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Full Length Research Paper

Exploring the contamination level by biogenic amines in fish samples collected from markets in Thuel – Saudi Arabia

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Biogenic amines are formed in some foods, especially fish and fish products as a result of the process of decarboxylation of free amino acids in food. The histamine (scombroid) poisoning arises from ingestion foods containing relatively high levels of histamine and other amines that act as histamine potentiators. Therefore, this study aims to explore the level of contamination by biogenic amines in fish and fish products in shore of Thuel – Saudi Arabia. One hundred and twenty of different fish samples were collected from Thuel shore markets. The biogenic amines histamine, cadaverine, putrescine and tyramine were determined in all samples and also in selected fish samples held iced for 0, 24 and 48 h. The effect of the commercial frying process on biogenic amines level was also tested using two different types of fish. Quantitative thin layer chromatography (TLC) analysis was used in biogenic amines determination. There are significant differences between the tested samples and the highest mean (mg/100 g) of putrescine. Cadaverine, histamine and tyramine were in loat fish (6.57), Baiad (3.348), Hareed (4.82) and Baiad (1.38), respectively. Significant increases in the level of biogenic amines were observed in fish held iced at 0, 24, and 48 h. A significant increase of histamine level was noticed in selected fish after commercial frying process.

Key words: Biogenic amines, fish, frying, hazard analysis critical control point (HACCP), thin layer chromatography (TLC).

INTRODUCTION

Biogenic amines are formed in fish and other food by microbial decarboxylation of amino acids. Some of biogenic amines like histamine, cadaverine, putrescine and tyramine are very important as a main cause of food intoxication and they also serve as chemical indicators of fish spoilage (Lehane and Olley, 2000; Kim et al., 2009).

The presence of free histidine and the formation of histidine decarboxylase by certain bacterial species present at appropriate temperature and time are considered as rudiments for histamine forming in foods (Kim et al., 2004).

Histamine (scombroid) poisoning is associated with the consumption of scombroid fish such as tuna, bonito and mackerel. However, non-scombroid fish such as herring, anchovies and mahi-mahi have also been concerned in outbreaks (Lehane and Olley, 2000; Huss et al., 2003).

Biogenic amines formation in foods is objectionable because it can result in toxicological effects to consumers such as hypertension, headache, diarrhea, rash, and localized inflammation when ingested in extreme amounts, cardiac palpitation and even death in very severe cases (Rawles et al., 1996). Biogenic amines are considered as precursors of carcinogenic amines such as N-nitrosamines, and they are also used as indicators of food decomposition (Mietz and Karmas, 1978).

The United States, Food and Drug Administration (USFDA) has set that histamine must be used in Hazard Analysis Critical Control Point (HACCP) programs for fish and has set the maximum action level of 50 ppm

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Table 1. Types of fish samples collected from markets in shore Thuel - Saudi Arabia.

Local name	Common name	Scientific name	References
Hareed	Green parrot fish	*Scarus frenatus*	Um El-Qura university-Saudi Arabia
Shuoor	Emperor fish	*Lethrinus minatus*	http://uqu.edu.sa/page/ar/108198
Baiad	Bluefin jack	*Caranx melampygus*	
Loat	Moontail seabass	*Variola louti*	
Bagha	Indian mackerel	*Rastrelliger kanagurta*	
Hamoor	Greasy grouper	*Epinefhelus chlorostigma*	
Seagan	Marbled spinefoot	*Siganus rivulatus*	
Araby	Blue spot grey Mullet	*Valamugil seheli*	
Nagel	Saddleback grouper	*Plectropomus maculates*	

(FDA, 1995). Enterobacteriaceae family has been found to be the most important histamine forming bacteria (HFB) in fish. *Morganella morganii, Klebsiella pneumoniae, Proteus vulgaris* and *Hafnia alvei* are known to originate from fish implicated incidents of histamine poisoning (Frank, 1985; Lehane and Olley, 2000; Huss et al., 2003).

Histamine represents the major and the main cause of scombroid (histamine) poisoning, and other biogenic amine, such as tyramine, cadaverine, and putrescine, acts as potentiates of histamine toxicity (Taylor, 1988; Al Bulushi et al., 2009; Joshi and Bhoir, 2011). Using histamine and other biogenic amines as indicators of product decomposition as these compounds are detected at non significant concentrations in fresh fish, and at the same time, their formation is frequently associated with bacterial spoilage. The HACCP implementation mandates specifically the monitoring of these safety indicators (Fernández-Salguero and Mackie, 1987).

A hazardous level of histamine for human health has been suggested as 500 mg/kg, although, low levels as 50 mg/kg (50 ppm) have been reported in histamine poisoning (FDA, 2001; Huss et al., 2003).

The objective of the current study was to explore the level of contamination by biogenic amines causing histamine poisoning in fish and fish products in shore of Thuel – Saudi Arabia and to discuss the level of safety for the consumer.

MATERIALS AND METHODS

Sampling

Since the objective of the current study was to explore the level of contamination by biogenic amines in fish and fish products in shore of Thuel – Saudi Arabia and to discuss the level of safety for the consumer. 120 samples of different 9 types of fish (Table 1) were collected from the fish markets in Thuel shore – Saudi Arabia. Biogenic amines (putrescine, cadaverine, histamine and tyramine) were determined in collected samples. Selected fish types were stored with crushed ice for storage time 0, 24, and 48 h. The effect of the commercial frying process on the level of the tested biogenic amines was also studied using different two types of fish samples (Mullet /Araby and Greasy grouper / Hamoor fish).

Chemicals and supplies

Amines (histamine, putrescine, cadaverine, and tyramine) as their crystalline hydrochlorides, dansyl chloride (5-dimethylaminonaphtalene-1- sulphonyl) and thin layer chromatography (TLC) plates (20 × 20 cm aluminium sheets coated with 0.20 mm silica gel G-60) were obtained from Merck company. All chemicals used were of analytical grades.

Extraction of biogenic amines

Histamine, putrescine, cadaverine, and tyramine, were extracted in all tested samples according to Mietz and Karmas (1978) and Maijala and Eerola (1993).

Biogenic amines derivatization

The dansylated derivatives of the extracted amines were formed by dissolving the sample residue with 0.5 ml of saturated $NaHCO_3$ solution, then 1 ml of dansyl chloride solution (500 mg/100 ml acetone) was added ,and the mixture was incubated at 55ºC for 45 min. The dasylamines were extracted using diethyl ether then evaporated under gentle stream of nitrogen and the sample residue reconstituted in known volume of methanol (Ayesh, 1993; Sultan, 2994).

Determination of dansylamines

One-dimensional TLC technique was used to separate the four dansylamines under investigation. The TLC plates was developed using chloroform: benzene: triethylamine (6: 4.5: 1) as a solvent system. The marked dansylamines areas were visualized (under UV lamp at 365 nm) and determined in Microanalysis Centre, Faculty of science, Cairo University using CS- 9000 Dual wavelength flying spot scanning densitometer (SHIMADZU) using wavelength 254 nm. Standard curve of each dansylamine was used to calculate the concentrations of biogenic amines in the tested samples.

Statistical analysis

The data were subjected to the proper statistical analysis using Mstat-

Table 2. Mean of biogenic amines concentrations (mg/100g) in fish samples collected from Thuel markets in Saudi Arabia.

Fish types	Putrescine	Cadaverine	Histamine	Tyramine
Hamoor	1.732^{bc}*	1.084^{bcde}	0.932^{b}	0^{e}
Loat	6.574^{a}	1.038^{cde}	1.387^{b}	0.827^{bc}
Nagel	1.092^{c}	3.285^{abc}	1.506^{b}	0.37^{de}
Seagan	0.892^{c}	1.299^{bcde}	0.633^{b}	0.576^{bcd}
Araby	0^{c}	3.079^{abcd}	3.586^{a}	0.658^{bcd}
Baiad	4.0755^{ab}	3.348^{ab}	0^{b}	1.375^{a}
Shuoor	1.839^{bc}	0.57^{e}	0.509^{b}	0.518^{cd}
Hareed	4.935^{a}	0.846^{de}	4.823^{a}	0.642^{bcd}
Bagha	0.715^{c}	0.355^{e}	0.222^{b}	0.557^{bcd}
LSD	2.838	2.292	1.804	0.409

*Means that do not share a letter are significantly different.

C program (MSTAT, 1988). For means comparison Duncan's multiple range test was applied at 5% level.

RESULTS AND DISCUSSION

Effect of storage time on biogenic amine level

Biogenic amine level in the collected fish samples

Data in Table 2 showed the mean of biogenic amines level in all studied fish types (Table 2). The obtained data showed that all tested fish types have highly significant differences for the level of putrescine, histamine and tyramine and only significant differences in case of cadaverine. The highest mean expressed as mg/100g of putrescine, cadaverine, histamine and tyramine was 6.57 in loat fish, 3.348 in Baiad, 4.82 in Hareed and 1.38 in Baiad, respectively.

In other words, Hareed fish has the highest mean of both histamine and total biogenic amines, with histamine levels as high as 48 ppm, very close to the action level established by USFDA (50 ppm), which means that this value may increase during storage and handling to pose a health risk for consumers. Also, the results showed a relative increase in the mean value of histamine and cadaverine in both Nagel and Araby fish meaning that attentions must be taken during handling and storage of these fish types. Therefore, the fish should not be exposed to temperatures >4.4ºC for more than 4 h after the initial chilling, also it was found that some of bacteria species can produce histamine in toxic levels even at 0 to 5°C(FDA, 2001 and Dalgaard et al., 2008).

Federal Register (1995) considered that the best quality fish has histamine values less than 10 ppm, while histamine values between 10 to 30 ppm are accepted as middle quality and 30-50 ppm histamine value is critical as it is close to the level of FDA regulation (50 ppm). According to the above-mentioned conclusion our data showed that Araby and Hareed fish represents the critical state regardless the other impact factors.

Also rapid chilling of fish directly after catching is most important factor for prevent histamine formation , especially for fish that are exposed to warmer water or air (FDA, 2001), these are in very close to the catching conditions that apply in our fish samples in the current study. The significantly differences -in biogenic amines contents – between all types of the studied fish may be due to that fish muscle is naturally rich in free amino acids and the content may increase even further post mortem. Also, amino acid formation depends beside fish type, on bacterial flora, the harvesting season and feeding activity prior to capture that affect on amino acid contents which acts as precursors of biogenic amines (Aksnes, 1988).

Effect of storage time on biogenic amine level

Figures 1, 2, 3 and 4 illustrated the development of the mean values of the tested biogenic amines during the storage times 0, 24 and 48 h held in iced four types of fish (Shuoor, Bagha, Sigan and Baiad).

A steady increase of the mean value of putrescine, cadaverine and histamine were detected during 48 h. Highly significant differences between the four types of fish were noticed regardless of the three treatments and the same trend applies to treatments regardless of the fish types. The obtained results also showed a significant difference between the mean concentration of histamine and putrescine during 0, 24 and 48 h, while in case of cadaverine and tyramine, the significant difference was noticed only between treatments 0 and 48 h.

Despite the earlier recorded values, histamine does not exceed the safe limits (50 ppm). While histamine represents the major and the main cause of scombroid (histamine) poisoning, the other biogenic amine, such as tyramine, cadaverine, putrescine, and tryptamine, acts as potentiates of histamine toxicity (Taylor, 1988; Al Bulushi et al., 2009; Joshi and Bhoir, 2011). In the meantime, the control of temperature lead to inhibition the growth of mesophilic microorganism during the storage of fish, the occurrence of psychotropic bacteria even in adequate

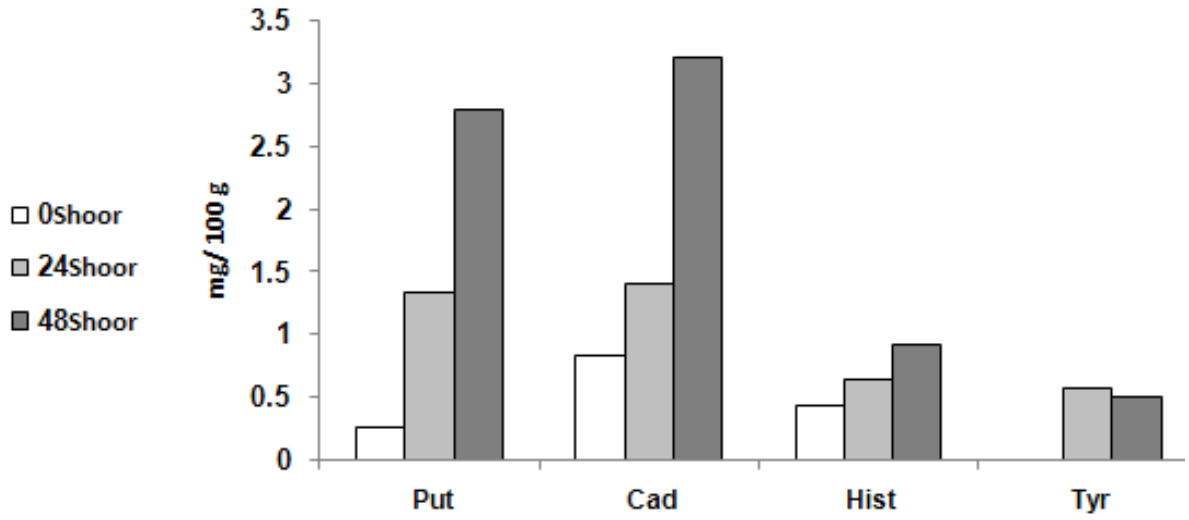

Figure 1. Biogenic amine putrescine, cadaverine, histamine and tyramine contents in shoor fish stored on ice for 0, 24 and 48 h.

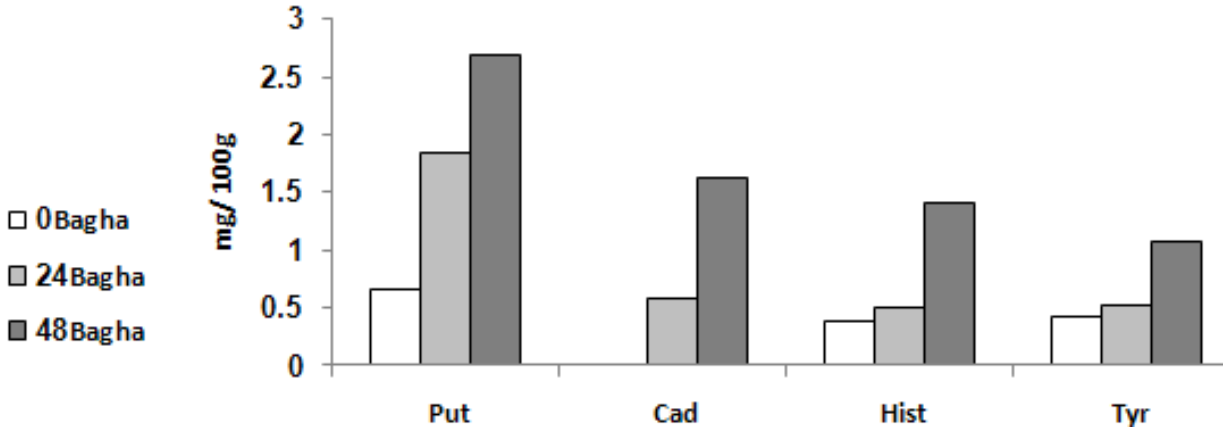

Figure 2. Biogenic amine putrescine, cadaverine, histamine and tyramine contents in Bagha fish stored on ice for 0, 24 and 48 h.

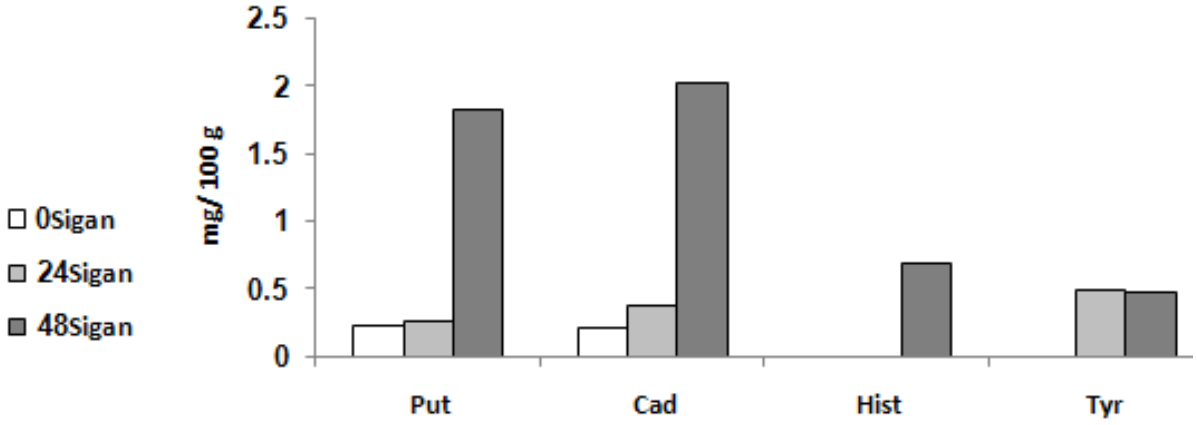

Figure 3. Biogenic amine putrescine, cadaverine, histamine and tyramine contents in Sigan fish stored on ice for 0, 24 and 48 h.

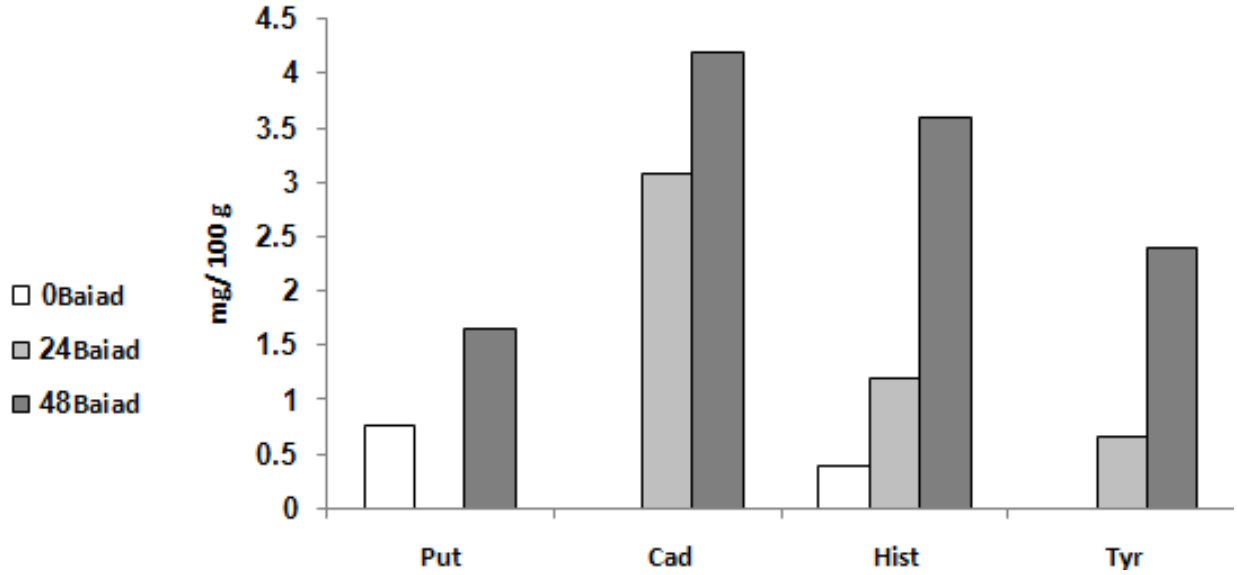

Figure 4. Biogenic amine putrescine, cadaverine, histamine and tyramine contents in Baiad fish stored on ice for 0, 24 and 48 h.

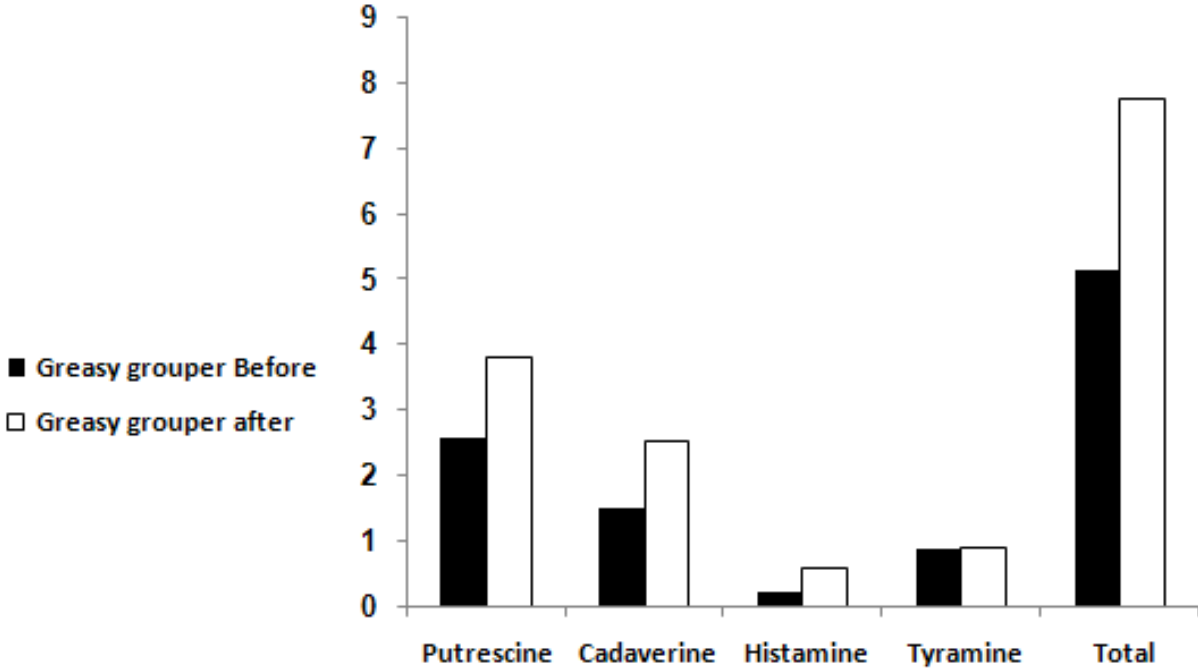

Figure 5. Biogenic amines level in Greasy/Hamoor grouper fish samples before and after commercial frying process.

chilling condition can result in biogenic amines formation (Latshmanan et al., 2002; Rezaei et al., 2007).

Effect of commercial frying process on biogenic amine level

Data in Figures 5 and 6 illustrated the effect of the local commercial frying process on the level of the tested biogenic amines (putrescine, cadaverine, histamine and tyramine) in two different types of fish (Mullet /Araby and Grease grouper/Hamoor). Apparent increase in the level of all tested amines was observed as a result of the frying treatment however, the statistical analysis showed that increase is significant only in histamine in both two tested fish.

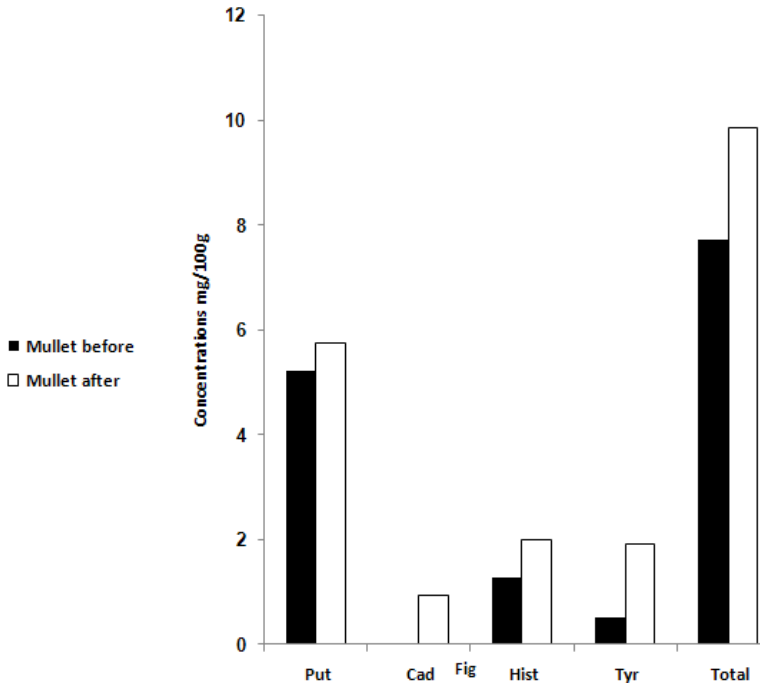

Figure 6. Biogenic amines level in Mullet/Araby fish samples before and after commercial frying process.

Since histamine is heat resistant, it can remain integral in canned or other processed fish products (Lopez-Sabater, et al., 1994, Hungerford, 2010). It is also known that once histamine is formed, it cannot be eliminated by heat treatments therefore, recontamination of the fish with the enzyme-forming bacteria is required for extra histamine to form (FDA, 2001). The increase of histamine level together with other biogenic amines in the current study may be due to: a) the handling period and preparation step before frying process under relatively high temperature, meaning that this step may be considered as critical control point (CCP) that must be monitored concerning time and temperature. In this regard, Kose (2010) reported that histamine development more likely occurs in raw, unfrozen fish therefore it is important to control histamine formation before processing in particular at raw material stage. b) The effect of frying process on histamine content where, Veciana-Nogues et al. (1989, 1997) and Ganowiak et al., (1991) found that thermal process increased the amount of histamine as result of protein breakdown. In the same direction Pan, (1985) reported that the optimal temperature for histidine decarboxylase are 55°C, therefore, it may be probable that during the come-up time in thermal processing, there was added degradation by endogenous and microbial enzymes to produce histamine.

Data in Figures 5 and 6 also showed that the maximum detected level of histamine not exceed the safe limits where histamine levels in illness- causing fish have been above 200 ppm. There are indications that decomposition can result in the production of other toxic amines, like putrescine and cadaverine that have likely to cause illness, even in the absence of histamine formation (FDA, 2001).

In general there are some types of food hazards like histamine that are not easy to control at incoming material stage or during Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP) applications. Therefore, certain preventive measures and monitoring procedures must be done starting from raw material stage until final consumer. Of these preventive measures, time/temperature control that mainly used for critical limit for monitoring histamine formation at raw fish stage. Since histamine development more likely occurs in raw and, unfrozen fish. Therefore, it is important to control histamine formation before processing by rapid chilling after fish catching and the time requisite to lower the internal temperature of fish after capture will be dependent upon a number of factors such harvest method, the size of the fish and the chilling method (FDA, 2001).

Conclusion

1. The scientific giving out of the biogenic amine determination in fish and the hazards associated with their ingestion, may lead to increase the attentiveness of the operators involved, increase the consumer awareness and a production of higher quality and safety.
2. The processors must apply several critical limits to

monitor histamine formation in fresh fish including checking the temperature of fish and water at arrival, checking sensory quality of fish (for example, odor, texture, eye liquid, gills color, etc), testing histamine levels at the arrival for suspected lot and set up critical histamine levels according to the type of processing method to be applied (Kose et al., 2007).

3. The increase of histamine level after frying process may be due to the handling period and preparation step before frying process under relatively high temperature, meaning that this step may be considered as critical control point (CCP) that must be monitored concerning time and temperature.

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REFERENCES

Aksnes A (1988). Location of enzymes responsible for autolysis in bulk-stored capelin (*Mallotus villosus*). J. Sci. Food Agric., 44: 263-271.

Al Bulushi I, Susan P, Hilton CD, Gary AD (2009). Biogenic Amines in Fish: Roles in Intoxication, Spoilage, and Nitrosamine Formation-A Review. Cri. Rev. Food Sci. Nutri., 49(4): 369 – 377.

Ayesh AM (1993). Fishmeal, production, biological evaluation and elimination of its toxic biogenic amines. Ph.D. Thesis, Food Sci. and Tech. Dept., Cairo Univ., Egypt.

Dalgaard P, Emborg J, Kjølby A, Sørensen ND, Ballin, NZ (2008). Histamine and biogenic aminesformation and importance in seafood. T Børresen (Ed.), Improving seafood products for the consumer, Woodhead Publishing Ltd., Cambridge, pp. 292–324.

FDA (1995). Procedures for the safe and sanitary processing and importing of fish and fishery products. Federal Register, 60: 65096-65202.

FDA (2001). Scombrotoxin (histamine) formation. Ch. In Fish and Fishery products Hazards and Controls Guidance.3nd ed., pp. 83-102. Food and Drug Adminsration, Center for Food Safety and Applied Nutrition, Office of seafood, Washington, DC.

Federal Register (1995). Decomposition and histamine--raw, frozen tuna and mahi-mahi; canned tuna; and related Species, 60(149): 39754-30956, CPG 540.525.

Fernández-Salguero J, Mackie IM (1987). Preliminary survey of the content of histamine and other higher amines in some samples of Spanish canned fish. Int. J. Food Sci. Technol., 22: 409-412.

Frank HA (1985). Histamine-forming bacteria in tuna and other marine fish. Pan B.S and James D (Eds), Histamine in marine products: production by bacteria, measurement and prediction of formation, FAO Fish Technical Paper, Rome, 252: 2-3.

Ganowiak Z, Gajewska R, Lipka, E (1991). Histamine content of fish and fish products on the Polish market in 1990. Przemysl-Spozywczy, 45(9): 233-235 (Abstract). Available at:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi.

Hungerford JM (2010). Scombroid poisoning: A review. Toxicon. August, 15, 56(2): 231-243.

Huss HH, Ababouch L, Gram L (2003). Assessment and Management of Seafood Safety and Quality, FAO Fisheries Technical Paper 444, Rome, 230 pp.

Joshi PA, Bhoir VS (2011). Study of Histamine Forming Bacteria in Commercial fish samples of Kalyan city. Int. J. Cur. Sci. Res., 1(2): 39 – 42.

Kim SH, Eun JB, Chen TY Wei CI Clemens RA, An H (2004). Evaluation of histamine and other biogenic amines and bacterial isolation in canned anchovies recalled by the USFDA. J. Food Sci., Vol.69,Nr.6, M157-M162.

Kim MK, Mah JH, Hwang HJ (2009). Biogenic amine formation and bacterial contribution in fish, squid and shellfish. Food Chem., 116: 87–95.

Kose S (2010). Evaluation of seafood safety health hazards for traditional fish products: Preventive measures and monitoring issues. Turkish journal of fisheries and aquatic sciences 10: 139-160.

Köse S, Koral S, Yaºar, A, Yaylı N, Tufan B, Üzen F, Genç S, Boran M (2007). Investigating some quality parameters of salted and fermented fish products of EU and Turkish origin in relation to food safety. Recent Advances in Food Analysis (RAFA conference). 7-9 November, Prague, Chez Republic (poster presentation).

Latshmanan R, Shakila RJ, Sekaran GJ (2002). Changes in the halophilic amine forming bacterial flora during salt-drying of sardines (*Sardinella gibbosa*). Food Res. Int., 35(6): 541-546.

Lehane L, Olley J (2000). Histamine fish poisoning revisited. Int. J. Food Microbiol., 58: 1-37.

Lopez-Sabater EI, Rodriguez-Jerez JJ, Roig-Sagues AX, Mora-Ventura MAT (1994). Bacteriological quality of tuna fish (*Thunnus thynnus*) destined for canning: Effect of tuna handling on prssssesence of histidine decarboxylase bacteria and histamine level. J. Food Protect., 57: 318–323.

Maijala RL, Eerola SH (1993). Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. Meat sci., 35: 387-395.

Mietz JL, Karmas E (1978). Polyamine and histamine content of rock fish, salamon, lobster and shrimp as an indicator of decomposition. J. AOAC, 16(1): 139-145.

MSTAT C (1988). MSTAT-C, a microcomputer program for the design, arrangement and analysis of agronomic research. Michigan State University, East Lansing.

Pan BS (1985). Histamine in canned products. In: Histamine in Marine Products: Production by Bacteria, Measurements and Prediction of Formation Pan BS and James D (Eds.) FAO: Rome.

Rawles DD, Flick GJ, Martin RY (1996). Biogenic amines in fish and shellfish. Adv. Food Nutr. Res. 39: 329-365.

Rezaei M, Montazeri N, Langrudi HE, Mokhayer B, Parviz M, Nazarinia A (2007) The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chem., 103: 150–154.

Taylor SL (1988). Marine toxins of microbial origin. Food Technology, March, pp. 94-98.

Veciana-Nogués MT; Vidal-Carou MC and Marine-Font A (1989). Histamine and tyramine in preserved and semipreserved fish products. J. Food Sci., 54: 1653-1655.

Veciana-Nogués MT, Mariné-Font A and Vidal-Carou MC (1997). Biogenic amines in fresh and canned tuna. Effects of canning on biogenic amine contents. J. Agric. Food Chem., 45: 4324-4328.