Enliven Archive
Intensifying Thoughts

Research Article www.enlivenarchive.org Enliven: Toxicology & Allied Clinical Pharmacology

ISSN: 2379-5719

Comparison of the HPLC and the TLC Techniques for the Determination of Biogenic Amines Spiked to Sausage and Smoked Herring Samples

M.M. Deabes*[1], Naguib, Kh[1], Ayesh, A M[1] , El-Damaty EM[2], and Rowayshed GH[2]

[1]Food Toxicology and Contaminants Department, National Research Center, Cairo, Egypt

[2]Food Science and Technology Department, Faculty of Agricultural, Al-Azhar Univeristy, Cairo, Egypt

*Corresponding author: M.M. Deabes, Food Toxicology and Contaminants Department, National Research Center, Egypt,
E-mail: mydeabes@yahoo.com

Received Date: 20th February 2018
Accepted Date: 19th March 2018
Published Date: 21st March 2018

Citation: M.M. Deabes, Naguib, KH, Ayesh, A M, El-Damaty EM and Rowayshed GH (2018) Comparison of the HPLC and the TLC Techniques for the Determination of Biogenic Amines Spiked to Sausage and Smoked Herring Samples. Enliven: Toxicol Allied Clin Pharmacol 5(1): 001.

Copyright: @ 2018 M.M. Deabes. This is an Open Access article published and distributed under the terms of the Creative Commons Attribution License, that permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.reproduction in any medium, provided the original author and source are credited.

Abstract

The aim of our study to comparative the suitability of chromatographic techniques such as thin layer chromatography (TLC)-densitometry and high performance liquid chromatography (HPLC) for the analysis of biogenic amines in fish and meat products was carried out. The recovery percent of the tested biogenic amines spiked to sausage and smoked herring samples was 92.0-102.2% and 94.5-100.3 % for HPLC versus 86.2-98.5 % and 88.0- 98.0 %for the recommended one-dimensioned TLC, respectively. It could be observed that there were not significant differences in the recovery percentages of tryptamine, β -phenylethylamine, putrescine, histamine and tyramine spiked to either sausage sample or smoked herring sample between the tested tow analytical methods. Therefore, the TLC-densitometry was found to be rapid and less expensive. In addition, this method is facile, fast, routine, cost effective and suitable method for the analysis of wide range eight biogenic amines and simultaneous screening of several samples at a time.

Keywords: Biogenic amines; Histamine; Tyramine; HPLC; TLC

Introduction

Biogenic amines (BAs) are low molecular weight organic bases with an aliphatic (putrescine, cadaverine, spermine, spermidine) aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structure. They arise as a consequence of metabolic processes in animals, plants and microorganisms [1].

Amines are naturally present in living organisms and, hence, in foods. These compounds play an important role in nucleic acid regulation and protein synthesis, and possibly in the stabilization of membranes [2,3]. Biogenic amines are produced by decarboxylation of free amino acids mediated by amino acid decarboxylase enzymes. Amino acid decarboxylation occurs through removal of the α-carboxyl group to give the corresponding amines [2-4]. Biogenic amines play essential roles in the normal development, metabolism and physiological functions of humans. However, when ingested in high concentrations they can cause a range of toxicological effects. The presence of some of these toxic compounds has been directly or indirectly related to a number of carcinogenic effects [5]. Moreover [6], demonstrated that polyamines stimulate the formation of mutagenic 1, N 2-propanodeoxyguanosine adducts from acetaldehyde, the first metabolite of ethanol, explaining the carcinogenicity of alcoholic beverage consumption, especially at the gastrointestinal tract level. Gerner and Meyskens [7] reported that a positive correlation between cancer occurrence and polyamine consumption has been extensively. For example, high putrescine levels in gastric carcinoma are caused by Helicobacter pylori [8]. Tyramine and histamine, the most toxic biogenic amines (BAs), are often found in high concentrations in certain foods. Surprisingly, tyramine had a stronger and more rapid cytotoxic effect than histamine. Their mode of action was also different, while tyramine caused cell necrosis, histamine induced apoptosis. To avoid health risks, the BA content of foods should be reduced and legal limits established for tyramine [9].

The occurrence of biogenic amines, as well as controlling its limits in foods and food products, would not be possible without the supporting analytical methods which should be standardized and harmonized by different analysts. The complex sample matrix, the presence of potentially interfering compounds and the occurrence of several biogenic amines

simultaneously in the same aliquot of an extract are typical problems encountered in the analysis of food for biogenic amines. Several methods have been reported for the analysis of histamine and other amines, which include fluorimetric, enzymatic and chromatographic techniques. Among these, only the chromatographic techniques have the capacity to separate the different biogenic amines. Chromatographic techniques such as thin layer chromatography (TLC) [10,11], gas chromatography GCand high performance liquid chromatography (HPLC) [12-20] have been used for the analysis of biogenic amines.In recently [21] developed a competitive fluorescent molecularly imprinted polymer (MIP) assay to detect biogenic amines in fish samples. The methods used for analysis of food for amines individually would be extremely difficult and time-consuming, scince they require large- scale and laborious purification, as well as the time required for separation and determination of biogenic amines is also so long [22]. So, an accurate, economic, and rapid analytical method is urgently needed. In general, TLC is simple and dos not require special equipment, but most of the published methods suffer from the excessive time needed for analysis and / or inaccuracy of the obtained results.

Therefore, the comparison study was carried out between the HPLC and the TLC techniques, for the determination of eight biogenic amines in the most acceptable samples of sausage and smoked herring fish.

Materials and Methods

Biogenic Amines Determination

Eight biogenic amines included histamine, tyramine, tryptamine, cadaverine, spermine, putrescine, spermidine and β-phenylethylamine were extracted and determined in all tested samples according to [20] as follows

Reagents

a- Chloroform, n-butanol, n-heptane, acetone, sodium hydroxide, sodium bicarbonate (NaHCO3), hydrochloric acid, trichloroacetic acid (T.C.A), benzene, triethylamine acetone. All chemicals used in the analytical analysis were produced by (Adwic- Co., El Nasr pharmaceutical chemicals, A.R.E.) .Methanol, acetonitrile, diethylether and acetic acid of HPLC grade, produced by (BDH, England).The pure standard biogenic amines and dansyl chloride were produced by (Sigma- Co., Louis, MO 63178 U.S.A). TLC plates aluminum sheets (20 x 20 cm) precoated with silica gel G 60 without Fluorescence indicator, 1.05553, Layer thickness 0.2 mm, (E.Merck, 64271 Darmstadt, Germany) Dansyl chloride (5- { Dimethylamino} naphthalene -1- sulfonyl chloride) (Sigma Co. Louis, Mo 63178 U.S.A).Histamine-2HCl, tyramine - HCl, cadaverine - 2 HCl, putrescine -2 HCl, tryptamine - 2 HCl, spermine - 4 HCl, spermidine -3HCl and β -phenylethylamine were purchased from (Sigma- Co. Louis, Mo 63178 U.S.A).b) Dansyl chloride solution: 500mg of dansyl chloride were dissolved in 100 ml acetone.

b- Standard solutions: Stock standard solutions of the tested amines: 25 mg of each standard pure amines histamine-2HCl, tyramine - HCl, cadaverine - 2 HCl, putrescine -2 HCl, tryptamine - 2 HCl, spermine - 4 HCl, spermidine - 3 HCl and β -phenylethylamine were dissolved in 50 ml distilled water individually andstored at -18°C until used.

c- Working standard solutions two milliliters of each stock standard solution were pipetted into 100 ml volumetric flask and diluted to volume with 5% trichloroacetic acid (TCA). This solution is prepared freshly (weakly) and stored in a refrigerator.

Mobile phase solvents consists of

Apparatus

High performance liquid chromatography (HPLC) was used to dansylamines determination. The system equipped with (Waters 600) delivery system.

HPLC column: Reverse phase C18 Nucleosil column 250 x 4 mm, 10 µm packing, (Macherey - Naggl). The detection was performed using U.V detector (waters 486) at 254 nm wavelength, using linear program of 25 min period and 1 ml / min constant solvent flow rate. Data were integrated and recorded using a Millennium Chromatography. Manger Software 2010 (Waters, Milford MA 01757).

Determination of dansylamines by TLC- densitometry

The chromatographic separation was carried out to separate the eight dansylamines by one- dimensional TLC.Ten microliters of standard dansylamine and the samples extract were spotted 2.0 cm from the base of the TLC plates using a Hamilton microsyring. The plate was developed in a different suggested solvent system included 1- benzene / triethylamine (5:1) 2-benzene: triethylamine: acetone (10:2:1) and 3-chloroform: benzene: triethylamine (6: 4.5:1) for 17 cm. highest. Then, the Rf values were determined. The plate was dried at room temperature until the excess of solvent disappeared. The resulting zones were examined and marked under long ultraviolet wavelength (360 nm). The marked areas were determined using CS- 9000 Dual wave length 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 in Research Center of poisons and Narcotics, Faculty of Science, Cairo University.

Extraction

Twenty five gram of homogenized smoked herring flesh or sausages was blended with 125 ml of 5% TCA for 3 min using a warning blender then filtration was achieved using filter paper Whatman No. (1) England. Ten milliliters of the extracts was transferred into a suitable culture tube with 4g NaCl and 1 ml of 50 % NaOH then shake and extracted three times by 5 ml n-butanol: chloroform (1:1 v/v) stoppered and shake vigorously for 2.0 min. followed by centrifugation for 5.0 min. at 3000 rpm and the upper layer was transferred to 50 ml separating funnel using disposable Pasteur pipette. To the combined organic extracts (upper layer), 15 ml of n-heptane was added and extracted three times with 1.0 ml portions of 0.2 N HCl, the HCl layer was collected in a glass stoppered tube. Solution was evaporated just to dryness using water bath at 95°C with aid of a gentle current of air.

Formation of dansylamines

Two hundred µl of each stock standard solution (or sample extract) were transferred to a culture tube and dried under vacuum. About 0.5 ml of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard). Stoppered and carefully mixed to prevent loss- due to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min. About 10 ml of distilled water were added to the reaction mixture, stoppered and shaked vigorously using vortex mixer, then the extraction of dansylated biogenic amines was carried out using three times of 5.0 ml portions of diethylether, stoppered, shake carefully for 1.0 min and the ether layers were collected in a culture tube using disposable pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry bath with aid of current air. The obtained dry film was dissolved in 1ml methanol, then 10 µl injected in HPLC.

Calibration

Two hundred of each stock standard solutions were transferred to glass stoppered tube. Using a current of air on dry bath, at 90°C the solution the solution was evaporated to < 200 µl. Dansyl derivatives were prepared above the residue was dissolved in 5.0 ml acetonitrile (1ml = 20 µg or 50 µl = 1µg each of the derivatives).Injection was carried out using 10- 20 µl of each calibrated.

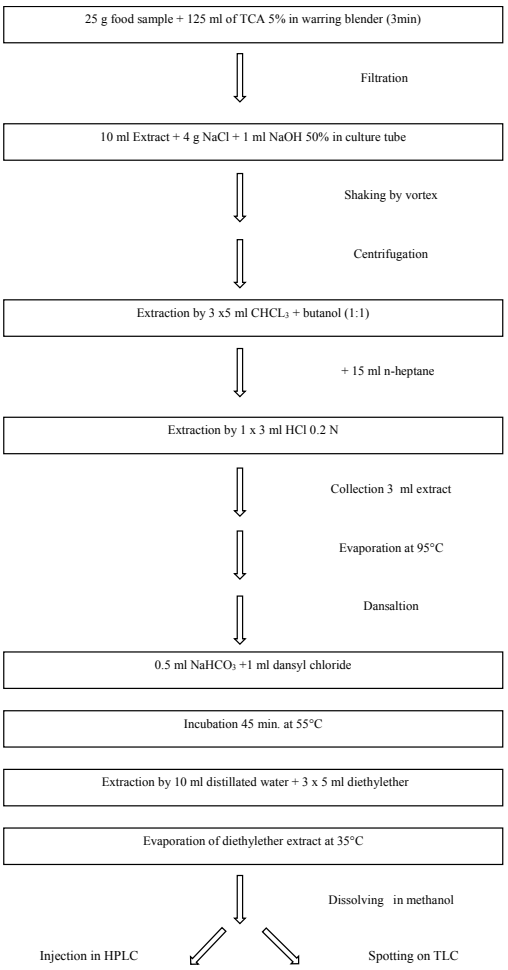


Fig.(1):Schematic Diagram of biogenic amines determination (Deabes2000).

Calculation

Peaks area of each of the eight dansylamines (standard or the examined sample) were obtained from the HPLC Millennium report and the concentrations were calculated according to the follow equation:

ppm of each dansylamine = (P /P*) x dilution factor of sample.

P* = peak area of standard.

Recovery determination

For the recovery assay, accuracy, a known amount of biogenic amines was added is mixture to a samples to the most acceptable of sausage and smoked herring fish from biogenic amines to a level of 20 mg /kg (3 samples of each). Extraction, separation, and quantitative procedures were done using TLC and HPLC as previously described.

Statistical Analysis

The experiment followed randomized complete block design. The obtained data were subjected to analysis of variance (ANOVA) according to [23] using Mstate Programmed. Least significant differences were used compare between means of treatments according to [24] at probability 5%.

Results and Discussion

The dansyl derivatives could be easily detected at a very low concentration under UV Light due to their fluorescent characteristics. The obtained results of (table 1) and (figures (3&4)) indicated that the R_f values calculated during the separation of mixture of the eight dansylamine (tryptamine, putrescine, cadaverine, spermidine, histamine, spermine, tyramine and β -phenylethamine) by the suggested 3 solvents system using one dimensional TLC technique. It is clear from them presented data that solvent system No.1 Benzene: Triethylamine (5:1), system No.2 Benzene: Triethylamine: Acetone (10:2:1) and system No. 3 chloroform: Benzene: Trimethylamine (6: 4.5: 1) showed different levels of separation depending on the tested dansylamine. However the first system was showed an obvious separation of only four dansylamines namely (spermidine, histamine, tyramine and β -phenylethamine), but the second system was resulted in marked separation of only six dansylamines (tryptamine, putrescine, cadaverine, spermidine, histamine, spermine,) from the tested eight compounds with an occurrence of an interference between histamine and spermine, which means that this system is not recommended for the detection of histamine and spermine. Also, the above data of (table 1) and (Figure 3&4) illustrated that the best separation of the tested eight amines was achieved by using the solvent system No.3chloroform: benzene: triethylamine (6: 4.5: 1).

The chromatogram of standard biogenic amines: spermine, tryptamine, β -phenylethamine, putrescine, cadaverine, histamine, tyramine and spermidine as (Figure 2) Indicated the efficiency of resolution with respect to retention time for the identification of the responded authentic samples.

ByusingthesolventsystemNo.3Chloroform:Benzene:Triethylamine(6:4.5:1). The comparison study between the accuracy (% recovery of amines) of the HPLC and the recommended one-dimensioned TLC technique (system No. 3) for determination the tested eight biogenic amines in spiked sausage and smoked herring (table 2) .

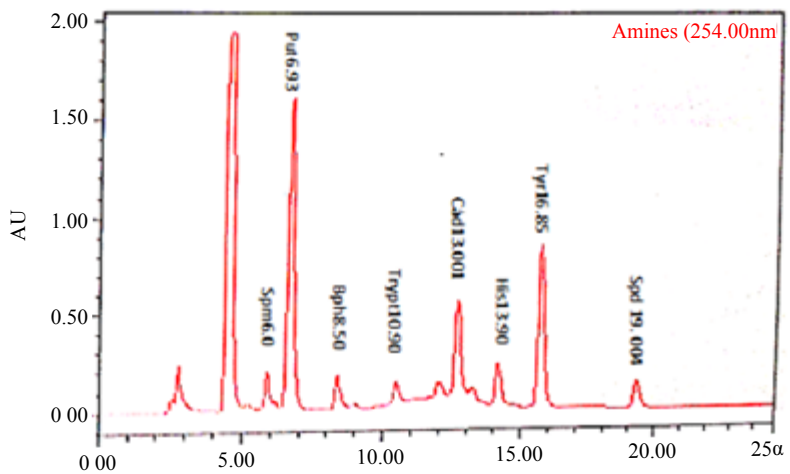
Mobile phase solvents consists of:
Solvent A: Acetonitrile: 0.02 N acetic acid (1:9) and
Solvent B: 0.02N acetic acid: acetonitrile: methanol (1: 9: 9).
Solvent A and B were used in gradient elution program as follow:

Time min.	Flow rate ml/min	Solvent		Curve
		A%	B%	
0	1	25	75	-
10	1	10	90	6
20	1	5	95	6
25	1	25	75	6

Table 1: R_f values of the tested biogenic amines

Developing system	R_f values							
	Trypt	Put	Cad	Spd	His	Spm	Tyr	β-ph
1-Benzene: TEA 5:1	0.09	0.06	0.08	0.09	0.20	0.13	0.33	0.14
2-Benzene: TEA: Acetone 10:2:1	0.41	0.30	0.34	0.36	0.37	0.24	0.48	0.50
3-Choloroform: Benzene: TEA 6: 4.5:1	0.66	0.72	0.79	0.82	0.86	0.89	0.93	0.95

TEA= triethylamine, Spm= spermine, Trypt= tryptamine, β-ph= β-phenylethylamine, put= putrescine, Cad= cadaverine, His= histamine, tyr= Tyramine, Spd= spermidine.



Spm=spermine,Tryp=tryptamine,3)Bph= β-phenylethamine, put= putrescine, cad=cadaverine, his=histamine,tyr- tyramine, spd=spermidine.

Fig.(2): Dansylated of standard biogenic amines overviewed by HPLC.

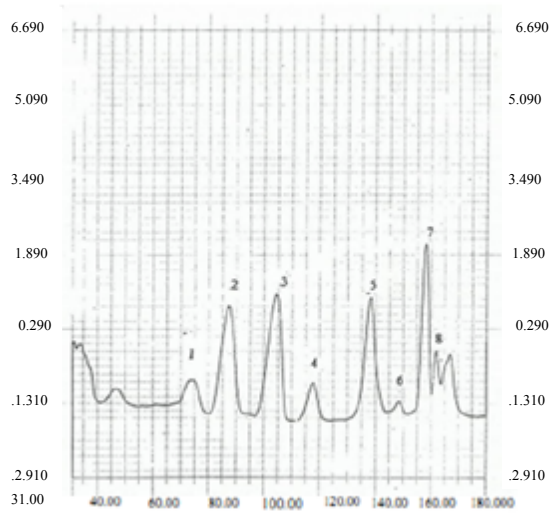


Fig.(3) Densitometric chromatogram of eight biogenic amines standard on silica gel TLC plate.
1-tryptamine, 2-putrescine,3-cadaverine, 4-spermidine, 5-histamine, 6- spermine, 7-tyramine and 8- β -phenylethylamine.

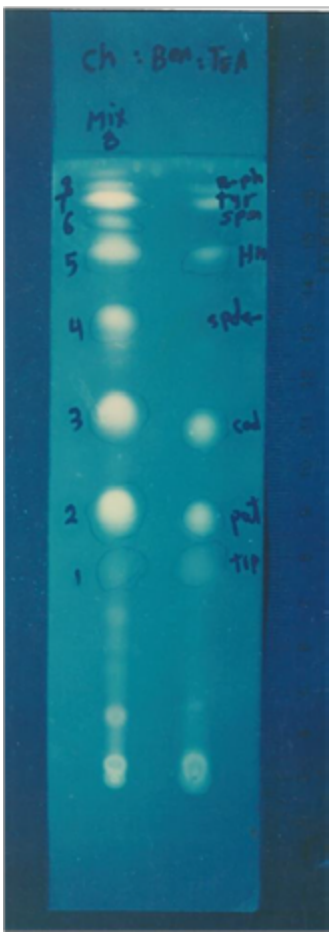


Fig.(4) TLC separation of the tested eight dansylamines using Chloroform: Benzene: Triethylamine (6: 4.5: 1).
1-tryptamine,2-putrescine, 3-cadaverine, 4-spermidine, 5-histamine, 6- spermine, 7-tyramine and 8- β -phenylethylamine.

Table 2: Recovery of biogenic amines from spiked sausage and smoked herring fish using HPLC and TLC.

Biogenic amines	Sausage				Herring			
	HPLC		TLC		HPLC		TLC	
	BA	%R	BA	%R	BA	%R	BA	%R
Spm	18.4	92.0[a]	17.2	86.2[b]	19.1	95.5[a]	18.1	90.4[b]
Trypt	19.2	96.2[a]	19.5	97.7[a]	18.9	94.5[a]	19.0	95.1[a]
β –ph	19.4	97.0[a]	19.7	98.5[a]	19.3	96.5[a]	19.4	96.9[a]
Put	20.0	100.0[a]	19.6	97.9[a]	19.8	99.0[a]	19.7	98.5[a]
Cad	20.5	102.5[a]	18.8	94.1[b]	20.1	100.3[a]	18.4	92.0[b]
His	19.9	99.5[a]	19.9	99.3[a]	19.8	98.8[a]	19.4	97.1[a]
Tyr	17.0	95.1[a]	19.0	95.1[a]	18.9	94.5[a]	18.7	93.7[a]
Spd	19.3	96.5[a]	18.5	92.7[b]	19.0	95.0[a]	17.6	88.0[b]

Spm= spermine, Trypt= tryptamine, β-ph= β -phenylethylamine, put= putrescine, Cad= cadaverine, His= histamine, tyr= Tyramine, Spd= spermidine, BA= biogenic amines, %R=% revovery

In order to evaluate the accuracy-recovery of the HPLC and the recommended one-dimensioned TLC (system No.3) for determination the tested eight biogenic amines, 20 mg/kg of each amine was spiked to both sausage and smoked herring samples. The obtained results (table 2) cleared that all the tested biogenic amines were detected by the two analytical methods. On the other hand, the recovery percent of the tested biogenic amines spiked to sausage and smoked herring samples was 92.0-102.2% and 94.5-100.3 % for HPLC versus 86.2-98.5 % and 88.0- 98.0 %for the recommended one-dimensioned TLC, respectively. In addition to, it could be observed that there were not significant differences in the recovery percentages of tryptamine, β -phenylethylamine, putrescine, histamine and tyramine spiked to either sausage sample or smoked herring sample between the tested two analytical methods. It is worth to mention that high-pressure liquid chromatography (HPLC) offer some advantages in accuracy and flexibility, yet there are some difficulties in its availability, cost and experience and also the apparatus needed are usually available to only the most sophisticated analytical laboratories due to their higher costs and they also require special experience for operators. On the other hand, the developed TLC technique system No. 3 Chloroform: Benzene: Triethylamine (6: 4.5: 1) that was reached through this study can assay numerous samples with a minimum of time, effort and cost and also it is simpler has satisfactory accuracy, therefore it can be available in all food testing laboratories for quantitative and qualitative determination of all tested biogenic amines.

Conclusion

The routine to analysis of fish and meat and their products, the TLC method using precoated with silica gel G 60 without Fluorescence indicator. The separation of eight biogenic amine on TLC system No. 3 Chloroform: Benzene: Triethylamine (6: 4.5: 1) and determination by densitometry. It can be used as a quick screening method to assess the presence of eight biogenic amines. This method can be easily adopted by regulatory agencies and food industries as a qualitative and quantitative tools for the simultaneous analysis of the quality.

Reference

1. Askar A, Treptow H (1986) Biogenic amines in food. Ung Eugen Ulmer Gmbh &co; Stuttgart. Germany.
2. Smith TA (1980) Amines in food. Food Chemistry 6: 169-200.
3. Bardocz S (1995) Polyamines in food and their consequences for food quality and human health. Trends Food Sci. Tech 6: 341-346.
4. Halasz A, Barath A, Simon-Sarkadi L, Holzapfel W (1994) Biogenic amines and their production by microorganisms in food. Trends Food Sci Tech 5: 42-49.
5. Ladero V, Calles-Enríquez M, Fernández M, Alvarez MA (2010) Toxicological effects of dietary biogenic amines. Curr Nut Food Sci 6: 145-156.
6. Theruvathu JA, Jaruga P, Nath RG, Dizdaroglu R, Brooks PJ (2005) Polyamines stimulate the formation of mutagenic 1, N2-propanodeoxyguanosine adducts from acetaldehyde. Nucl Acid Res 33: 3513-3520.
7. Gerner EW, Meyskens FL (2004) Polyamines and cancer: Old molecules, new understanding. Nat Rev Cancer 4: 781-792.
8. Shah P, Swiatlo E (2008) A multifaceted role for polyamines in bacterial pathogens. Mol Microbiol 68: 4-16.
9. Linares DM, del Rio B, Martin MC, Fernandez M, Alvarez MA, et al. (2016) Comparative analysis of the in vitro cytotoxicity of the dietary biogenic amines tyramine and histamine. Food chem 197: 658-663.
10. Chin H, Koehler PE (1983) Identification and estimation of histamine, tryptamine, phenethylamine and tyramine in soy sauce by Thin-Layer Chromatography of dansyl derivatives. J of Food Science 48: 1826-1828
11. Naguib K, Ayesh AM, Shalaby AR (1995) Studies on the determination of biogenic amines in foods. 1. Development of a TLC method for the determination of eight biogenic amines in fish. J. Agric. Food Chem. 43: 134-139.

12. Mietz JL, Karmas E (1977) Chemical quality index of canned tuna as determined by high pressure liquid chromatography. Journal of Food Science 42: 155-158.
13. Yen, GC, and Hsieh CL (1991) Simultaneous analysis of biogenic amines in canned fish by HPLC. J. Food Sci 56: 158-160.
14. Luten JB, Gouyou JP, Landrein A, Ritchie A, Leclerq M, et al (1992) Biogenic amines in fishery products: standardization methods within EC. H.H. Huss et al (Eds) Quality Assurance in the Fish Industry Elsevier Science publishers 427-439.
15. Eerola S, Hinkkanen R, Lindfors E, Hirvi T (1993) Liquid chromatography determination of biogenic amines in dry sausage. J of AOAC Int. 76: 575-577.
16. Ayesh AM, Nadir AS, Ibrahim SH (1997) Effect of frozen storage on the content of biogenic amines of the main edible parts of chicken. The Egyptian J Food Sci 25: 83-97.
17. Deabes MM (2000) Studies on some biogenic amines in some meat and fish products with respect to other quality attributes. Food Sci Tech Dep, Faculty of Agriculture, Al-Azhar University, Egypt.
18. Karovicova J, Kohajdova Z (2005) Biogenic amines in food. Chem Pap. 59: 70-79
19. Chen H, Huang Y, Hsu H, Lin C, Chen W, et al. (2010) Determination of histamine and biogenic amines in fish cubes (Tetrapturusangustirostris) implicated in a food borne poisoning. Food Control 21:13-18.
20. Deabes MM, Ahmed SA, Hayam A, Mansour Laila A, Mohamed et al. (2013) Liquid chromatographic determination of histamine in traditionally salted, smoked and frozen fish with relation to microbial load. Toxicology letters 221: S122-S123
21. Mattsson L, Xu J, Preininger C, Bui BTS, Haupt K (2018) Competitive fluorescent pseudo-immunoassay exploiting molecularly imprinted polymers for the detection of biogenic amines in fish matrix. Talanta 181: 190-196.
22. Shalaby AR (1994) Separation, identification and estimation of biogenic amines in foods by thin-layer chromatography. Food Chemistry 49:305-310.
23. Snedecor Gw, Cochran WG (1980) Statistical Methods, 7th ed. Iowa State Unive. Press, Iowa, USA.
24. Walter A, Duncan DB (1969) Multiple range and multiple test. Biometrics 11:1-24.
25. Ayesh AM, Amra HA, Abu-Arab AAK, Naguib K (1995) Detection and determination of eight biogenic amines in frozen mackerel combromorous Sp.), and sardine (Sardinella Sp.) using HPLC". J Egypt Eer Soc Zool 17: 121-135.
26. Shalby AR (1996) Significance of biogenic amines to food safety and human health, Food research International 29:675-690.

Submit your manuscript at
http://enlivenarchive.org/submit-manuscript.php

New initiative of Enliven Archive

Apart from providing HTML, PDF versions; we also provide **video version** and deposit the videos in about 15 freely accessible social network sites that promote videos which in turn will aid in rapid circulation of articles published with us.