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Microbiology of the food chain — Detection and quantification of histamine in fish and fishery products — HPLC method

Microbiologie de la chaîne alimentaire — Détection et quantification de l'histamine dans le poisson et les produits de la pêche — Méthode CLHP

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Introduction

Histamine is a causative agent of scombroid poisoning or histamine fish poisoning. Histamine can be present mainly in *Scombridae* (tuna, mackerel) and *Clupeidae* (herring, sardine), species which contain a high level of free histidine. Histamine is formed through the decarboxylation of histidine by microbiological histidine decarboxylase.

Histamine [2-(1H-imidazol-5-yl)ethanamine] is defined as a biologically active low molecular weight basic nitrogenous molecule. The consumption of food containing significant concentration of histamine can cause symptoms similar to those associated to food allergies.

This document was developed in response to the need to standardize a method for histamine detection and quantification in fish and fishery products, in particular for European Regulation 2073/2005[1] on microbiological criteria for foodstuffs.

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Microbiology of the food chain — Detection and quantification of histamine in fish and fishery products — HPLC method

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

1 Scope

This document specifies a high performance liquid chromatography (HPLC) method to analyse histamine in fish and fishery products (fish sauces, fish maturated by enzyme in brine, etc.) intended for human consumption.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

4 Principle

This method enables the separation of histamine among biogenic amines in fish and fishery products. The sample is extracted by mixing with perchloric acid. Precolumn derivatization is performed using dansyl chloride. The biogenic amines and the components in the solution are separated by HPLC with an appropriate column, using UV detection. Histamine mass concentration is calculated from the peak area ratio of histamine and internal standard with a calibration curve.

5 Reagents and materials

Use only reagents of recognized analytical grade and water complying with grade 1 of ISO 3696, unless otherwise specified. Solvents shall be of quality for HPLC analysis, unless otherwise specified.

5.1 Acetone, CH_3COCH_3.

5.2 Acetonitrile, CH_3CN.

5.3 Toluene, C_7H_8.

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