cultures are essential for lowering the amount of biogenic amine in the chemicals used to protect processed foods (García-Díez and Saraiva, 2021). This study aimed at estimation of biogenic amines in *Lates niloticus*, canned tuna, smoked herring, and salted sardine. Further, the application of the bacterium Bacillus polymyxa for the reduction of such harmful amines in fish was also studied.

MATERIALS AND METHODS

Collection of samples

A total of 120 random samples of fresh fish (*Lates niloticus*), tuna canned, smoked herring, and sardine salted (30 for everyone) were collected during October 2022 to February 2023 from different markets in Shibin Elkom, Menoufia, Egypt. The collected samples were kept individually in an insulated ice box and directly transferred to the Food Safety Central Laboratory at Faculty of Veterinary Medicine, Benha University, Egypt for biogenic amines estimation.

The collected samples were subjected to examination for determination of their contents of biogenic amines (histamine, putrescine, cadaverine, and tyramine).

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Determination of biogenic amines by using HPLC

Four biogenic amines including histamine, cadaverine, putrescine, and tyramine were determined in all examined samples based on the protocol recommended by Krause *et al*. (1995) and Pinho *et al*. (2001).

Reagents preparation

All chemicals and biogenic amines standards were purchased from Merck, Darmstadt, Germany unless specified.
Dansyl chloride solution: 500 mg of dansyl chloride was dissolved in 100 ml acetone
Standard solutions: Stock standard solutions of the tested amines were prepared as the following: add 25 mg of each standard pure amine (histamine-2HCl, cadaverine– 2HCl, putrescine-2HCl) were dissolved in 25 ml distilled water individually.

Extraction of samples

Twenty-five grams of each sample were blended with 125 ml of 5% Tri chloro acetic acid (TCA) for 3 min using a warning blender then filtration was achieved using filter paper What man No1. Thus, 10 ml of the filtrate was transferred into a suitable glass tube with 4g NaCl and 1 ml of 50 % NaOH. The filtrate was extracted 3 times (2 min each) using 5 ml n-butanol: chloroform (1:1 v/v) and the upper clear layer was transferred to a 100 ml separating funnel by using a disposable Pasteur pipette. To combine the organic extracts (upper layer), 15 ml of n-heptane was added in a separating funnel and extracted three times with 1.0 ml portions of 0.2 N HCl, the HCl layer was collected in a glass Stoppard tube. The solution was evaporated just to dryness using a water bath at 95°C with aid of a gentle current of air.

Experimental part

The effect of Bacillus polymyxa as a biological test for reducing the concentrations of biogenic amines as histamine and cadaverine experimentally added to fresh fillets (*Lates niloticus*) was demonstrated as follows:

Preparation of bacterial suspension (Eom et al., 2015)

Bacillus polymyxa strain was cultivated in Brain Heart Infusion (BHI) Broth (Fluka, Sigma-Aldrich Chemie GmbH) for 24 hours at 37ºC to prepare an overnight culture. One ml of the cultivated bacterial suspension was decimally diluted in sterile peptone water (0.1%, w/v) (Merck, Darmstadt, Germany).

Throughout the plate count method the number of colonies of the Bacillus polymyxa strain was measured (A volume of the culture broth corresponding to approximately 2×10^7 bacteria was centrifuged (500 rpm, 15 minutes at 5ºC) and the bacterial pellets were washed twice with deionized water.

Binding assay (Halttunen et al., 2008)

The bacterial pellets were suspended in 2 Kg fresh fish fillets. The tested samples were divided into 3 groups; the 1st and 2nd ones were controls inoculated separately with the standard solution (50 mg/Kg histamine and 30 mg/Kg cadaverine). The 3rd group was inoculated with a mixture adjusted to reach a final concentration of 2×10^7 bacteria plus 50 mg/Kg histamine standard solution and the 4th one was inoculated with 2×10^7 bacteria and 10 mg/Kg cadaverine. Bacterial pellets and biogenic amine solution were vortexed for 5 seconds (Stuart, Staffordshire, U.K) and incubated for 24 hours on a Finemixer SH2000 orbital shaker (Finepcr, Seoul, Korea) with soft agitation.

Accordingly, the tested samples contaminated with histamine and cadaverine (without Bacillus polymyxa) served as a control assay. However, the test group represented fish fillets contaminated with histamine and cadaverine as well as treated with Bacillus polymyxa were served as treated groups. The samples were acidified with ultrapure HNO_3 and examined at zero, 8, 16 and 24-hour time points for measuring their histamine and cadaverine using A Shimadzu® model LC/10 AS, coupled to UV detector SPD/10 AVHPLC system.

RESULTS

Values for the mean histamine concentrations in Fresh fish, canned tuna, herring smoked and sardine salted were 10.74±0.59, 15.08±0.76, 17.93±0.81and 26.12±0.89 mg%, in order, however, the putrescine levels ranged from 1.0 to 21.9, the mean value was 7.61±0.49 mg% for fresh fish, 1.7 to 26.4, the average was 11.13±0.57 mg% for canned tuna, 2.1 to 30.2, the mean value was 14.35±0.64 mg% for herring smoked and 2.8 to 39.5 with an average 19.59±0.81 mg% for sardine salted. However, the cadaverine average concentrations were 5.96±0.43, 10.22±0.49, 12.41±0.58 and 16.85±0.62 mg% fresh fish, tuna canned, smoked herring, and salted sardine respectively. Finally, The tyramine averages were from 0.4 to 3.7 mg/100g, with an average of 2.05±0.10 mg % for fresh fish, 0.9 to 15.4mg/100g, with an average of6.37±0.19 mg % for tuna canned, from 1.1 to 21.3 mg/100g, with an average of7.9±0.24mg% for smoked herring and from 1.4 to 23.9 mg/100g, with an average of 10.44±0.27 mg% for sardine salted.

Additionally, using *B. polymyxa* culture ($2x10^7$) reduced histamine and cadaverine levels injected experimentally to fish fillets (50 mg/Kg, 30 mg/Kg) with reduction percent of 37.2%, 61.0%, and 74.4% after 8, 12 and 24 hours for histamine, and was 26.3%, 48.7%, and 67.7% after 8, 12 and 24 hours, respectively.

Table 1. Statistical analysis of histamine levels (mg %) in the examined samples of fish and fish products (n=30).

Fish products	Min	Max	Mean±S.E
Fresh fish (*Lates niloticus*)	1.1	24.5	10.74±0.59C
Canned Tuna	2.3	31.2	15.08±0.76B
Smoked herring	2.9	35.8	17.93±0.81B
Salted Sardine	4.1	52.6	26.12±0.89A

*Means with different letters in the same column are significantly different (P<0.05).

Table 2. Acceptability of fish products according to their histamine residues (n=30).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fresh fish (*Lates niloticus*)	20	26	86.7	4	13.3
Canned Tuna	20	23	76. 7	7	23.3
Smoked herring	20	21	70	9	30
Salted Sardine	20	16	53.3	14	46.7
Total (120)		86	71.7	34	28.3

* Maximum Residual Limit (EOS, 2010).

Table 3. Statistical analysis of putrescine levels (mg %) in the examined samples of fish and fish products (n=30).

Fish products	Min	Max	Mean±S.E
Fresh fish	1	21.9	7.61±0.49D
Canned Tuna	1.7	26.4	11.13±0.57C
Smoked herring	2.1	30.2	14.35±0.64B
Salted Sardine	2.8	39.5	19.59±0.81A

DISCUSSION

The most prevalent BAs that are discovered in fish are histamine, cadaverine, putrescine, and tyramine, which are formed when bacteria decarboxylate the corresponding free amino acids. The amount of them that accumulate depends on the existence of precursor amino acids, the growth or activity of decarboxylating bacteria, and an appropriate environment (Visciano *et al.*, 2020).

As shown in Table 1, the concentration of histamine in the investigated fresh samples was from 1.1 to 24.5 mg/Kg with average10.74±0.59, while tuna canned from 2.3 to 31.2 with average15.08±0.76 mg%, herring smoked from 2.9 to 35.8 with average 17.93±0.81 and sardine salted from 4.1 to 52.6 with average 26.12±0.89. These outcomes were more than what was mentioned by Nada (2021) who reported that the mean histamine level in sardine salted ranged from 2.5 to 38.1 with average 21.93±0.40, herring smoked from 2.1 to 32.4 with an average of 18.07±0.29 and tuna canned from 1.3 to 27.9 with average12.61±0.23 mg%. The outcomes were decreased than results from El-Dahman-Dalia (2016), who found that the amounts of histamine in smoked herring and sardine were 28.14 1.02 mg% and 23.12 0.86 mg%, respectively.

HFP (histamine food poisoning), formerly called "scombroid fish poisoning" as its initial involvement recorded from Scombrid members such as tunaand bonito, is associated with it, histamine is the BA that is most frequently examined. Although it was thought that 1 g of histamine per kilogram of food was required to cause a hazardous reaction (Kočar *et al.,* 2021).

Results in Table 2 showed that the number of accepted samples for histamine was 26, 23,21, and 16 with a percent 86.7%, 76.7%, 70%, and 53.3% and unaccepted samples were 4, 7, 9, and 14 with percent 13.3 %, 23.3%, 30% and 46.7% in fresh fish, tuna canned, smoked herring andsalted sardine examined samples, respectively. The maximum acceptable value for histamine was 20 mg/100 g according to EOS (2010). Consumption of raw fresh or processed fish has been linked to several cases of histamine toxicity around the world (canned tuna, smoked, fermented, salted).

In this study, the range of putrescine levels in the fresh fish samples that were tested was 1.0 to 21.9 mg/100g, with a mean value of 7.61±0.49 mg/100g. As well, the putrescine level in the examined tuna canned was from 1.7 to 26.4 mg/100g, with an average of 11.13±0.57 mg/100g. Concerning herring smoked putrescine level in the tested samples was from 2.1 to 30.2 mg/100g, with meanvalue14.35±0.64 mg/100g. Farther more, the concentration of putrescine in the examined samples of sardine salted was 2.8 to 39.5 mg/100g, with a mean value of 19.59±0.81 mg/100g. Nada (2021) obtained fewer results and demonstrated that the putrescine concentration in the samples analyzed of sardine salted was from 1.7 to 27.2 mg/100g, with a mean value 14.45±0.26 mg/100g, in the herring smoked putrescine level was 1.4 to 25.8 mg/100g, with mean value 12.78±0.22mg/100g. Finally, the concentration of putrescine in the Tuna canned examined samples was 1.0 to 22.1 mg/100g, with a mean value of 9.10±0.15 mg/100g (Table 3).

In the present study, the number of accepted samples for putrescine were 28, 25,23, and 19 with a percent 93.3%, 83.3%, 76.7%, and 63.3% and unaccepted samples were 2, 5, 7, and 11 with a percent 6.7 %, 16.7%, 23.3%, and 36.7% in the tested samples of fresh (latus niloticus), tuna canned, smoked herring and sardine salted respectively. El-Sayed (2010) produced less impressive results, revealing that the putrescine average of tuna canned, smoked herring and sardine was5.16±0.31 mg/100g, 8.17±0.46 mg/100g and 4.09±0.25 mg/100g respectively (Table 4). Several microbes, including Lactobacilli, E. coli, Enterobacter, 88 Pseudomonas, Micrococci Streptococci and aerobic species that can be generated by bacteria or auto-enzymes, have been connected to the production of putrescine (Nada, 2021).

Findings in Table 5 demonstrated that the level of cadaverine in the tested samples of fresh fish was 1.0 to 20.2 with an average of 5.96±0.43, while tuna canned was from 1.2 to 24.6 with an average of 10.22±0.49, smoked herring was from 1.5 to 27.9 with an average12.41±0.58 and sardine salted was from 2.3 to 33.1 with an average16.85±0.62mg%. These results outperformed those from (El-Dahman-Dalia, 2016) and showed that the samples un-

Table 4. Edibility of the examined fish and fish products based on their putrecine levels (n=30).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fresh fish	20	28	93.3	2	6.7
Canned Tuna	20	25	83.3	5	16.7
Smoked herring	20	23	76.7	7	23.3
Salted Sardine	20	19	63.3	11	36.7
Total (120)		95	79.2	25	20.8

* Maximum Residual Limit (EOS, 2010).

der study had higher average cadaverine levels in sardine salted was 11.05±0.46 and in herring smoke was 7.78±0.41(mg%). Lower results were obtained by Nada (2021) who mentioned that the concentration of cadaverine in tuna canned was from 1.0 to 11.9 with an average 5.47±0.15 mg%, smoked herring was from 1.0 to 20.6 with an average of 8.93±0.19 mg% and sardine salted was from 1.3 to 21.2 with an average 9.81±0.20 mg%.

As presented in Table 6, the accepted samples for cadaverine 29, 26, 25 and 22 represented by 96.7%, 86.7%, 83.3% and 73.3%, while the unaccepted samples were 1, 4, 5, and 8 represented by 3.3%, 13.3% 16.7% and 26.7% for fresh fish, tuna canned, smoked herring and salted sardine examined samples, respectively. Putrescine and cadaverine are significant factors in food toxicity because they can make histamine more harmful (Kočar *et al* (2021).

The findings listed in Table 7 represented the level of tyramine in the tested samples which was from 0.4 to 3.7 mg/100g, with a mean value 2.05±0.10 mg/100g for fresh fish, in canned tuna was from 0.9 to 15.4mg/100g, with mean value 6.37±0.19 mg/100g. Moreover, tyramine level in smoked herring was from 1.1 to 21.3 mg/100g, with a mean value 7.90±0.24 mg/100g, and in sardine salted tyramine level was from 1.4 to 23.9 mg/100g, with mean value 10.44±0.27 mg/100g. El-Sayed (2010) noted that, the tyramine mean values in the studied samples of tuna canned, smoked herring and sardine, were 11.45±0.85 mg/100g, 14.52±0.91 mg/100g and 10.67±0.63 mg/100g respectively, which were higher results. Nada (2021) showed lower results that the concentration of tyramine in tuna canned was from 1.0 to 4.9mg/100g, with a mean value of 2.95±0.09 mg/100g, in smoked herring was from 1.0 to 9.7 mg/100g, with mean value 3.74±0.14mg/100 and Finally, tyramine level in sardine was from 1.0 to 20.1 mg/100g, with an average of 6.08±0.15mg/100g.

Table 5. Statistical analysis of cadaverine levels (mg %) in the examined samples of fish and fish products (n=30).

Fish products	Min	Max	Mean±S.E
Fresh fish	1	20.2	5.96±0.43^{D}
Canned Tuna	1.2	24.6	10.22±0.49^{B}
Smoked herring	1.5	27.9	12.41±0.58^{B}
Salted Sardine	2.3	33.1	16.85±0.62^{A}

*Means with different letters in the same column are significantly different (P<0.05).

Table 6. Edibility of the examined fish and fish products based on their cadaverine levels (n=30).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fresh fish	20	29	96.7	1	3.3
Canned Tuna	20	26	86.7	4	13.3
Smoked herring	20	25	83.3	5	16.7
Salted Sardine	20	22	73.3	8	26.7
Total (120)		102	85	18	15

* Maximum Residual Limit (EOS, 2010).

Table 7. Statistical analysis of tyramine levels (mg %) in the examined samples of fish and fish products (n=30).

Fish products	Min	Max	Mean±S.E
Fresh fish	0.4	3.7	2.05±0.10^{D}
Canned Tuna	0.9	15.4	6.37±0.19^{B}
Smoked herring	1.1	21.3	7.90±0.24^{B}
Salted Sardine	1.4	23.9	10.44±0.27^{A}

*Means with different letters in the same column are significantly different (P<0.05).

In the current study, the unaccepted samples for tyramine in smoked herring and sardine salted were 3.3% and 10%, respectively (Table 8), but all of the fresh fish and tuna canned samples that were analyzed were approved according to Egyptian Organization for Standardization (EOS, 2010). If someone using monoamine oxidase inhibitor medication consumes 60 mg/kg of dietary tyramine, they may experience migraines, whereas someone who consumes 100 to 250 mg/kg may experience a hyper-

Table 8. Edibility of the examined fish and fish products based on their tyramine levels (n=30).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fresh fish	20	30	100	0	0
Canned Tuna	20	30	100	0	0
Smoked herring	20	29	96.7	1	3.3
Salted Sardine	20	27	90	3	10
Total (120)		116	96.7	4	3.3

* Maximum Residual Limit (EOS, 2010).

Table 9. Effect of *B. polymyxa* culture (2x10^{7}) on the levels of histamine experimentally inoculated to fish fillets (50 mg/Kg).

Group / Storage time	Control (mg/Kg)	*B. polymyxa* Treated group (mg/Kg)	Reduction %
Zero time	50	50	-------
8 hours	50	31.4	37.2
12 hours	50	19.5	61
24 hours	50	12.8	74.4

tensive crisis. Kočar *et al*. (2021)

Results in Table 9 showed the effect of Bacillus polymyxa culture ($2x10^7$) on histamine level inoculated in the lab to fish fillets (50 mg/Kg) was reduced to 31.4 mg/kg after 8 hours, 19.5 mg/kg after 12 hours and 12.8 mg/kg after 24 h with reduction percentage 37.2% after 8 h, 61.0% after 12 hours and 74.4% after 24 h. Lee *et al*. (2016) reported lower results, noting that infected samples had a 34% drop in histamine at the end of the test compared to controlled samples.

Table 10. Effect of *B. polymyxa* culture ($2x10^7$) on the levels of cadaverine experimentally inoculated to fish fillets (30 mg/Kg).

Group / Storage time	Control (mg %)	*B. polymyxa* Treated group (mg %)	Reduction %
Zero time	30	30	-------
8 hours	30	22.1	26.3
12 hours	30	15.4	48.7
24 hours	30	9.7	67.7

The obtained results in Table 10 declared the impact of Bacillus polymyxa culture ($2x10^7$) on cadaverine levels inoculated into fish fillets in an experiment (30 mg/Kg) was reduced to 22.1 mg/kg after eight hours, 15.4 mg/kg after twelve hours, and 9.7 mg/kg after 24 h with a reduction percentage 26.3%, 48.7%, and 67.7%, respectively. The main use of starter cultures is to quickly produce lactic acid, which lowers pH, inhibits the growth of pathogenic, and increases the shelf life of processed food (García-Díez and Saraiva, 2021).

CONCLUSION

The current investigation revealed that some samples of fresh *Lates niloticus*, tuna canned, smoked herring and sardine salted contains residues of histamine, cadaverine, putrescence, and tyramine. The concentration of putrescence, histamine, tyramine, and cadaverine are the highest in sardine salted whereas they are the lowest in the fresh fish. It is useful to use Bacillus polymyxa starting culture in fish to lower biogenic amine production and to improve safety of the final products.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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