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Trials for controlling of biogenic amines in fish products

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ABSTRACT

One hundred random samples of fesiekh, sardine,smoked herring and canned tuna (25 of each) obtained from various fish markets in Menoufia governorate, Egypt. All collected samples were investigated for their harmful biogenic amines residues (histamine, putrescine cadaverine and tyramine). Additionally, trials to control such serious residues using biological techniques were applied. The mean values of biogenic amines in the examined samples of fesiekh , sardine, smoked herring and canned tuna were 26.48 ± 0.52 , 21.93 ± 0.40 , 18.07 ± 0.29 and 12.61 ± 0.23 mg % for histamine, 17.69 ± 0.31, 14.45 ± 0.26, 12.78 ± 0.22 and 9.10 ± 0.15 mg% for putrescine, 13.56 ± 0.23, 9.81 ± 0.20, 8.93 ± 0.19 and 5.47 ± 0.15 mg% for cadaverine, respectively. On the other hand, the average concentrations of tyramine were 8.92 ± 0.21 mg % for fesiekh, 6.08 ± 0.15mg % for sardine, 3.74 ± 0.14mg % for smoked herring and 2.95 ± 0.09 mg % for canned tuna. The effect of *B. polymyxa* culture ($2x10^7$) on the levels of histamine experimentally inoculated to sardine fillets (40 mg/Kg) was excellent where its level was decreased to 22.1mg/kg after 8 hours, 14.2 mg/kg after12 hours and 8.9mg/kg after 24 hours with reduction percentages of 44.7%, 64.5% and 77.8%, respectively. Keywords: biogenic amines; fish products; histamin.

1. INTRODUCTION

Fish has a high-quality protein, excellent source of phosphorus and calcium and the provision of ß-complex vitamins are considered to be the preferred source of high nutritional value and highly desired food (Hassan *et al*., 2007).

The majority of marine food items are wholesome, nutritious, safe, and appealing. May be contain potent natural toxins that pose a significant threat to consumers' health (Sindermann, 1996).

Biogenic amines have typically been produced by decarboxylation of free amino acids by certain bacteria. In addition, the quantification of biogenic amines in foods can be used for the quality and freshness of foods (Awan and Thomas, 2008).

High concentrations of histamine are found in the muscles of some species, such as tuna fish and bonito and sardines. They also mentioned that biogenic amine content in fish varies depending on the season, climate, food, sex, genetics, physiological state, storage and sample tissue (Lee *et al.* ,2012).

Histamine is one of the amines implicated in the toxicity of food, however at low levels histamine is not toxic; the presence of cadaverine and putrescine which have five times higher levels than histamine will contribute to histamine toxicity (Emborg and Dalgaard ,2006).

Biogenic amines may result in nausea, breathing problems, hot flashes, swollenness, heart palpations, bright red rash, oral burn, hypo, or hypertension and other problems for sensitive users. Apart from histamine, tyramine has been linked to adverse reactions such as headache and hypertension in MAOI (Mono Amine Oxidase Inhibitors) patients (Seyed et al., 2009).

Hypertensive crises have been associated with dietary tyramine because it induces catecholamine release. They also said that tyramine toxicity is thus genetically determined due to differences in individual metabolism removal (Niwa et al., 2011).

Putrescine and cadaverine seem to be pharmacologically less powerful than histamine and tyramine activity. Hypotension, bradycardia, lockjaws and extremity paresis are the adverse effects identified. The most important food-related problem of putrescine and cadaverin may be the increasing of toxicity of other amines, particularly histamine (Halasz and Barath, 2002).

Stater cultures play a key role in reducing the content of biogenic amine and polycyclic aromatic hydrocarbons in the chemical protection of fermented foods (García-Díez and Saraiva, 2021).

2. MATERIAL AND METHODS

2.1 Application of HPLC for determination of biogenic amines:

The techniques recommended by Krause et al. (1995) and Pinho et al. (2001) were carried out.

2.1.1 Preparation of reactants:

- Dansyl chloride solution: dissolved in 100 ml acetone 500 mg of danzyl chloride.
- Standard solutions: standard solutions of the amines tested were prepared as follows: add 25 mg of each

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standard pure amine, each of which had been distilled with 25 ml individually (histamine-2HCl, cadaverine–2HCl, putrescine-2HCl).

2.1.2. Samples Extraction:

An alarm blender used for three minutes was used in 25g of each sample with 125 ml of five percent tri chloro acetic acid (TCA) and a filtration was then made using Whatman No1 filter paper. 10 ml of the filtrate has thus been moved to the required 4g NaCl glass tube and 50 percent NaOH 1 ml. Usage of 5 ml n-butanol to remove the filtrate, 3 times (2 min): chloroform (1:1 v/v), and 100 ml separating funnel were transferred to the upper clear layer with disposable Pasteur pipette. The HCl layer was collected into a stoppard glass tube to combine organic (upper) extracts in a separating funnel of 15 ml n-heptane and extracted threefold, with 1.0 ml parts of 0.2 NHCl. With the aid of a gentle air current, the solution was evaporated only to dryness with the 95°C water bath.

2.2 Experimental part:

The effect of *Bacillus polymyxa* as a biological trial for reduction the concentrations of histamine experimentally inoculated into sardine fillets was studied as follow:

2.2.1. Preparation of bacterial suspension:

In Brain Heart Infusion (BWI) Broth (Fluka, Sigma - Aldrich Chemie GmbH) were cultivated individually for 24 hours at 37ºC to establish an overage culture in the strains of *Bacillus polymyxa*. One ml (1%), diluted in sterile peptone water in the bacterial suspension was cultivated (0.1%, w/v) (Merck, Darmstadt, Germany). Accordingly, the viable count of *Bacillus polymyxa* strains was carried out according to the plate count method (A volume of the culture broth corresponding to approximately 2×10^7 for *Bacillus polymyxa* was centrifuged (500 rpm, 15 minutes at 5ºC) and the bacterial pellets were washed twice with deionized water (Halttunen et al., 2007).

2.2.2. Binding assay

The bacterial pellets were suspended in 1 Kg sardine fillets. The mixture was adjusted to reach a final concentration of 5×10^6 bacteria and 30 mg/Kg histamine level according to Halttunen et al. (2008) with some modifications. Bacterial pellets, histamine and lead standard solutions were vortexed for 5 seconds (Stuart, Staffordshire, U.K.) and incubated for 24 hours on a Fine mixer SH2000 orbital shaker (Finepcr, Seoul, Korea) with soft agitation. Histamine (without cultural bacteria) infected sardine fillets was used as a control test. The control group however described the contamination of fish fillets with histamine and treatments with *polymyxa Bacillus* The samples were acidenced to ultrapure HNO3 and tested for histamine levels as previously stated at 0-, 8-, 16-, and 24-hour times.

3. RESULTS

Histamine concentrations mean values in Fesiekh, Sardine, Smoked herring and Canned tuna were 26.48 ± 0.52 , 21.93 ± 0.40 , 18.07 ± 0.29 and 12.61 ± 0.23 mg %, respectively, while the putrescine levels were varied from 2.2 to 31.5 ,the average was 17.69 ± 0.31 mg % for fesiekh, 1.7 to 27.2 ,the average was 14.45 ± 0.26 mg % for sardine , 1.4 to 25.8 ,the average was 12.78 ± 0.22mg % for smoked herring and 1.0 to 22.1 with an average of 9.10 ± 0.15 mg % for canned tuna. On the other hand, the average concentrations of cadaverine were 13.56 ± 0.23, 9.81 ± 0.20, 8.93 ± 0.19 and 5.47 ± 0.15 mg % respectively for Fesiekh , sardine, smoked herring and canned tuna samples and The tyramine levels were varied from 1.3 to 21.6 with an average of 8.92 ± 0.21 mg % for Fesiekh, 1.0 to 20.1 with an average of 6.08 ± 0.15mg % for sardine , 1.0 to 9.7 ,the average was of 3.74 ± 0.14mg % for smoked herring and 1.0 to 4.9 with a mean average of 2.95 ± 0.09 mg % for canned tuna.

Furthermore, the using of *B. polymyxa* culture ($2x10^7$) decreased the levels of histamine experimentally inoculated to sardine fillets (40 mg/Kg) with percentages of 44.7%, 64.5% and 77.8% after 8, 12 and24 hours, respectively.

Table (1): Incidence and levels of histamine residues (mg/Kg) in fish products (n=25).

Fish products	+ve samples		Min	Max	Mean ± S.E
	No	%			
Fesiekh	25	100	3.6	45.7	26.48 ± 0.52
Sardine	23	92	2.5	38.1	21.93 ± 0.40
Smoked herring	22	88	2.1	32.4	18.07 ± 0.29
Canned tuna	19	76	1.3	27.9	12.61 ± 0.23

Table (2): Acceptability of fish products according to their histamine residues(n=25).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fesiekh	20	11	44	14	56
Sardine	20	12	48	13	52
Smoked herring	20	15	60	10	40
Canned tuna	20	17	68	8	32
Total (100)		55	55	45	45

* EOS (2010)

Table (3): Incidence and levels of putrescine residues in fish products (mg/Kg) (n=25).

Fish products	+ve samples		Min	Max	Mean ± S.E
	No	%			
Fesiekh	18	72	2.2	31.5	17.69 ± 0.31
Sardine	17	68	1.7	27.2	14.45 ± 0.26
Smoked herring	15	60	1.4	25.8	12.78 ± 0.22
Canned tuna	11	44	1.0	22.1	9.10 ± 0.15

Table (4): Acceptability of the examined fish products depending on their putrescine residues (n=25).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fesiekh	20	16	64	9	36
Sardine	20	19	76	6	24
Smoked herring	20	21	84	4	16
Canned tuna	20	22	88	3	12
Total (100)		78	78	22	22

* EOS (2010)

Table (5): Incidence and levels of cadaverine residues (mg/Kg) in fish products (n=25).

Fish products	+ve samples		Min	Max	Mean ± S.E
	No	%			
Fesiekh	14	56	1.5	24.7	13.56 ± 0.23
Sardine	12	48	1.3	21.2	9.81 ± 0.20
Smoked herring	11	44	1.0	20.6	8.93 ± 0.19
Canned tuna	8	32	1.0	11.9	5.47 ± 0.15

Table (6): Acceptability in fish products according to their cadaverine residues (n=25).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fesiekh	20	22	88	3	12
Sardine	20	23	92	2	8
Smoked herring	20	23	92	2	8
Canned tuna	20	25	100	0	0
Total (100)		93	93	7	7

* EOS (2010)

Table (7): Incidence and levels of tyramine residues (mg/Kg) in fish products (n=25).

Fish products	+ve samples		Min	Max	Mean ± S.E
	No	%			
Fesiekh	10	40	1.3	21.6	8.92 ± 0.21
Sardine	7	28	1.0	20.1	6.08 ± 0.15
Smoked herring	5	20	1.0	9.7	3.74 ± 0.14
Canned tuna	2	8	1.0	4.9	2.95 ± 0.09

4. DISCUSSION

Biogenic amines are organic compounds of low molecular weight and a fundamental nature which, by decarboxilation of precursor free amino acids or by amination and aldehyde and ketone deamination, are synthesized into the metabolism of plants, animals and microorganisms (Rodriguez *et al.*, 2014).

The concentrations of histamine in fesiekh samples as explained in table (1) and Figure (1) were higher than the results obtained by El - Sayed (2010) who showed that mean histamine concentrations of sardine, smoked herring and canned tuna were 6.91 ± 0.46 mg/100g, 10.06± 0.70 and 7.24 ± 0.39 mg/100g respectively, and Azza Hassan and Weam Baher (2011) who reported that the mean average of histamine in Fesiekh and sardine were 21.5 and 17.2 mg/ 100gm respectively, and Huda El- Sayed (2014) who reported that mean histamine value was 4.44± 0.98 in examined smoked herring.

These results were lower than those of Dalia Anter (2016) which reported that the histamine concentrations in Fesiekh, sardine and smoked herring were 33.12± 1.15, 28.14± 1.02 and 23.12± 0.86 mg% respectively and Huda El- Sayed (2014) which mentioned that the mean histamine values were 126.65± 14.77 in Fesiekh samples.

In this study the acceptability of fish products according to their histamine residues was illustrated in table 2 and the acceptability of the examined fish products depending on their putrescine residues was described intable 4.

E.O.S (2010) stated that the maximal acceptable limit for histamine was 20mg/100gm. Accordingly, the numbers of accepted samples were 11, 12,15 and 17by 44%, 48%, 60% and 68% and unaccepted samples are 14, 13, 10 and 8 by 56%, 52%, 40% and 32% in the examined.

The available free amino acids, presence of microorganisms that can decarboxylase amino acids, and favorable conditions to develop such microorganisms and to produce decarboxylase enzymes are the factors affecting histamine output (El- Mossalami and El- Agizy, 2005).

The concentration of putrescine in Fesiekh ranged between 2.2 to 31.5 mg/100g, and an average of 17.69 ± 0.31 mg/100g, in Sardine were from 1.7 to 27.2 mg/100g, and an average of 14.45 ± 0.26 mg/100g, in smoked Herring ranged between 1.4 to 25.8 mg/100g, and an average of 12.78 ± 0.22mg/100g and in canned tuna ranged from 1.0 to 22.1 mg/100g, with a mean concentration of 9.10 ± 0.15 mg/100g as explained in table (3) and Fig. (1).

Lower results were obtained by Kerr et al., (2002) who mentioned that the level of putrescine as a biogenic amine residue in canned tuna was ranged from 1.00 to 3.50 mg/100g, with a mean value of 1.40±0.16 mg/100g and El - Sayed (2010) who showed that the mean putrescine value of Sardine, smoked herring and canned tuna was 4.09 ± 0.25 mg/100g, 8.17 ± 0.46 and 5.16 ± 0.31 mg/100g.mg/100g, respectively.

According to EOS (2010), 9, 6, 4 and 3 samples represented as 36%, 24%, 16% and 12% of fesiekh, sardine, Smoked herring and canned tuna samples exceed the permissible limits, respectively.

Productions of putrescine were linked to the existence of enzymes such as *E. coli*, *Enterobacter*, *Lactobacilli*, *Pseudomonas*, *Streptococci*, *Micrococci* and aerobic species that can be synthesized by auto-enzymes or bacteria (Maijala et al., 1993 and Roig- Sagues et al., 1997).

Table (5) and Figure (1) showed that the concentrations of cadaverine in the examined samples of Fesiekh ranged from 1.5 to 24.7 with a mean value 13.56 ± 0.23 mg%., while in sardine ranged from 1.3 to 21.2 with a mean value 9.81 ± 0.20 mg%., in smoked herring ranged from 1.0 to 20.6 with a mean value 8.93 ± 0.19 mg% and Canned tuna ranged from 1.0 to 11.9 with a mean value 5.47 ± 0.15 mg%.

These results were similar to those reported by Dalia Anter (2016) who showed that the mean values of Cadaverine in the examined samples of Feisiekh 13.80±0.54, while lower results than that obtained in salted sardine 11.05± 0.46 and higher results than were obtained in smoked herring 7.78± 0.41(mg%).

Table (8): Acceptability of the examined fish products according to their tyramine residues (n=25).

Fish products	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Fesiekh	20	23	92	2	8
Sardine	20	24	96	1	4
Smoked herring	20	25	100	0	0
Canned tuna	20	25	100	0	0
Total (100)		97	97	3	3

* EOS (2010)

Table (9): Effect of B. polymyxa culture ($2x10^7$) on the levels of histamine experimentally inoculated to sardine fillets (40 mg/Kg).

Group / Storage time	Control (mg/Kg)	*B. polymyxa* Treated group (mg/Kg)	\ Reduction %
Zero time	40	40	-------
8 hours	40	22.1	44.7
12 hours	40	14.2	64.5
24 hours	40	8.9	77.8

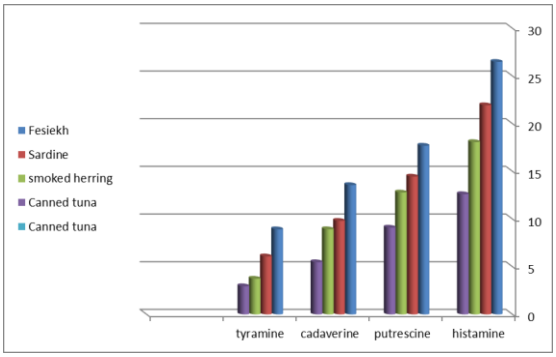

Figure (1): The mean average of concentrations of histamine, putrescine cadaverine and tyramine residues in the examined fish products (mg/Kg).

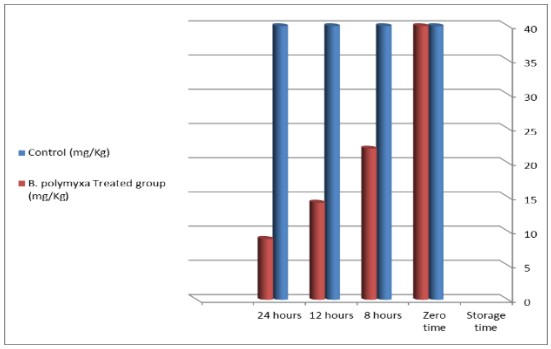

Figure (2): histamine levels (mg/Kg) in the control and *B. polymyxa* treated sardine fillet samples.

Table (6) showed that the accepted samples were22, 23, and 23 by 88%, 92% and 92% and the unaccepted samples were 3, 2, and 2 by 12%, 8% and 8% of the examined Feisiekh, salted sardine and smoked herring samples, respectively, according to E.O.S (2010) which stated that the maximal acceptable limit for Cadaverine was (20 mg/100g, while all the examined samples of canned tuna were accepted.

The concentration of cadaverine is a strong indicator of spoilage and has a significant effect on the post-processing of fish products or on the management of fresh fish after harvest (Flick et al., 2001).

The results as shown in table (7) and Fig.(1) revealed that the concentration of tyramine in Fesiekh samples ranged between 1.3 to 21.6 mg/100g, and an average of 8.92 ± 0.21 mg/100g, in Sardine were from 1.0 to 20.1 mg/100g, and an average of 6.08 ± 0.15mg/100g. , in smoked Herring ranged from 1.0 to 9.7 mg/100g, with an average of 3.74 ± 0.14mg/100g and in canned tuna ranged from 1.0 to 4.9mg/100g, and an average of 2.95 ± 0.09 mg/100g.

Higher results were obtained by El - Sayed (2010) who mentioned that mean average of tyramine in the examined samples of Sardine, smoked Herring and canned tuna were 10.67 ± 0.63 mg/100g, 14.52 ± 0.91 mg/100g and 11.45 ± 0.85 mg/100g respectively.

Biogenic amine residue in fish by thin-layer chromatography (TLC) and recorded that the detected limit was 10 mg/100g (Lapa-Guimarães and Pickova 2004).

According to EOS (2010), the unaccepted examined samples of Fesiekh and sardine were 8% and 4% respectively based on their tyramine residues content, while all of the examined samples of smoked herring and canned tuna were accepted Table (8).

Table (9) and Figure (2) showed that the effect of *B. polymyxa* culture ($2x10^7$) on the levels of histamine experimentally inoculated to sardine fillets (40 mg/Kg) was decreased to 22.1mg/kg after 8 hours, 14.2 mg/kg after12 hours and 8.9mg/kg after 24 hours by a percentage of reduction 44.7%,64.5% and 77.8% respectively. Lower results were obtained by Lee et al. (2016) who recorded the reduction of histamine in inoculated samples was 34% at the end of fermentation, compared to control samples.

5. CONCLUSION

The present study declared that some samples of Fesiekh, sardine, smoked herring and canned tuna obtained from various fish markets in Menoufia governorate, Egypt, exposed for consumption were contaminated with different chemical residues such as biogenic amines residue (histamine, putrescine, cadaverine and tyramine), Feisiekh contained the highest level of histamine, Cadaverine, tyramine and Putrescine, while canned tuna contained the lowest level of these biogenic amines. Application of *B. polymyxa* as a starter culture in salted fish products fermentation is effective to inhibit biogenic amines accumulation and to enhance the safety of salted and fermented fish products.

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