Estimation of Biogenic Amines by HPLC Method

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Biogenic amines have been defined chemically as aliphatic, alicyclic and heterocyclic organic bases of low molecular weight. They are formed by the action of bacterial enzymes and decarboxylases, on substrates such as free amino acids. *Enterobacter aerogenes and Morganella morganii* are two strong bacteria forming histamine from histidine. Important biogenic amines in fish are histamine, putrescine, cadaverine and tyramine and these are formed from histidine, ornithine, lysine and tyrosine respectively. Spermine and spermidine are formed from putrescine by further reaction.

Histamine is formed in fish during bacterial spoilage due to the decarboxylation of amino acid histidine by bacteria with the enzyme histidine decarboxylase. Putrescine and cadaverine are not particularly toxic, but may increase the potential toxicity of histamine. Putrefactive amines such as putrescine and cadaverine are also formed in fish during bacterial spoilage by the decarboxylation of ornithine and lysine.

Histamine poisoning results from ingestion of foods that contain usually high levels of histamine. It is a rather short, mild illness. Important symptoms of histamine poisoning are gastrointestinal (nausea, vomiting, diarrhoea, abdominal cramps), cutaneous (rash, urticaria, edema), haemodynamic (hypotension), and neurological (flushing, itching, burning, tingling, headache) disorders. Most patients suffering from histamine poisoning will experience only a few of these symptoms and their severity will depend on the dose of histamine and the individual's susceptibility. Histamine poisoning occurs world-wide, particularly in countries where scombroid and related types of fish like, tuna, mackerel, bonito, Spanish mackerel, and saury are frequently consumed. However, non scombroid fish belonging to the families *Pomatomidae* (blue fish), *Cophenaeidae*

(Mahi mahi), *Carangidae* (jack mackerel, amberjacks, yellow tail), *Clupeidae* (herring, sardines) and *Engraulidae* (anchovies) are occasionally implicated. The presence of biogenic amines is a useful indicator of the condition of the fish. Only spoiled fish can cause histamine poisoning. Fish containing hazardous level of histamine are not always detected by organoleptic examination.

Biogenic amines are generally either psychoactive or vasoactive. Former group of amines act as the neutral transmitters in the central nervous system, while the vasoactive amines act, either directly or indirectly on the vascular system. Biogenic amines present in fish do not cause any hazard to individuals unless large quantities are ingested or the natural mechanisms for catabolism of one of the amines are inhibited.

Following guidelines are given in UK for the safe consumption of fish to avoid histamine poisoning.

< 5 mg histamine/100 g fish : Safe for consumption

5-20 mg histamine/100 g fish : Possibly toxic

20-100 mg histamine/100 g fish : Probably toxic

> 100 mg histamine/100 g fish : toxic and unsafe for consumption.

The European Community has recently proposed that the average content of histamine in nine samples of fish may not exceed a level of 10 mg/100 g. Two samples may have a value of more than 10 mg/100 g but less than 20 mg/100 g. No sample may contain more than 20 mg/100 g.

Controlling the limits of histamine needs standardised and harmonized analytical methods for determination. Histamine and other putrefactive amines can be detected in fish by a variety of techniques including HPLC, gas liquid and thin layer chromatography. 'Gradient HPLC with pre or post derivatisation is a good method for determination of biogenic amines in fish'. It gives reproducible and accurate results for histamine, putrescine, cadaverine and tyramine.

Biogenic amines in trichloroacetic acid extracts of fish are stable during storage.

Determination of biogenic amines by HPLC

This method is suitable for the separation and quantification of histamine, cadaverine, putrescine, spermine, spermidine and tyramine and may be suitable for other amines.

Reagents

Trichloroacetic acid (TCA) 5% w/v, 25 g. TCA made upto 500 ml with distilled water.

Sodium carbonate (anhydrous)

Dansyl (5-dimethyl amino naphthalene-1-sulphonyl)

Chloride, 0.5% w/v in acetone: 0.05 g made upto 10 ml with acetone. Prepare fresh proline, 15% w/v, 7.5 g made upto 50 ml with distilled water, refrigerate up to 2 weeks.

Ethyl acetate (HPLC grade)

Water (HPLC grade)

Methanol (HPLC grade)

Standards

Amines are required at 5 mg/100 ml free base. Keep frozen in small amounts ready for use. Using amine hydrochlorides the following amounts give 50 mg/100 ml solutions when dissolved in 100 ml water.

Histamine .2 HCl	0.0828 g
Putrescine .2 HCl	0.0914 g
Cadaverine .2 HCl	0.0857 g
Spermine .4 HCl	0.0860 g
Spermidine .3 HCl	0.0877 g
Tyramine (as base)	0.0500 g

To use as a single standard, dilute to 5 mg% by adding 1 volume of stock solution to 9 volumes of water. For preparation of a mixed

standard, mix 1 volume of each standard required and make upto 10 volumes with water.

Sample preparation

Before mincing, the sample should be kept frozen and only defrosted shortly before use. Once minced, histamine levels are likely to change even more rapidly and it is essential to keep the mince frozen until needed. Mixing with TCA renders the histamine level stable for at least several hours.

Mince the sample finely and mix thoroughly to ensure even distribution of amines. After sampling, the remaining mince should be frozen quickly to as low a temperature as practicable, certainly below -5°C. Stored at -20°C, amine levels should not change significantly for several weeks.

Extraction

Weigh out 1.0 g of minced sample and add 9 ml of 5% trichloroacetic acid (alternatively increase sample size and volume of acid added i.e. 5 g sample + 45 ml of TCA). Mix well. Vigorous shaking or blending are both adequate. Leave the extract for 10-15 minutes, then filter.

Derivatisation

Pipette 0.2 ml of sample extract into an Eppendor vial and add an excess of sodium carbonate to neutralise.

Add 0.5 ml of dansyl chloride solution and shake well. Check that there is still sodium carbonate at the bottom of the vial; if not, add more. Leave at ambient temperature for 1 h. Add 0.5 ml of ethyl acetate and shake well. After the layers have separated, remove the upper (non aqueous) layer into a clean tube taking care not to include any of the lower layer. A mixed standard should be prepared in the same way, substituting 0.2 ml of standard solution in place of the sample solution.

HPCL separation

Column	25 cm x 4.6 mm, S5-C8 maintained at 25°C
Detector	UV at 254 nm
Sample volume	50 µl
Flow rate	1.2 ml min^{-1}
Solvent system	Gradient using methanol and water

Gradient

Time, min.	% methanol	% water
0	70	30
7	80	20
11.5	100	0
19	100	0
25	70	30

Quantification

Absorption peaks are integrated electronically or by hand. The results should be calculated taking into consideration the dilution factor, ie 1 g fish muscle in 9 ml TCA.