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A rapid gas chromatographic method for the determination of histamine in fish and fish products

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Abstract

A gas chromatographic (GC) method, which reduced the time for determination of histamine in fish and fish products to less than 20 min, was demonstrated. Contrary to traditional GC method, histamine in sample was initially extracted with alkaline methanol and injected into a GC column (CP-SIL 19CB) for analysis without derivatization. Internal standard used in this protocol was 1,9-nonanediol. Detection limit for histamine by this method was about 5 μg/g. Standard addition test indicated 98–111% (CV: 2.7–7.8%) of recovery for tuna flesh and 99–102% (CV: 2.7–8.9%) for shrimp meat after adding with authentic compound, suggesting that using direct GC analysis for histamine determination was feasible.

Keywords: Histamine; Fish; Gas chromatography

1. Introduction

Histamine is a potentially hazardous compound and historically referred to be the major causative agent in scombroid fish poisoning (Arnold & Brown, 1978; Hwang, Chang, Shiau, & Cheng, 1995). The formation of histamine in fish and shellfish was mainly derived from decarboxylation of histidine by exogenous decarboxylase released from microflora associated with the specimens or surrounding seawater (Rawles, Flick, & Martin, 1996). Immediately after catching, fresh fish contains very low levels of histamine, but the content increases with the progress of fish decomposition (Fernandez-Salguero & Mackie, 1987; Frank, Yoshinaga, & Nip, 1981). Therefore, histamine has also been proposed as a chemical index of freshness of fishes (López-Sabater, Rodríguez-Jerez, Roig-Sagues, & Mora-Ventura, 1994) and poor hygienic quality of raw materials used and/or poor manufacturing conditions (Hui & Taylor, 1983). The guideline established by the US Food and Drug Administration (FDA, 1996) for histamine in edible fish is 5 mg/100 g and the fish with histamine above that level are prohibited from being sold for human consumption. In order to provide a basis for practicing hazard analysis critical control point (HACCP) in fish and fish product processing industry, a rapid, precise, and reliable method for quantification of histamine is urgently required.

Many quantification methods for histamine have been developed. The current official method (AOAC, 1995) using a fluorometric procedure is sensitive and reproducible (Stratton & Taylor, 1991) but complex and time consuming. Besides, a variety of chromatographic methods have also been applied to separate histamine and determine the content of histamine in foods, which include: gas chromatography (GC) (Du, Huang, Kim, Marshell, & Wei, 2001; Farn & Sims, 1987; Staruszkiewicz & Bond, 1981); thin-layer chromatography (Naguib, Ayesh, & Shalaby, 1995), liquid chromatography (Fernandez-Salguero & Mackie, 1987; Veciana-Nogués, Hernández-Jover, Mariné-Font, & Vidal-Carou, 1995), and high performance liquid chromatography (HPLC) (Hui & Taylor, 1983; Hwang, Chang, Shiau, & Chai, 1997; Vázquez-Ortiz, Caire, Higuera-Ciapara, & Hernández, 1995 Yen & Hsieh, 1991). Other techniques which have been used to measure histamine are, for example, oxygen-sensor based assay (Ohashi, Nomura, Suzuki, Otsuka, Adachi, & Arakawa, 1994),

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copper chelation assay (Bateman, Eldrige, Wade, McCoy-Messer, Jester, & Mowdy, 1994), enzyme-based screening assay (Ben-Gigirey, Craven, & An, 1998; Lerke, Porcuna, & Chin, 1983), dipstick test (Hall, Eldrige, Saunders, Fairclough, & Bateman, 1995), capillary electrophoretic analysis (Mopper & Sciacchitano, 1994), and enzyme-linked immunosorbent assay (ELISA) (Serrar, Brebany, Bruneau, & Denoyel, 1995), etc.

Prior to analysis, preparation of histamine derivative, such as pentafluoropionic anhydride derivatization, was traditionally considered a necessary step when using GC method (Du et al., 2001; Staruszkiewicz & Bond, 1981). The hesitation of direct analysis of histamine or other biogenic amines by GC without derivatization was probably attributed to less volatility of the compounds. With improvement of the quality of commercial GC column, our laboratory has established several rapid methods to determine less volatile compounds in foodstuff using GC, such as organic acids (Choong, Ku, Wang, & Lee, 1997; Choong, Wang, Chou, & Fan, 1999; Wang, Lin, Lee, & Choong, 1999), and food preservatives (Lin & Choong, 1999), and sterols (Choong, Lin, Chen, & Wang, 1999). The successful application of GC on measuring less volatile compounds shed a light on that this method may be also working on the determination of histamine. The aim of this study was, therefore, to test the possibility of using GC to directly determine histamine content in fish and fish products. In this study, the GC column selection and extraction protocol for obtaining histamine from sample were also discussed.

2. Materials and methods

2.1. Samples and reagents

The sample of fish (including *Thunnus thynnus*, *Coloabis saira*, *Scomber australasicus*, *Engraulis japonicus*, and *Pampus argenteus*), shrimp (*Penaeus monodan*) and fish products (including fish ball, fish cake, and tempura) were purchased from supermarket located at Pingtung, Taiwan. Storage condition for fish and shrimp samples in supermarket was by freezing (−18 to −20 °C) and that for fish products was by refrigeration (4–6 °C). These samples were carried in icebox back to laboratory. All samples were stored at −20 °C before analysis. Two cultured fishes in Taiwan, *Tilapia* sp. and *Chanos chanos*, were also examined for displaying the level of histamine in very fresh samples. The sample of *Tilapia* was obtained from instantly sacrificed fish, and that of *C. chanos* was from the fish still in rigor mortis. Chemicals, such as histamine and 1,9-nonanediol, were purchased from TCI (Tokyo Chemical Industry Co., Japan). The solvents used in this study were liquid chromatographic grade and purchased from ALPS Chemical Co., Taiwan. Standard solutions of histamine and internal standard were made up with 50 mg in 100 ml methanol, respectively.

2.2. GC and GC/MS analysis

In order to analyze histamine directly by GC, several megapore capillary columns with different polarity (CP-SIL 5CB, CP-SIL 8CB, CP-SIL 19CB) were compared. These columns were purchased from ChromPack (Netherlands) and with specifications as follows: length: 30 m, I.D.: 0.53 mm, and film thickness: 1.5 μm. The efficiency of column on analysis of histamine was examined with authentic compound and alkaline methanol extract from tuna meat or shrimp meat spiked with histamine, followed by GC and GC/MS analysis. The GC analysis was conducted in a GL Science Model G-390B GC (Tokyo, Japan) equipped with a megapore capillary column and a flame ionizing detector (FID; H_2: 30 ml/min and air: 300 ml/min). Carrier gas was nitrogen flushed at the rate of 3 ml/min. The temperature at injector port and detector was set at 260 and 290 °C, respectively, and splitless injection (0.5 μl for each injection) was used. Oven temperature was controlled with a temperature elevation program during analysis, which was initially set at 160 °C for 2 min, elevated to 220 °C at the rate of 10 °C/min and then to 280 °C at the rate of 40 °C/min. The detection limit of histamine by GC analysis was also determined using diluted authentic histamine when the settings of FID range was at 1, attenuation at 1, and signal/noise ratio > 2. With above condition, the detection limit was found to be about 5 μg/g.

During the early stage of developing this method, the proposed histamine peak shown in GC profile was also confirmed by GC/MS analysis, which was conducted in a Perkin-Elmer GC (Turbo Mass ™) connected with a Perkin-Elmer Mass Selective Detector (600 Series). The column used for GC/MS was a 30 m CP-SIL 19CB (I.D. 0.32 mm). Carrier gas was helium flushed at the rate of 1.5 ml/min. Temperature of injector port was set at 260 °C and that of connecting interface was 280 °C. Oven temperature was elevated following with a program initially set at 140 °C for 2 min, then increased to 250 °C at the rate of 10 °C/min and maintained for 2 min. Split ratio at 1/25 was used for injection mode. The condition for mass spectrometry was set at 70 eV for electron impact mode, 4.0 min for solvent delay, autotune voltage for EM voltage, and scan for data acquisition mode. The mass spectrum of the sample peak was then compared with that in database.

2.3. Calculation of relative response factor (RRF) of histamine to 1,9-nonanediol

Standard solution of histamine and 1,9-nonanediol (0.5 mg/ml each) in methanol were mixed in the following

combinations: 1:2, 1:1, or 2:1 (v/v), then subjected to GC analysis. Peak area obtained from above analysis was applied to Eq. (1) for RRF calculation.

$$\mathrm{RRF} = (A_{\mathrm{his}})/(W_{\mathrm{his}}) \div (A_{\mathrm{IS}})/(W_{\mathrm{IS}}) \quad (1)$$

where A_{his}, peak area of histamine obtained from GC analysis; A_{IS}, peak area of 1,9-nonanediol obtained from GC analysis; W_{his}, weight (μg) of histamine used in analysis; and W_{IS}, weight (μg) of 1,9-nonanediol used in analysis.

2.4. Effect of pH on histamine extraction and detection efficiency by GC

Firstly, the flesh of tuna (*T. thynnus*) was thawed and exposed to room temperature for 2 h to produce a slightly decomposed sample. Histamine in samples was extracted with methanol modified from AOAC (1995). The tuna flesh was initially homogenized in a blender and weighted about 1 g into a capped test tube. The homogenate was added with 400 μl 1,9-nonanediol (0.5 mg/ml) and 3 ml methanol with or without a drop of 0.1 N NaOH, and then ultrasonication extraction in an ice-bathing sonicator for 5 min. Added with NaOH would make the pH of methanol extract elevate to above 9 or even to 10. Solid residue in samples was removed by centrifugation at 3000 × *g* for 1 min. The supernatant of methanol extract was collected for GC analysis. The content of histamine in samples was calculated by the following equation:

$$\text{Histamine } (\mu g/g) = \frac{[(A_S/A_{IS}) \times (W_{IS}/\mathrm{RRF})]}{(\text{weight of sample})}$$

2.5. Recovery determination

Amount of 48, 96, 191, 239 μg authentic compound was added to 1 g homogenized tuna flesh (*T. thynnus*) or shrimp meat (*P. monodan*) in a capped test tube. Then, extraction and determination of histamine content was conducted as described earlier.

3. Results and discussion

When examining with standard solution containing histamine and internal standard (1,9-nonanediol) alone, the GC profiles from all tested columns displayed two separate peaks. Among these tests, histamine standard displayed shorter retention time (RT) than internal standard when CP-SIL 5CB (histamine: 4.66 min and 1,9-nonaediol: 5.22 min) and CP-SIL 8CB (histamine: 4.77 min and 1,9-nonaediol: 5.56 min) were used. However, elution sequence of two standards was reversed when using CP-SIL 19CB (histamine: 5.24 min and 1,9-nonaediol: 4.90 min). Furthermore, the GC analysis on alkaline methanol extract suggested only CP-SIL 19CB column could resolve three distinct peaks identical to internal standard (RT: 4.90 min), histamine (RT: 5.24 min) and an unknown compound (RT: 5.88 min) (Fig. 1). The proposed histamine peak found in above GC analysis was further confirmed by GC/MS. As shown in Fig. 2, molecular ion peak derived from the proposed histamine peak displays an identical number (*m*/*z*: 111) to authentic compound. Due to the setting for mass scanning of sample peak beginning at 35 *m*/*z*, molecular ion peak below 30 *m*/*z*, which showed on the spectrum of database, were not found in that of sample. Conclusively, the result of GC/MS confirmed that the peak with retention time at 5.24 min was histamine, when CP-SIL 19CB column was applied. Therefore, in this study, we used CP-SIL 19CB column to analyze histamine and 1,9-nonanediol as internal standard.

The regression of the ratios of peak area of histamine to that of 1,9-nonanediol on the concentrations of histamine standard fitted well to a linear relationship ($r^2 = 0.998$) between 4 and 4000 μg/ml. The result of RRF value calculated from Eq. (1) in Section 2 was 0.54.

The pH of methanol extract from samples obtained in this study was between 4 and 5. With two pKa at 6.04 and 9.75, histamine contained in this pH range shall be partially in ionized form. Charged molecules have been known of less volatility and are usually adsorbed in the glass liner or column. Consequently, charged molecule always displayed very poor or even no response during direct GC analysis. Therefore, the effect of pH on

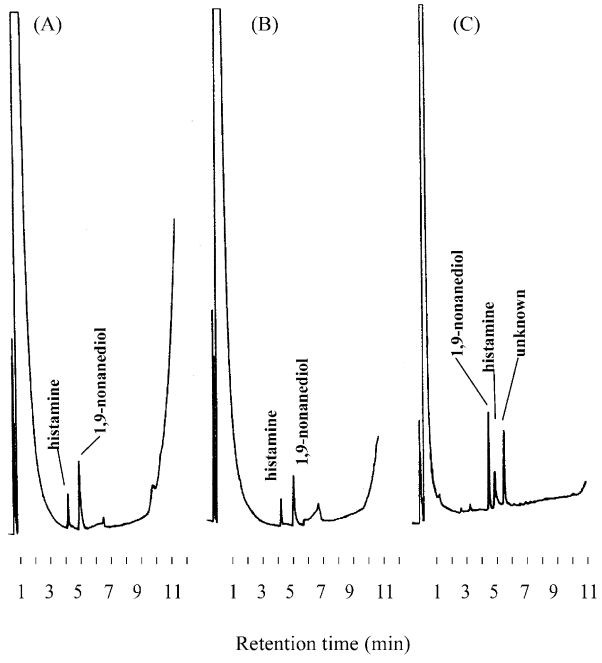

Fig. 1. GC profiles of alkaline methanol extract from slightly decomposed tuna flesh by the columns with different polarity. (A) CP-SIL 5 CB; (B) CP-SIL 8 CB; (C) CP-SIL 19 CB.

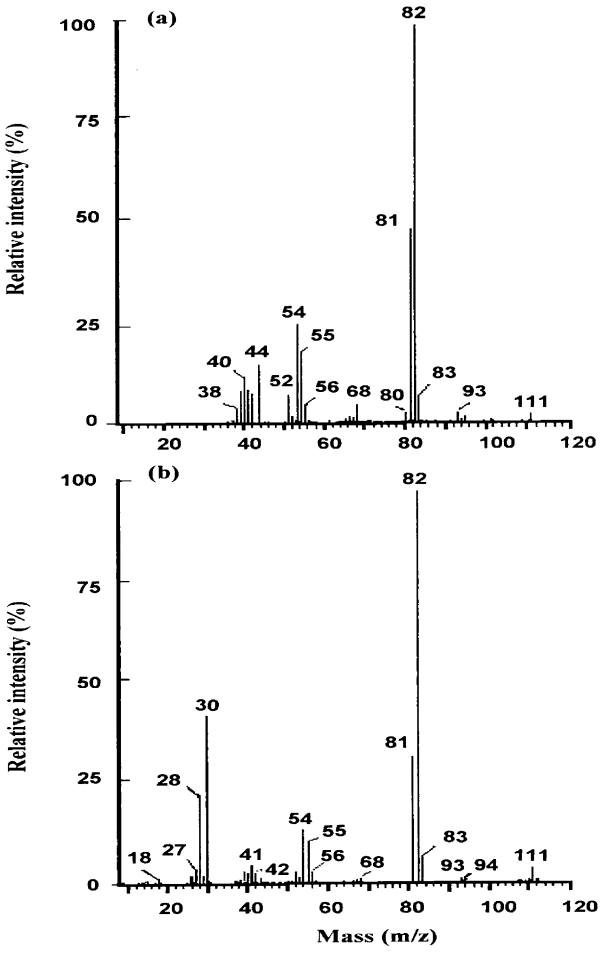

Fig. 2. Mass spectrum of the proposed histamine peak (a) and authentic compound obtained from database (b) by GC/MS analysis.

detection efficiency of histamine using direct GC analysis was also examined. Not well consistent with our speculation, Table 1 shows that the sample extracted with untreated methanol only displayed about 15% less amount of histamine than that with alkaline methanol. However, the experiments with untreated methanol performed over five times higher variation between replicates than that with alkaline methanol. When contacting with methanol, the homogenized fish meat would shrink vigorously due to dehydration and denature of proteins. Trapping of soluble compounds including histamine by physical constraint would very probably happen, and this might account for the high variation of data in the treatment with methanol. The samples shrank as well when mixed with alkaline methanol, but the data obtained from this treatment showed a very low variation (2.8%). This is probably due to muscle proteins have higher solubility in alkaline pH which make shrunk meat porous and leaky (Cheftel, Cuq, & Lorient, 1985). Consequently, the trapped compounds could move freely during ultrasonic extraction. Examining the data and their variation in Table 1, this result suggested that decrease of response in the treatment with methanol per se might be mainly attributed to physical trapping during dehydration of sample by methanol. Due to the pH of fish extract usually as low as 4–5, using alkaline methanol to extract histamine from fish samples was recommended.

Table 1
Effect of alkaline methanol on extraction and detection efficiency of histamine from tuna flesh by GC method

Replicates	Histamine detected (μg/g)	
	Untreated methanol	Alkaline methanol
1	104	148
2	150	151
3	145	159
4	113	153
5	143	157
Mean±S.D. (CV%)[a]	131±21 (15.9%)	154±4 (2.8%)

[a] CV, coefficient variation.

Table 2
Recovery determination of histamine from tuna flesh and shrimp meat by GC method

Sample	Histamine content (μg)			Mean of recovery[b] (%)	CV[c] (%)
	Initial	Added	Finally detected[a]		
Tuna flesh	325	48	378±12	110.9	3.1
		96	421±16	100.8	3.8
		191	514±14	97.9	2.7
		239	557±43	97.5	7.8
Shrimp meat	ND[d]	48	50±4	102.1	8.8
		96	93±4	98.5	4.1
		191	201±5	102.4	2.7
		239	251±22	101.5	8.9

[a] Each standard addition was conducted in triplicates, and final detection of histamine contents were displayed in mean±S.D.

[b] Recovery of added histamine from samples was calculated as follow: [(finally detected histamine content−initial histamine content)/(added histamine content)] × 100%.

[c] CV, coefficient variation.

[d] ND, not detectable.

Recovery of histamine from fish or shrimp meat was also examined using alkaline methanol extraction and GC analysis. As shown in Table 2, tuna flesh used in this experiment initially contained 325 μg histamine, but that of shrimp meat was not detectable. The mean recovery of histamine calculated from this experiment ranged from 97.5 to 110.9% for tuna flesh sample and 98.5 to 102.4% for shrimp meat sample, respectively. Both standard additions showed a low coefficient of variation (CV < 9%). The results of above experiments strongly indicated that direct GC analysis could obtain reliable measurement of histamine content in the fish and shrimp meat.

Furthermore, we used this methodology to survey the histamine content in fish and fish products bought in a

Table 3
Histamine contents of various species of fish and fish products from local supermarket

Samples	Histamine content (μg/g)[a]
Frozen fish	
Thunnus thynnus	267±11
Coloabis saira	168±8
Scomber australasicus	149±8
Engraulis japonicus	115±9
Pampus argenteus	10±1
Fresh fish	
Chanos chanos	Trace
Tilapia sp.	Trace
Shrimp meat	
Penaeus monodan	Trace
Fish products	
Fish ball	26±1
Tempura	24±1
Fish cake	Trace

[a] The data are expressed in mean±S.D. with triplicates.

local supermarket. In total, five species of frozen fish, two species of fresh fish, one species of shrimp, and three kinds of fish products were used for the measurement of histamine content. Consistent with previous studies (Fernandez-Salguero & Mackie, 1987; Frank et al., 1981), histamine content in the extremely fresh *Tilapia* sp. and *C. chanos* was almost not detectable (Table 3). Table 3 also shows that most frozen fishes including *T. thynnus*, *C. saira*, *S. australasicus*, and *E.japonicus*, on the other hand, contained comparatively high amounst of histamine ranging from 114 to 267 μg/g. Only frozen *P. argenteus* contained histamine as low as 10 μg/g. Histamine content in frozen shrimp (*P. monodan*) was also almost not detectable. The histamine contents of three kinds of fish products (fish ball, tempura, and fish cake) were also very low, ranging from trace to 26 μg/g.

In conclusion, the application of GC to directly determine histamine not only reduces the time for analysis, but also provides possibility to detect the compound itself and avoid errors derived from the reaction of derivative synthesis. This method also has comparable level of detection limit with HPLC and almost as quick as using dipstick test. Further application of this method to determine histamine and other biogenic amines in other food stuffs is quite possible and more research is being undertaken in our lab.

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References

AOAC. (1995). *Fish and other marine products. Official methods of analysis* (Vol. 2). Virginia: Association of Official Analytical Chemists.

Arnold, S. H., & Brown, W. D. (1978). Histamine (?) toxicity from fish products. *Advances in Food Research*, *24*, 113–154.

Bateman, R. C. Jr., Eldrige, D. B., Wade, S., McCoy-Messer, J., Jester, E. L. E., & Mowdy, E. D. (1994). Copper chelation assay for histamine in tuna. *Journal of Food Science*, *59*, 517–518.

Ben-Gigirey, B., Craven, C., & An, H. (1998). Histamine formation in albacore muscle analyzed by AOAC and enzymatic methods. *Journal of Food Science*, *63*, 210–214.

Cheftel, J. C., Cuq, J. L., & Lorient, D. (1985). Amino acid, peptide and proteins. In O. R. Fennema (Ed.), *Food chemistry* (pp. 245–370). New York: Marcel Dekker.

Choong, Y. M., Ku, K. L., Wang, M. L., & Lee, M. H. (1997). Simple and rapid method for simultaneous determination of levulinic acid, sorbic acid, and benzoic acid in foods. *Journal of Chinese Agricultural Chemistry Society*, *35*, 26–39.

Choong, Y. M., Lin, H. J., Chen, C. W., & Wang, M. L. (1999). A rapid gas chromatographic method for direct determination of free sterols in fats and oils. *Journal of Food and Drug Analysis*, *7*, 279–290.

Choong, Y. M., Wang, M. L., Chou, C. H., & Fan, J. J. (1999). Rapid method for the determination of sorbic acid, dehydroacetic acid and benzoic acid in butter, cheese, margarine and salad dressing by gas chromatography. *Journal of Chinese Agricultural Chemistry Society*, *37*, 166–178.

Du, W. X., Huang, T. S., Kim, J., Marshell, M. R., & Wei, C. I. (2001). Chemical, microbiological, and AromaScan evaluation of mahi-mahi fillets under various storage conditions. *Journal of Agricultural and Food Chemistry*, *49*, 527–534.

Farn, G., & Sims, G. G. (1987). Chemical indices of decomposition in tuna. In D. K. Kramer, & J. Liston (Eds.), *Seafood quality determination* (pp. 175–183). New York: Elsevier.

Fernandez-Salguero, J., & Mackie, I. M. (1987). Technical note: Preliminary survey of the content of histamine and other higher amines in some samples of Spanish canned fish. *International Journal of Food Science and Technology*, *22*, 409–412.

Food and Drug Administration. (1996). Decomposition and histamine in raw, frozen tuna and mahi-mahi, canned tuna; and related species. *Compliance Policy Guides*, *7108.240* (Section 540.525).

Frank, H. A., Yoshinaga, D. H., & Nip, W. K. (1981). Histamine formation and honeycombing during decomposition of skipjack (*Katsuwonus pelamis*) at elevated temperature. *Marine Fishery Review*, *43*, 9–14.

Hall, M., Eldrige, D. B., Saunders, R. D., Fairclough, D. L., & Bateman, R. C. (1995). A rapid dipstick test for histamine in tuna. *Food Biotechnology*, *9*, 39–57.

Hui, J. Y., & Tayor, S. L. (1983). High pressure liquid chromatographic determination of putrefactive amines in foods. *Journal of the Association of Official Analytical Chemists*, *66*, 853–857.

Hwang, D. F., Chang, S. H., Shiau, C. Y., & Cheng, C. C. (1995). Biogenic amines in the flesh of sailfish (*Istiophorus platypterus*) responsible to scombroid poisoning. *Journal of Food Science*, *60*, 29–31.

Hwang, D. F., Chang, S. H., Shiau, C. Y., & Chai, T. J. (1997). High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *Journal of Chromatography B*, *693*, 23–30.

Lerke, P. A., Porcuna, M. N., & Chin, H. B. (1983). Screening test for histamine in fish. *Journal of Food Science*, *48*, 155–157.

Lin, H. J., & Choong, Y. M. (1999). Simple method for the simultaneous determination of various preservatives in liquid foods. *Journal of Food and Drug Analysis*, *7*, 291–304.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Roig-Sagues, A. X., & Mora-Ventura, M. T. (1994). Bacteriological quantity of tuna fish (*Thunnus thynnus*) destined for canning: effect of tuna handling on presence of histidine decarboxylase bacteria and histamine level. *Journal of Food Protection*, *57*, 318–323.

Mopper, B., & Sciacchitano, C. J. (1994). Capillary zone electrophoretic determination of histamine in fish. *Journal of the Association of Official Analytical Chemists International*, *77*, 881–884.

Naguib, K., Ayesh, A. M., & Shalaby, A. R. (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. *Journal of Agricultural and Food Chemistry*, *43*, 134–139.

Ohashi, M., Nomura, F., Suzuki, M., Otsuka, M., Adachi, O., & Arakawa, N. (1994). Oxygen-sensor-based simple assay of histamine in fish using purified amine oxidase. *Journal of Food Science*, *59*, 519–522.

Rawles, D. D., Flick, G. J., & Martin, R. E. (1996). Biogenic amines in fish and shellfish. *Advances in Food and Nutrition Research*, *39*, 329–364.

Serrar, D., Brebany, R., Bruneau, S., & Denoyel, G. A. (1995). The development of a monoclonal antibody-based ELISA for the determination of histamine in food: application to fishery products and comparison with the HPLC assay. *Food Chemistry*, *54*, 85–91.

Staruszkiewicz, W. F. Jr., & Bond, J. F. (1981). Gas chromatographic determination of cadaverine, putrescine, and histamine in foods. *Journal of the Association of Official Analytical Chemists*, *64*, 584–591.

Stratton, J. E., & Taylor, S. L. (1991). Scombroid poisoning. In D. R. Ward, & C. Hackney (Eds.), *Microbiological marine products* (pp. 331–351). New York: Avi Publishing.

Vázquez-Ortiz, F. A., Caire, G., Higuera-Ciapara, I., & Hernández, G. (1995). High performance liquid chromatographic determination of free amino acids in shrimp. *Journal of Liquid Chromatography*, *18*, 2059–2068.

Veciana-Nogués, M. T., Hernández-Jover, T., Mariné-Font, A., & Vidal-Carou, M. C. (1995). Liquid chromatographic method for determination of biogenic amines in fish and fish products. *Journal of the Association of Official Analytical Chemists International*, *78*, 1045–1050.

Wang, M. L., Lin, H. L., Lee, M. H., & Choong, Y. M. (1999). A rapid method for direct determination of levulinic acid in soy sauce. *Journal of Food and Drug Analysis*, *7*, 143–152.

Yen, G. C., & Hsieh, C. L. (1991). Simultaneous analysis of biogenic amines in canned fish by HPLC. *Journal of Food Science*, *56*, 158–160.