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Development of a Spectrophotometric Method to Estimate Histamine Content in Fish

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ABSTRACT. *Histamine is a biogenic amine produced in fish as a result of spoilage. Consumption of fish contaminated with histamine may cause histamine intoxication. Development of rapid and simple methods to detect histamine in fish has been of interest to many involved in research and quality control work. A spectrophotometric method was developed to estimate histamine, by measuring the absorbance of bromo-cresol purple added to histamine in pure solution. The method was next attempted in Niven's broth inoculated with histamine producing bacteria and then in fish stored under controlled conditions.*

Niven's broth was inoculated with histamine producing bacteria and incubated at 37°C. After seven days, sub-samples (5 ml) were drawn twice daily to measure absorbance at 585 nm and to estimate the histamine concentration by thin layer chromatography (TLC). A plot of histamine concentrations of the Niven's broth estimated by TLC vs absorbance showed a positive linear relationship in the range of 50-1000 ppm histamine.

Market skipjack fish samples were stored at 0°C. Sub-samples (5 g) were tested daily, after 5 days, for histamine by TLC. To measure absorbance, 0.5% bromo-cresol purple solution (20 ml) was added to methanol extract (20 ml). A plot of histamine concentrations estimated by TLC vs absorbance showed a positive linear relationship in the range of 50-1000 ppm histamine. A plot of absorbance values vs histamine concentrations could be used to estimate histamine concentrations of fish. This method can be recommended as a simple spectrophotometric method to estimate histamine in fish.

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INTRODUCTION

Histamine is a biogenic amine produced in fish as a result of spoilage. Consumption of fish contaminated with histamine may cause histamine intoxication. Development of rapid and simple methods to estimate histamine in fish has been a need for many interested in histamine. Over the past few years several methods have been developed for rapid detection of histamine in fish and a few methods for estimation of histamine in microbiological culture media. The most accurate current method of estimation of histamine is a fluorometric analysis (AOAC, 1990). This method involves expensive chemicals, instruments and is time consuming. The lengthy procedure of AOAC is simplified in another method, which still requires expensive chemical o-phthalaldehyde and spectrofluorometer (Perkin Elmer model 203) (Taylor *et al.*, 1978). Other methods indicated in the AOAC official methods of analysis include a chemical method which involves coupling of diazonium reagent and a biological method.

A rapid screening method based on spectrophotometry uses diamine oxidase to catalyze the conversion of histamine to hydrogen peroxide (Lerke *et al.*, 1983). Hydrogen peroxide is detected by formation of crystal violet from leuco crystal violet. The spectrophotometric method detects the colour intensity of crystal violet formed. An enzymic method has been developed to estimate histamine production in culture media (Lopez Sabater *et al.*, 1994). The method was based on the action of diamine oxidase enzyme at pH 6.8 on histamine. As products of enzymic activity, immidazole acetaldehyde, ammonia and hydrogen peroxide are formed. An added enzyme, peroxidase in the presence of leuco crystal violet caused its oxidation to crystal violet. Appearance of the colour of crystal violet is measured using a spectrometer at 596 nm. The method shows a good correlation ($r^2 = 0.99$) between the histamine content and the increase of absorbance in the concentration range of 3 to 30 ppm. Recently a rapid dipstick method was described using the coupling of diamine oxidase to a peroxidase/dye system (Hall *et al.*, 1995). Good agreement between the dipstick method, the official reference and modified AOAC fluorometric methods has been reported.

This study investigated the possibility of estimating the histamine concentrations in Niven's broths inoculated with histamine producing bacteria, by spectrophotometric method and then extending the method to estimate histamine in fish.

MATERIALS AND METHODS

Measurement of absorbance for standard histamine solutions

Preparation of standard histamine solutions

Crystalline powder of histamine acid phosphate was used to prepare the standards. Standards of 50, 100, 200, 300, 400 and 500 ppm were prepared by dissolving 0.07, 0.14, 0.28, 0.42, 0.64 and 0.70 g of histamine acid phosphate respectively in 10 ml of 0.1 N hydrochloric acid in a 50 ml volumetric flask and making up to the mark with AR Grade methanol.

Bromo-cresol purple (0.5% w/v) solution (20 ml) was added in duplicate to 20 ml of standard histamine solutions and shaken. The absorbance of the mixture was measured using a spectrophotometer (model WPA S 110 Auto Zero) in the visible range of the spectrum at intervals of 5 nm, to identify absorption maxima. Absorbance values of the standard histamine-bromo-cresol purple mixtures were plotted against the histamine concentrations of the standard solutions.

Estimation of histamine in Niven's broth

Preparation of culture media

Niven's medium (200 ml) was prepared in duplicate for each of the ten cultures of bacteria to be tested (Niven et al., 1981). The broth was adjusted to pH 5.3 by adding 12.0 ml of 0.1 N hydrochloric acid solution and sterilized in flat glass bottles with screw caps at 121°C for 10 min.

Inoculation of culture media

Previously isolated histamine producing bacteria maintained on nutrient agar slants were activated by inoculating into 5 ml of sterile Trypticase Soy Broth supplemented with histidine (TSBH) at (25±2)°C for 24 h. Each activated culture (1 ml) was inoculated in duplicate into sterile Niven's broth, incubated at 37°C and observed daily for colour change from yellow to purple. The cultures were shaken daily to allow uniform distribution of bacteria in the broth.

Measurement of absorbance

Seven days after incubation, subsamples of 5 ml were drawn twice daily from each culture to measure absorbance and estimate the histamine concentration. Absorbance was measured using 2 ml of the Niven's broth, at 585 nm.

Estimation of histamine

Histamine in Niven's broth was extracted by blending 5 g of the broth with 50 ml of methanol in a Waring mini-blender at low speed for 2 min. The extract was maintained at 60°C for 15 min to enhance extraction. The filtrate was used to spot (20 μl) on Thin Layer Chromatography (TLC) plates pre-coated with silica gel G 60 and activated at 105°C for 30 min. Histamine standards 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 ppm were spotted (20 μl) on TLC plates. The TLC plates were developed in methanol : chloroform : conc. ammonia (12:7:1). The developed plates were left to air dry for 5 min warmed in the oven at 50°C for 5 min and visualized using ninhydrin spray. The colour intensities of the unknown extracts and the standards were compared for estimation of histamine (Lieber and Taylor, 1978; Baranowski, 1985; Gunaratne and Samarajeewa, 1994). Estimation of histamine and measurement of absorbance were carried out until the broth lasted. The mid-point of the range of histamine concentrations estimated by TLC for the cultures were plotted against their respective absorbance values.

Estimation of histamine in fish

Preparation of samples of fish and estimation of histamine

Ten samples (10 × 1 kg) of skipjack fish (*Katsuwonus pelamis*) were obtained from the market and a subsample of 20 g drawn at random was cut into pieces of approximately 1 cm^3. Histamine was estimated in the fish pieces (5 g) by TLC. Each sample of fish was stored in the freezer at 0°C for 45 days. After 5 days of storage subsamples of 20 g were drawn daily from each sample, cut into small pieces and histamine estimated as described above.

Measurement of absorbance

Bromo-cresol purple (0.5% w/v) solution (20 ml) was added to 20 ml of methanol extract of fish and shaken well. The absorbance of the mixture was measured at 585 nm. Absorbance values of the resulting solution were plotted against the TLC estimates of histamine concentrations of fish.

RESULTS AND DISCUSSION

Absorbance values of standard histamine solutions

The maximum absorbance values for standard histamine solutions were observed at 585 nm (Figure 1). The plot between the absorbance values of the standard histamine solutions and the histamine concentrations showed a linear relationship, with correlation coefficient (r^2) = 0.99 (Figure 2). This indicated that the colour change in bromo-cresol purple due to histamine could be used in estimating histamine.

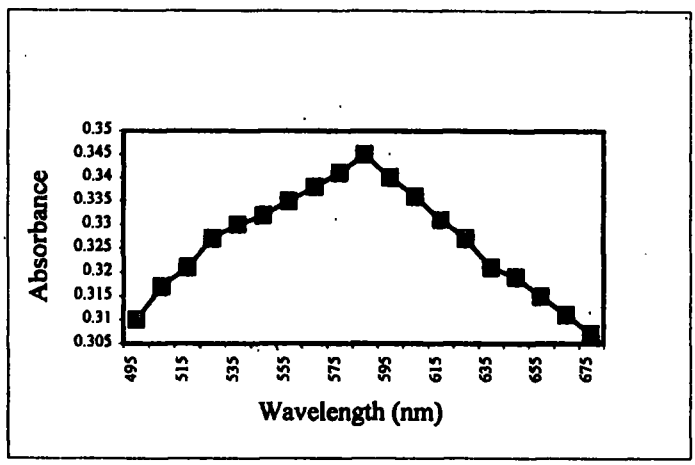

Figure 1. Absorption spectrum for standard histamine solution of 50 ppm.

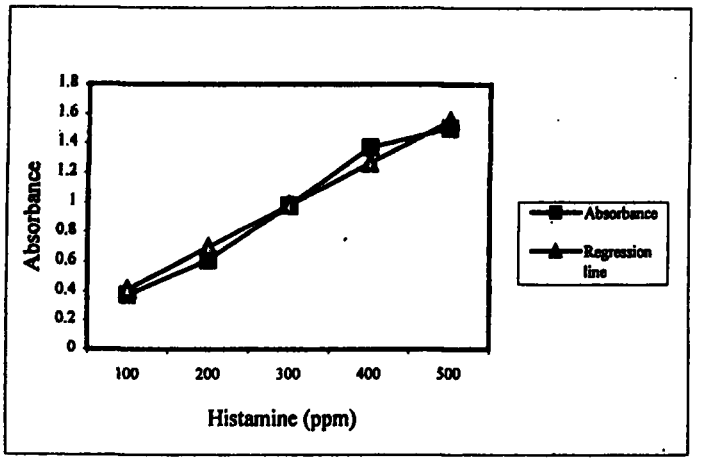

Figure 2. Correlation between absorbance *vs* histamine concentration of standard histamine solutions.

Histamine concentrations of Niven's broth and fish

The mean histamine concentration of Niven's broth as estimated by TLC showed a positive linear relationship ($Y = 0.0025 X + 0.3379$) with the absorbance in the range of 50-1000 ppm histamine. The correlation coefficient (r^2) was 0.97 (Figure 3). Even though the accuracy of the absorbance measurements is confined to the region of absorbance from 0.2 to 0.7, the linear relationship of the histamine concentrations and the absorbance values showed that absorbance values even above 0.7 could be used to estimate histamine concentrations. This was evident from the fact that the linear relationships of the histamine concentrations and absorbance values in the range of absorbance from 0.5 to 0.7 and 0.7 to 1.9 were $Y = 0.0020 X + 0.3401$ and $Y = 0.0026 X + 0.2897$. As this study is investigating the feasibility of the spectrophotometric method, it is useful to study the full range of the absorbance values that correlate with histamine concentrations. However if a research is solely dependent on spectrophotometric readings for estimation of histamine concentrations, it is advisable to use the absorbance values between 0.2 and 0.7 only. The spectrophotometric method is applicable for estimation of histamine produced in Niven's broth.

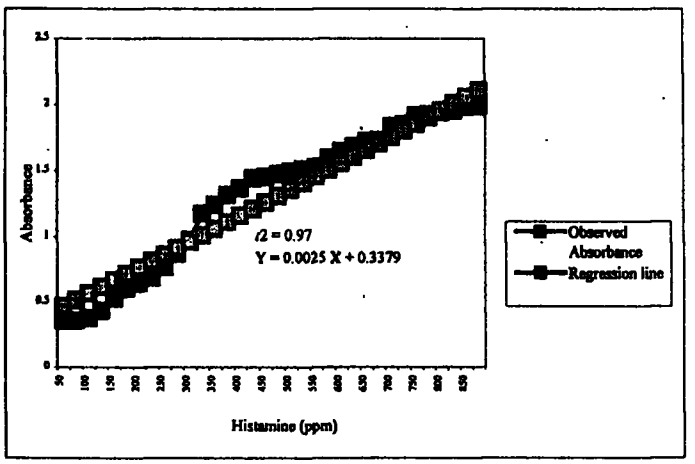

Figure 3. Correlation between absorbance and histamine concentration of histamine producing bacteria in Niven's broth.

The mean histamine concentration of fish as estimated by TLC showed a positive linear relationship (Y = 0.0017 X + 0.5727) with the absorbance in the range of 50-1000 ppm histamine. The correlation coefficient was 0.97 (Figure 4). The spectrophotometric method could be used to estimate the histamine concentrations in market fish.

This simple and inexpensive method provides a means of estimating histamine in fish without using complicated analytical techniques. The other simple method used, the TLC, could only provide a semi-quantitative estimation as the final reading is taken by visual comparison of the colour intensities of samples with the standards. The spectrophotometric method is therefore more reliable and provides an accurate estimate sufficient in taking the decisions in processing or export of fish. The test takes less than one hour to perform, thus making the results available rapidly. The lower limit of 50 ppm detectable by this method provides sufficient information to decide on safety of fish as the regulatory limits specified are 50 ppm and 100 ppm in USFDA regulations and Sri Lanka food standards respectively.

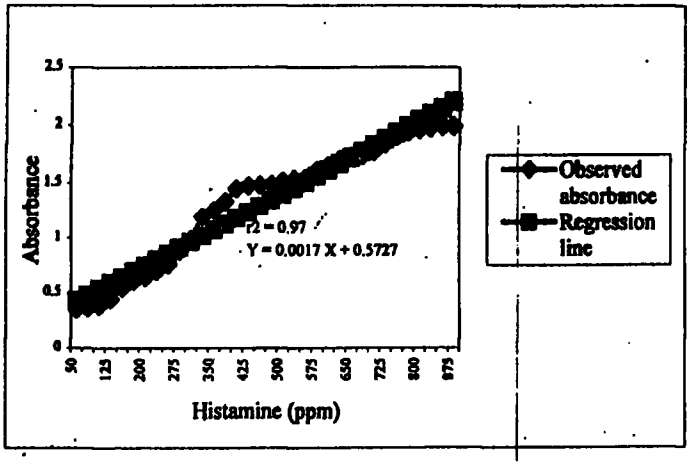

Figure 4. Correlation between absorbance and histamine concentration of skipjack fish samples stored at 0°C.

CONCLUSIONS

Absorbance values could be used to estimate the histamine concentrations of Niven's medium inoculated with histamine producing bacteria and fish contaminated with histamine. This spectrophotometric method can be used for routine estimation of histamine, and is less cumbersome and less expensive compared to methods based on fluorometry, HPLC or GC.

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REFERENCES

AOAC. (1990). Histamine in fish. Methods of analysis. Association of Official Analytical Chemists. Washington DC. pp. 875-877.

Baranowski, J. (1985.) Methodology for histamine analysis. pp. 4-9. *In*: Pan, B.S. and James, D. (Eds). Histamine in Marine Products: Production by Bacteria, Measurement and Prediction of Formation. AO Technical Paper 252.

Gunaratne, S. and Samarajeewa, U. (1994). Histamine and histamine producing bacteria in fish from Sri Lanka. Trop. Agric. Res. 6: 52-59.

Hall, M., Eldridge, D.B., Saunders, R.D., Fairclough, D.L. and Bateman, R. C. (1995). A rapid dipstick test for histamine in tuna. Food Biotech. 9(182): 39-57.

Lerke, P.A., Werner, S.B., Taylor, S.L. and Guthertz, L.S. (1983). Scombroid poisoning. Report of an outbreak. Western J. Med. 129: 381-382.

Lieber, E.R. and Taylor, S.L. (1978). Thin layer chromatographic screening methods for histamine in tuna fish. J. Chromatography. 153: 143-152.

Lopez-Sabater, E.I., Rodriguez-Jerez, J.J., Hernandez Herrero, M. and Mora-Ventura, M.T. (1994). Evaluation of histidine decarboxylation activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymic method. Lett. Appl. Microbiol. 19: 70-75.

Niven, C.F., Jeffrey, M.B. and Corlett, D.A. (1981). Differential plating medium for quantitative detection of histamine producing bacteria. Appl. Environ. Microbiol. 4(1): 321-322.

Taylor, S.L., Lieber, E.R. and Leatherwood, M.A. (1978). A simplified method for histamine analysis of foods. J. Food. Sci. 43: 247-250.