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A simple and rapid method for the analysis of fish histamine by paper electrophoresis

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ABSTRACT: The described analytical method for histamine determination in fish and seafood consists of sample extraction, adsorption onto a paper disc, application of the paper disc onto electrophoresis paper, electrophoresis for only 10 min, drying, and color developing by Pauly's reagent. Histamine can be satisfactorily detected and completely separated from histidine, carnosine and other Pauly reagent-positive compounds. This method does not require expensive instrumentation and any tedious pretreatment to eliminate potential interference by other imidazole compounds, such as histidine or carnosine. This method can be used to detect histamine in multiple fish and seafood samples simultaneously that contain as little as 15 p.p.m. histamine (1.5 mg/100 g).

KEY WORDS: **determination, fish, histamine, paper electrophoresis, Pauly reagent.**

INTRODUCTION

Histamine is known as a chemically harmful substance that can induce allergy-like food poisoning when ingested fish and seafood.[1–3] Therefore, the histamine content in seafood is of great concern in food hygiene.[4] In most cases, histamine levels in illness-causing fish have been above 200 p.p.m., often above 500 p.p.m.[5] Histamine is contained in various types of fresh and processed fish.[6–8] To prevent histamine-induced seafood poisoning, the U.S. Food and Drug Administration (FDA) established a hazards analysis and critical control point program and set up guidelines for histamine at 5 mg/100 g (50 p.p.m) for scombroid fish species.[9] Histamine monitoring has now been globally accepted for safety confirmation of fish and seafood products. A simple and rapid method for histamine analysis is now required all over the world.

Different determination methods have been used for the quantification of histamine, including a bioassay, thin-layer chromatography, gas chromatography, gas chromatography/mass spectrometry, colorimetric assay, fluorometric assay, enzymatic isotopic assay, immunological assay and high-performance liquid chromatography (HPLC).[1] A very serious problem in histamine determination is to separate histamine completely from a very large amount of interfering compounds, such as histidine or carnosine. Therefore, a careful and tedious pretreatment is required to eliminate potential interfering substances.[10,11]

In 1995, Sato *et al*. reported a simple method based on the one-step derivatization and HPLC separation;[12] however, this method required an expensive instrument and skilled operator. A rapid method by paper electrophoresis was also developed.[13] However, there are some points to be improved in this analytical method; for example, the spotting procedure of the sample solution on the electrophoresis paper and the spraying procedure of the electrophoresis buffer on the electrophoresis paper after sample spotting. In this present study, the authors describe a simple and rapid method for fish and seafood histamine analysis by paper electrophoresis and Pauly reagent development which can be possibly used during the postcatching contamination periods, such as in the processing plant, the distribution system, restaurants or the home.

MATERIALS AND METHODS

Materials

L-Histamine, L-histidine, sodium nitrite and sulfanilic acid were purchased from Wako Pure

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Chemical Industries (Osaka, Japan). Imidazole, 2- and 4-methylimidazole, L-carnosine and urocanic acid (imidazole-4-acrylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and all other reagents used were of analytical grade. Pauly reagent (A and B) for the development of histamine was prepared by the method of Sato *et al*.[13] Reagent A also consisted of two reagents, A-1 and A-2. Reagent A-1, a 20 mM sulfanilic acid in a 1 M HCl solution and reagent A-2, a 200 mM sodium nitrite solution. Reagents A-1 and A-2 were equally mixed just before spraying. Reagent B, a l0% anhydrous sodium carbonate in a 5% ethanol solution. Paper electrophoresis apparatus (Histamine Checker; JBB, Saitama, Japan) and electrophoresis paper with H-shape hole (30 cm length × 9 cm or 15 cm width) were purchased from JBB (Saitama, Japan).

Preparation of sample extract

Fish flesh sample of approximately 200 mg was homogenized with 5 volume (v/w) of 80% ethanol using a Polytron homogenizer. Each homogenate was centrifuged at 10 000 ×*g* for 5 min. Ten microliters of supernatant (10 μL) was applied to paper disc (6 mm in diameter).

Paper electrophoresis

Paper electrophoresis was conducted using Histamine Checker (JBB) during this experiment. The electrophoresis paper was set on the electrophoresis holder and immersed in the electrophoresis buffer, consisting of pyridine, acetic acid and water (1 : 10 : 289, pH 3.7). The electrophoresis paper holder was set into the electrophoresis apparatus chamber. Then, the paper disc containing the sample solution was set at the center of the H-shape hole of electrophoresis paper. The electrophoresis was conducted at 800 V for 10 min. The paper was then dried at 100°C using a forced convection oven (FC-410; ADVANTEC, Tokyo, Japan).

Color developing for histamine and quantification

Histamine on the electrophoresis paper was developed by spraying with Pauly reagent A (equal mixture of A-1 and A-2) and then regent B. The developed paper was then set on the light box (FUJICOLOR, Tokyo, Japan) and the color density of histamine spot was recorded using a digital camera CP-800 (EPSON, Tokyo, Japan). The histamine spot was digitized and calculated by image processing method software, Lane & Spot Analyzer (ATTO, Tokyo, Japan). A standard histamine solution, 9 nmol (1.0 μg)/10 μL, was used as the internal standard.

RESULTS AND DISCUSSION

Separation of histamine, histidine and other Pauly reagent-positive compounds

Histamine gave a red spot after Pauly reagent development and was completely separated from histidine (Fig. 1). It has been confirmed that histamine was also perfectly separated from other Pauly reagent positive compounds, such as imidazole, 2- and 4-methylimidazole, L-carnosine and urocanic acid, by 10 min electrophoresis, as mentioned in a previous report.[13]

Linearity of histamine determination

The linearity of the histamine estimation using this imaging processing method was good within the range from 30 to 1000 ng (1 μg) of histamine (Fig. 2).

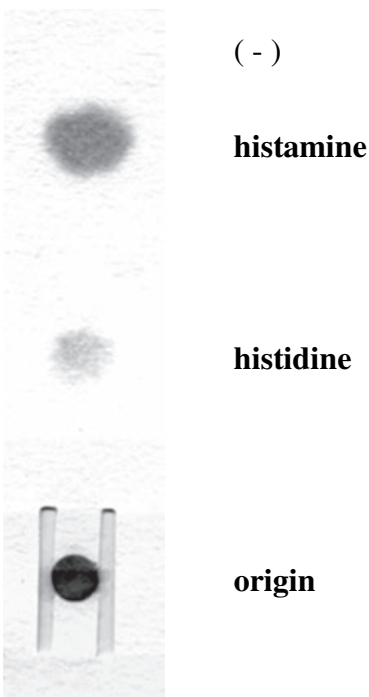

Fig. 1 Typical electrophoresis pattern of standard histamine and histidine on the electrophoresis paper with H-shape hole.

Table 1 Comparison of the histamine contents obtained by paper electrophoresis and high-performance liquid chromatography method

	Incubation time (h) (p.p.m.)†				
	0	3	6	24	36
Electrophoresis method	ND‡	32 ± 4	113 ± 7	2150 ± 20	4503 ± 38
HPLC method	ND	38 ± 2	120 ± 5	2006 ± 8	4480 ± 19

†Mackerel flesh with skin was incubated at 25°C; ‡not detected. §data are shown mean ± standard deviation by three determinations. HPLC, high-performance liquid chromatography.

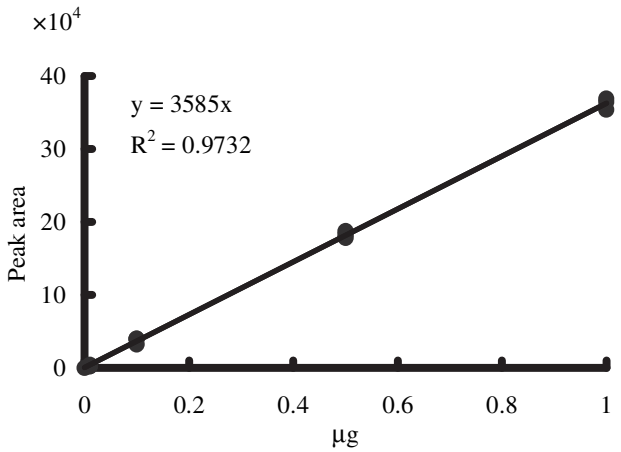

Fig. 2 Calibration curve of histamine.

Method comparison

The histamine levels in mackerel flesh with skin incubated at 25°C were compared using this electrophoresis method and the HPLC method.[12] The histamine contents in the mackerel flesh samples determined by the two methods were highly comparable in samples taken at all incubation times (Table 1).

Overall estimation

The improved method for histamine analysis by electrophoresis separation and Pauly reagent is more straight-forward and quicker than the method reported previously.[13] It eliminates the somehow nervous and awkward steps, such as the spotting procedure of the sample solution on the electrophoresis paper and the careful spray procedure of the electrophoresis buffer on the electrophoresis paper upon which the sample solution had been previously spotted. Most importantly, the procedure can be conducted by individuals with minimal technical skills.

Although this method is less sensitive than the HPLC method, it can simultaneously determine multiple samples in less than 30 min. It is noteworthy that the entire estimation time of one sample is quite short compared with the HPLC and other previously reported methods. The detection limit for histamine was 15 p.p.m. (1.5 mg/100 g), which is more sensitive than the guidance level of 50 p.p.m. set by the FDA.[9] It is well known that histamine is generally not uniformly distributed in decomposed fish. If 50 p.p.m. is found in one section, there is the possibility that other sections may exceed 500 p.p.m.[9] It is advisable to check several sections simultaneously. This new method can be therefore used in various contexts, such as in the fish market, processing plant, distribution center or restaurants.

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