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Biogenic Amines in Salted and Smoked Herring Fish

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Abstract: A total of 60 samples of feisiekh, salted sardine and smoked herring fish (20 of each) were collected from markets in kafr El- Sheikh and subjected to determine biogenic amines and NaCl% contents using HPLC. Mean values for Histamine content (mg/100g) were (33.12, 28.14, 23.14) for feisiekh, salted sardine and smoked herring respectively, meanwhile the mean values for Cadaverine content were (13.80, 11.05 and 7.78) for feisiekh, salted sardine and smoked herring respectively and (9.99, 8.81 and 6.12) for Putrescine respectively. Mean values for NaCl% of Feisiekh, Salted sardine and Smoked herring were (9.46%, 8.71% and 8.65%) respectively. Such results can propose that biogenic amines as histamine, Cadaverine and Putrescine are the most objective quality indicators among fish products.

Key words: Biogenic Amines · Feisiekh · Salted Sardine · Smoked Herring Fish · HPLC

INTRODUCTION

Salting is a method of preservation of fish and salted fish constitute an important part of the diet of great portion of consumers and have subjected to many risks [1]. Feisiekh, a traditional Egyptian salted fish, has been considered as a popular part of the Egyptian diet especially in certain celebration times as spring day. Since 1991, WHO/FAO recorded the first documented outbreak of food poisoning in Egypt due to consumption of uneviscerated salted fish, feisiekh, so pressure on the government, industry and the scientific community to seek new safe alternatives to control the microbial growth in uneviscerated salted fish [2]. The fatty fish species such as herring (*Clupea harangues pallasi)* and sardine (*Sardina pilchardus)* are very popular and important for domesticconsumption because of the good nutritional properties of polyunsaturated fatty acids. Products are fresh, frozen, salted, dried and canned fish. The salted herring has become one of the most popular traditional foods and is in high demand. But the fatty fish tends to be more susceptible to spoilage compared to other species because most of them are small and are caught in large quantities, rarely eviscerated immediately on capture and often chilled inadequately. The polyunsaturated fat is easily spoiled (becomes rancid). Delayed cooling, with environmental temperatures between 15 °C and 20 °C, will shorten the shelf life by several days [3].

Biogenic amines are low molecular weight organic bases, they can be formed and degraded as a result of normal metabolic activity in animals, plants and microorganism, in latter case, biogenic amines may be used as an indicator of food spoilage. These amines are usually produced by decarboxylation of amino acids as a result of decarboxylase enzyme activity of bacteria present in the food, or that contaminate the food during transporting, handling, processing and marketing [4]. Consumption of low concentration of biogenic amines in the average diet is not dangerous but a high concentration is dangerous especially in Histamine, Putrescine and Cadaverine [4, 5].

Histamine, Putrescine and Cadaverine have been used as a quality indicators for fresh fish and meat [6]. So the aim of this work of this study to investigate the hygienic quality of salted fish (Feisiekh and salted sardine) and smoked herring fish in kafr El-Sheikh and Mehalla El-Kubra city by screening the biogenic amines (histamine, Cadaverine, Putrescine) and NaCl% (Sodium chloride) by HPLC and comparing with EOS standards [7].

MATERIALS AND METHODS

Collection of Samples: Sixty random pickled and smoked herring fish samples represented by feisiekh, sardine and herring (20 of each) were collected from different fish

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markets at various localities in Kafr El-sheikh city, Mehalla EL-Kubra Egypt. Each sample was separately packed in sterile plastic bag and immediately transferred to the laboratory in an insulated ice box for determination of its contents of biogenic amines and NaCl%.

Determination of Biogenic Amines by Using HPLC: Three biogenic amines including histamine, Cadaverine and Putrescine were determined in all examined samples according to the protocol recommended by Krause *et al.* and Pinho *et al.* [8, 9].

Determination of Sodium Chloride "NaCl %" AOAC [10]: Actually, 10 g of the examined sample were weighed to which 10 ml of 0.1 Ag NO_3 (silver nitrate) solution were added to precipitate all chloride as Ag-Cl (silver chloride). Therefore, 20ml of Nitric acid were put to the mixture and boiled gently on hot plate or sand bath until all solids except silver chloride were dissolved (usually 15 min) then cooled. Then, 50 ml of distilled water and 5ml of ferric ammonium sulphate indicator were added and shaken well. Titration with 0.1 N of Ammonium thiocyanate standard solution was carried out until the appearance of permanent light brown.

Calculation

$$NaCl\% = \frac{(A - B) \times 0.0058 \times 100}{\text{wt of sample}}$$

A= amount of 0.1 n silver nitrate
B= amount of 0.1 N ammonium thiocyanate
1 ml of silver nitrate 0.1 N = 0.0058 sodium chloride

RESULTS

Statistical analytical results of biogenic amines (Histamine, Cadaverine and Putrescine) levels (mg %) in fish products are shown in Table1, while results of NaCl % in fish products are presented in Table 2 and correlation coefficient (r) between NaCl% vs. biogenic amine levels in the examined samples of salted and smoked herring fish is shown in Table 3.

DISCUSSION AND CONCLUSION

Salted fish as feisiekh (*Mugil cephalus)*, salted sardine (*Sardina Pilchardus*) and smoked herring (*Clupea harangues pallasi)* are most popular between fish products in Egypt and consumed on a large scale in some occasions and feasts. Biogenic amines are naturally found in many foods including fish and can be produced in high amounts by microorganisms through the activity of amino acid decarboxylase enzyme or by autolytic proteolysis of meat enzyme [11]. Biogenic amines formation in food is objectionable due to their ability to have a direct or in direct effect on the human vascular and nervous systems, toxicological effects such as hypertension, headache, diarrhea, rash and localized inflammation and when ingested in extreme amounts, cardiac palpation, intracerebral hemorrhage and even death in very severe cases [12].

Table (1) showed that the concentrations of histamine in the examined samples of feisiekh ranged from 6.2 to 82.7 with a mean value 33.12± 1.15, while salted sardine ranged from 4.6 to 37.2 with a mean value 28.14±1.02 and smoked herring ranged from 4.1 to 58.7 with a mean value 23.12± 0.86 mg%. These results are higher than that reported by Ebtsam [13] which showed that mean histamine values of examined samples were 18.06±0.99 and 23.51±1.21 in examined feisiekh and salted sardine respectively and also higher than the results from Azza and Weam [14] which reported that mean value of histamine in examined samples feisiekh and salted sardine were 21.5 and 17.2 mg/ 100gm respectively. These results are lower than results from Huda [15] which showed that mean histamine values of examined samples were 126.65±14.77 and 4.44±0.98 in examined feisiekh and smoked herring respectively. The result of mean histamine value of smoked herring is higher than from El- Sayed [16] which showed that mean histamine value of smoked herring was 10.06± 0.70. EOS [7] recommended that the maximal permissible limit for histamine was 20 (mg/100gm). Scombroid poisoning (scombrotoxicosis) is a worldwide problem of food borne intoxication caused by the consumption of sea food containing large quantity of

Table 1: Statistical analytical results of biogenic amines (Histamine, Cadaverine and Putrescine) levels (mg %) in fish products

	Histamine			Cadaverine			Putrescine		
Fish products	Min	Max	Mean ± S.E*	Min	Max	Mean ± S.E*	Min	Max	Mean ± S.E*
feisiekh	6.2	82.7	33.12±1.15	4.9	41.2	13.80±0.54	2.8	32.5	9.99±0.52
Salted sardine	4.6	73.2	28.14±1.02	3.8	35.7	11.05±0.46	2.5	29.9	8.81±0.44
Smoked herring	4.1	58.7	23.12±0.86	3.2	27.6	7.78±0.41	1.7	27.4	6.12±0.41

S.E* = standard error of mean

Table 2: Statistical analytical results of NaCl % in fish products

Fish Products	Min	Max	Mean ± S.E*
Feisiekh	7.2	11.6	9.46 ± 0.06
Salted sardine	5.8	10.3	8.71 ± 0.05
Smoked herring	6.4	10.1	8.65 ± 0.06

Table 3: Correlation coefficient (r) between NaCl% vs. biogenic amine levels in the examined samples of salted and smoked herring fish

Salted fish	Feisiekh	Salted Sardine	Smoked herring
NaCl %:			
1.Histamine	-0.57*	-0.51*	-0.69**
2. Cadaverine	-0.26	-0.19	-0.38*
3. Putrescine	-0.41*	-0.12	-0.45*

* Significant correlation ** High significant correlation

histamine [17]. High amount of histamine may be founding in food as a consequence of the use of poor quality raw materials, contamination and inappropriate conditions during food processing and storage [18]. The quantity of histamine is supposed to be a marker for microbiological contamination level in the food [19]. Factors affecting histamine production are the availability of free amino acids, presence of microorganisms that can decarboxylase amino- acids and favorable conditions for the growth of such microorganisms and production of decarboxylase enzyme [20].

Table (1) showed the concentrations of Cadaverine in the examined samples of feisiekh ranged from 4.9 to 41.2 with a mean value 13.80±0.54, while salted sardine ranged from 3.8 to 35.7 with a mean value 11.05±0.46 and smoked herring ranged from 3.2 to 27.6 with a mean value 7.78±0.41(mg%). These results lower than results reported by Ahmed *et al.* [21] which showed that mean Cadaverine values (mg/100g) of feisiekh and smoked herring were 803±156 and 895± 408.8 respectively and lower than results that reported by Huda [15] which showed that mean Cadaverine values in examined feisiekh and herring samples were 111.79±15.84 and 153.22± 25.47 respectively. These results nearly similar to results by Seham *et al.* [22] which reported that mean Cadaverine values in examined samples were 10.26±1.93 and 5.42±1.31 in low salted and high salted feisiekh and 11.69±2.12 and8.49±1.62 in low salted and high salted sardine respectively. EOS [7] recommended that the maximal permissible limit Cadaverine was 20(mg/100gm). The concentration of Cadaverine is a good indicator of spoilage and it significantly related to post processing handling of fish products or post-harvest handling of fresh fish [23].

Table (1) showed the concentrations of Putrescine in feisiekh ranged from 2.8 to 32.5 with a mean value 9.99±0.52 while salted sardine ranged from 2.5 to 29.9 with a mean value 8.81±0.44 and smoked herring ranged from 1.7 to 27.4 with a mean value 6.12±0.41(mg%). These results are lower than that reported by Ahmed *et al.* [21] which showed a mean value of Putrescine in feisiekh and smoked herring were 130.3±SE and 33.25±SE respectively, Huda [15] which reported the mean value of Putrescine in Feisiekh and smoked herring were 53.9± 8.56 and 30.5± 4.65 respectively and Zhai *et al.* [24] who found that Putrescine level within arrange of (0.19- 45.16 mg/kg) at all tested samples of fish products in Southern China. But these results nearly similar to that reported by El-Sayed [16] which showed that a mean value of Putrescine in pickled fish (sardine) was 4.09±0.25 mg/100g and 8.17±0.46 mg/100g in smoked herring, results by Fatih and Yesim [25] and that reported by Lapa-Guimarães and Pickova [26] who reported Putrescine in fish at level of 5 mg/100g. The production of Putrescine has been related to the presence of lysine decarboxylase enzymes which can be synthesized by auto-enzymes or by bacteria as *E. Coli, Enterobacter, lactobacilli and Pseudomonas, Streptococci, Micrococci and Aerobic spore-former species* [27, 28].

Table (2) showed that the concentrations of NaCl% in feisiekh ranged from 7.2 to 11.6 with a mean salt percentage 9.46± 0.06 while in salted sardine ranged from 5.8 to 10.3 with a mean salt percentage 8.71± 0.05 and in smoked herring ranged from 6.4 to 10.1 with a mean salt percentage 8.65±0.065%.These results nearly similar to results by Azza–and Weam [14] which reported that a mean salt percentage 7.3±0.8 and 7.1±0.6 in feisiekh and sardine respectively. These results lower than that reported by Seham *et al.* [22] showed mean values of sodium chloride% ranged from 10.98±3.47 to 14.47±4.57 at low salted sardine and high salted sardine while ranged from 12.29±3.88 to 16.12±5.09 in low salted and high salted feisiekh respectively. But these results higher than results reported by Ebtsam [13] which reported mean values of sodium chloride% were 5.45±0.13 and 5.9±0.17 in feisiekh and salted sardine respectively. EOS [7] which recommended that permissible limit % of sodium chloride (NaCl) in feisiekh and salted sardine not less than 6% and in smoked herring not more than 8%.

Table (3) showed the correlation coefficient between NaCl % and biogenic amines levels. Histamine was highly significant correlated to NaCl % among smoked herring and significant among salted sardine and feisiekh while Cadaverine was significant correlated to NaCl among smoked herring and Putrescine was significant to NaCl among feisiekh and smoked herring. Sodium chloride

(NaCl) plays an important role in microbial growth and therefore influences the activity of their amino acids decarboxylase [29] as salt concentration significantly affects lactic acid bacteria (LAB) since all types of bacteria are generally tolerant of moderate salt concentration [30]. The present study concluded that the examined samples (Feisiekh, Salted sardine, Smoked herring fish) contamination higher with mean values of biogenic amines (Histamine, Cadaverine, Putrescine). To prevent or reduce the contamination of salted and smoked herring fish with biogenic amines should be adapted the education on hygienic handling and manufacturing of raw materials.

REFERENCES

1. Kassem, G.M.A., 1996. Health hazard due to marketed salted fishes. M.V. SC. Thesis (Meat hygiene) Fac. Vet. Med., Cairo University.
2. Ahmed, A.M. and W.M. EL-Kazzaz, 2005. Control of Halotorerant Bacteria in Salted Fish (Feisiekh) using Trisodium Phosphate. Pak. J. Biol. Sci., 8(6): 882-887.
3. Burt, J.R. and R. Hardy, 1992. Compositionand deterioration of pelagic fish. In Pelagic fish: The resource and its exploitation. Fishing News Books-Division of Blackwell Scientific Publications Ltd., pp: 115-141.
4. Shalaby, A.R., 1996. Significance of biogenic amines to food safety and human health. Food Research. Int., 29: 213-231.
5. Chiacchierini, E., D. Restuccia and G. Vinci, 2005. Evaluation of two different extraction methods for chromatographic determination of Bioactive amines in Tomato products. Tanta, 69(3): 548-555.
6. Komprda, T., 2004. Obecnà hygiene potravin. Brno: Mendelovazemědělskà a lesnickàuniverzita, 145 p., ISBN 978-80-7157-757-7.
7. Egyptian Organization for Standardization "EOS", 2005. Reports related to No 1-1725/2005 for salted fish (Feisiekh), 2-1725/2005 for salted fish (Sardine) and No 288/2005 for smoked fish. Egyptian Standards, Ministry of Industry, Egypt.
8. Krause, I., A. Bockhardt, H. Neckerman, T. Henle and H. Klostermeye, 1995. Simultaneous determination of amino acids and biogenic amines by reversed- phase high performance liquid chromatography of the dabsyl derivatives. J. Chromatogr. A, 715: 67-79.
9. Pinho, O., I. Ferreira, E. Mendes, B. Oliviera and M. Ferreira, 2001. Effect of temperature on evolution of free amino acid and biogenic amine contents during storage of Azeitao cheese. Food Chem., 75: 287-291.
10. Association of Official Analytical Chemists "AOAC", 2000. Official methods of analysis. 13th Ed., W. Horwitz., (Editor), Academic press, Washington, D.C., USA.
11. Pipek, P., F. Bauer and G. Seiwald, 1992. Formation of histamine in vacuum packed fermented sausages. Proc. 38th ICOMST, (4): 819-822.
12. Rawles, D.D., G.J. Flic and R.E. Martin, 1996. Biogenic amines in fish and shellfish. Advances in Food and Nutrition Research, 39: 329-365.
13. Ebtsam, M. Abd El Fatah, 2014. Conformity of Hygienic Status of Retailed Salted Fish with Egyptian Standards. M.V. SC. Food control department (Meat Hygiene) Vet. Med. Banha University.
14. Azza, E.A. Hassan and Weam Baher, 2011. Chemical and Microbiological Evaluation of Some Marketed Fish in Mansoura. Assiut. Vet. Med. J., 57(131): 14-25.
15. Huda, K. El-Sayed, 2014. Biogenic amines in some fish product. Master of Veterinary Medical Science (Meat Hygiene) Zagazig University.
16. El- Sayed, E.A., 2010. Studies on chemical residues in fish and its products. Ph.D. SC., Meat Hygiene. Veterinary Medicine. Banha University.
17. Food and Drug Administration (FDA), 1998. Scombrotoxin (Histamine) formation in fish and fishery products, hazard and control guide, L2 edj. Washington, DC: FDA, center of food safety and applied Nutrition, office of seafood, pp: 73-90.
18. Halasz, A., A. Barath, L. Simon-Sarkadi and W. Halzapel, 1994. Biogenic amines and their production by micro-organisms in food-Review. Trends in food science and technology, 5: 42-49.
19. Leuschner, R.G., M. Kuthara and W.P. Hammes, 1999. Formation of biogenic amines by proteolytic enterococci during cheese ripening. J. Science and Food Agriculture, (79): 1141-1144.
20. El-Mossalami, H.H.A. and S.A.E. El-Agizy, 2005. Hazard of some toxic biogenic amines and improvement the quality of some fish and fish products in Alexandria city J. Beni-Suef Vet Med., K., pp: 83-90.
21. Ahmed, S.A., A. Hayam, Mansour Leila, A. Mohamed, M. Deabes and Doha, A. Salah Eldin, 2012. Biogenic Amines and its Relation with Microbial Load in Some Fish Products. Food hygiene and control. Global Veterinaria, 8(6): 583-590.
22. Seham, N. Hamouda and Ghada A.K. Kirrella, 2013. Quantitative Analysis of some Biogenic amines in Salted fish and its Correlation with NaCl Concentration. Kafr El- Sheikh University Vet. Med. J., (11) (2): 19- 37.

23. Flic, J.G., M.P. Oria and L. Douglas, 2001. Potential hazardous in cold smoked fish: Biogenic amines. J. Food Sci., 66: 1088-1099.
24. Zhai, H., X. Yang, L. Li, G. Xia, J. Cene, H. Huang and S. Hao, 2012. Biogenic amines in commercial fish and fish products sold in southern china. Food control, 25: 303-308.
25. Fatih, Ö. and Ö. Yesim, 2006. Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. J., Food Chemistry, 99(3): 574-587.
26. Lapa-Guimarães, J. and J. Pickova, 2004. New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. J. of chromatography A, 1045, 1-2, (6): 223- 232.
27. Maijala, R.L., S.H. Eerela and E. Nurmi, 1993. Contaminant lactic acid bacteria of dry sausage produce histamine and tyramine. J. Meat Science, 35: 387-3954.
28. Roig-Sagues, A.X., M. Hemand-Herrero and M.T. Mora-Ventura, 1997. Histidine decarboxylase activity during the production of ripened sausage and its influenced on the formation of Cadaverine. Journal of food protect, 60(4): 430-432.
29. Zaman, M.Z., A.S. Abdulamir, F.A. Bakar, J. Salamat and J.A. Bakar, 2009. Microbiological, Physicochemical and health impact of high level of Biogenic amines in fish sauce. American J. of Applied Sci., 6(6): 1199-1211.
30. Sanchez, P.C., 2008. Philippine Fermented Foods Principle and Technology. The University of the Philippines press, Quezon City.