Australian Standard®

Method of test for determination of histamine levels in fish and fish products/seafoods by fluorometric method

PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products.

The objective of this Standard method is to establish a quantitative method for the determination of histamine levels in fish and fish products by fluorometric method.

FOREWORD

Histamine, although having a physiological role in fish, is also associated with decomposition. The presence of histamine in fish is an indicator of decomposition and has been linked to scombroid poisoning. The occurrence of high levels of histamine in decomposed fish correlates well with outbreaks of scombroid poisoning. The natural levels of histamine in fresh fish is less than 50 mg/kg and the higher levels in decomposing fish are due to the decarboxylation of histidine, i.e. certain bacteria produce the enzyme histidine decarboxylase that reacts with free histidine to produce histamine.

Histidine is present in greater amounts in scombroid fish such as tuna, mackerel and bluefish increasing their vulnerability to excessive histamine (scombrotoxin) formation during handling and storage.

METHOD

1 SCOPE

This Standard sets out a quantitative method for the determination of histamine levels in fish and fish products/seafoods by fluorometric methods.

NOTE: This method has been tested using amines relevant to seafood such as Cadaverine, Putrescine and Spermine and has shown insignificant fluorescence response compared to histamine response. Therefore, it has been concluded that the detection was specific to histamine.

2 PRINCIPLE

Homogenized seafood is extracted with methanol. The extract is centrifuged, the supernatant collected and aliquot loaded onto an anion exchange column for cleanup. The sample is eluted with water and made to volume, then the histamine is derivatized with o-phthaldialdehyde (OPA) to form fluorescent histamine derivatives. The histamine derivative is determined using standard HPLC equipment (pump, autosampler and fluorescence detector).

3 REAGENTS AND STANDARD SOLUTIONS

3.1 Reagents

3.1.1 *Methanol, HPLC grade*

3.1.2 *Sodium hydroxide, 2 M*

Dilute 133 mL of 60% NaOH solution to 1L with deionized water or dissolve 80 g of NaOH in 1 L of deionized water.

3.1.3 *Sodium hydroxide, 0.1 M*

Dilute 50 mL of sodium hydroxide (3.1.2) to 1 L with deionized water.

3.1.4 *Hydrochloric acid, 0.1 M*

Dilute 9 mL of concentrated hydrochloric acid to 1 L with deionized water.

3.1.5 *o-phthaldialdehyde (OPA), 1%*

Dissolve 250 mg of solid or equivalent in methanol and dilute to 25 mL. Prepare fresh weekly and store at 4°C.

3.1.6 *Citric acid, 0.2M*

Dissolve 42 g of citric acid in 400 mL deionized water and make up to 1 L.

3.1.7 *Ion-exchange resin (Solid phase extraction column containing a quaternary ammonium functional group), e.g. Biorad poly-prep filled chromatography column 1 – X8 resin 100 – 200 mesh cat no. 731 – 6211 or equivalent.*

3.1.8 *Histamine dihydrochloride*

3.2 Standard solutions

3.2.1 *Histamine, 1000 mg/L (stock solution)*

Weigh accurately 0.1656 g histamine dihydrochloride into a 100 mL volumetric flask and make up to volume with deionized water. Store in a refrigerator. Prepare fresh at least monthly.

3.2.2 *Histamine, 100 mg/L (spiking solution)*

Dilute 10 mL of histamine stock solution (3.2.1) to 100 mL with deionized water. Prepare fresh at least monthly. Store in refrigerator.

3.2.3 *Histamine, 0.4 mg/L (working standard)*

Dilute 4.0 mL of histamine spiking solution (3.2.2) to 1 L with 0.1 M HCl. Store in a refrigerator. Prepare fresh at least monthly.

4 APPARATUS

4.1 50 mL graduated tubes with lids

4.2 Centrifuge

4.3 10 mL graduated tubes with lids

4.4 Vacuum manifold

4.5 Vortex mixer

4.6 HPLC apparatus Including pump, autosampler, fluorescence detector and computerized data system.

5 SAMPLE PREPARATION

5.1 General

Samples shall be prepared as given below and analysis shall be commenced immediately to minimize sample decomposition of frozen samples.

NOTE: Ensure homogeneity of sample, as this is a critical control point.

5.2 Liquid samples, for example fish sauce

Gently invert the sample container several times immediately before opening. If the samples contain suspended matter, this must be well mixed throughout the sample. These samples can be blended using a hand-held blender where appropriate.

Due to frequent precipitate formation, the final extracts are prepared in water in place of methanol (see Clause 6.2.1(f)).

5.3 Raw and cooked fish

Trim the inedible parts, e.g. bones, skin, fins and offal and blend the remaining tissue in a food processor. Include the skin if this is usually consumed.

5.4 Dried fish

Cut the sample into small pieces and place the pieces in a plastic beaker. Add liquid nitrogen. When the pieces are frozen, transfer to a food processor and blend to a powder.

5.5 Canned fish

Drain the liquid from the can and blend the remaining solids in a food processor.

Store all samples at –20°C until required for analysis.

6 PROCEDURE

6.1 Ion exchange resin conversion

The prepacked solid phase ion exchange resin columns are supplied in different forms, but the desired form is hydroxide. To convert the resin to hydroxide form, the procedure shall be as follows:

(a) Set up columns in the vacuum manifold unit and fill with 2 M NaOH to near the top of the reservoir.

(b) Apply vacuum, and open stopcocks depending on how many can be managed at once. Adjust vacuum to give a drip rate of 2 to 3 drops per second. As level of liquid approaches resin bed, refill with hydroxide.

(c) Watch carefully and do not allow liquid level to fall below top of resin bed and avoid running dry.

(d) For each column, it is necessary to refill the reservoir five times to ensure complete conversion.

(e) After the conversion step, wash columns with deionized water until pH of effluent is les than 9 (test with pH paper).

NOTE: Perform caustic wash 5 times and ensure that effluent pH to be less than 9 as this is a critical control point.