



EAST AFRICAN STANDARD

Quick frozen fish fillets — General specification



EAST AFRICAN COMMUNITY

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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East African Community

P O Box 1096

Arusha

Tanzania

Tel: 255 27 2504253/8

Fax: 255-27-2504481/2504255

E-Mail: eac@eachq.org

Web: www.each.int

Introduction

In the preparation of this East African Standard, the following sources were consulted extensively:

CODEX STAN 190:1995, *General Standard for Quick Frozen Fish Fillets*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp

USDA Foreign Agricultural Service website: <http://www.mrlatabase.com>

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSv1.0/Standards>

European Union: http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm

Assistance derived from these sources is hereby acknowledged.

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Quick frozen fish fillets — General specification

1 Scope

This standard applies to quick frozen fillets of fish as defined below and offered for direct consumption without further processing. It does not apply to products indicated as intended for further processing or for other industrial purposes.

2 Normative references

The following referenced documents are indispensable for the application 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.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CD/K/572:2010, *Fish and fisheries products — Methods of sampling*

CAC/RCP 52[CD/K/521:2010], *Code of practice for fish and fishery products*

EAS 35, *Edible salt — Specification*

EAS 12, *Drinking (potable water) — Specification*

EAS 38, *Labelling of prepackaged foods — Specification*

EAS 41, *Fruits, vegetables and derived products — Sampling and methods of test*

EAS 103, *Schedule for permitted food additives*

EAS 123, *Distilled water — Specification*

ISO 4831, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 degrees C*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7937, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique*

ISO 13720, *Meat and meat products — Enumeration of Pseudomonas spp.*

ISO 17239, *Fruits, vegetables and derived products — Determination of arsenic content — Method using hydride generation atomic absorption spectrometry*

ISO 6634, *Fruits, vegetables and derived products — Determination of arsenic content — Silver diethyldithiocarbamate spectrophotometric method*

ISO 21567, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.*

ISO/TS 21872-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 1: Detection of Vibrio parahaemolyticus and Vibrio cholerae*

ISO/TS 21872-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 2: Detection of species other than Vibrio parahaemolyticus and Vibrio cholerae*

ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method*

ISO 11290-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 2: Enumeration method*

3 Description

3.1 Product definition

Quick frozen fillets are slices of fish of irregular size and shape which are removed from the carcass of the same species of fish suitable for human consumption by cuts made parallel to the backbone and sections of such fillets cut so as to facilitate packing, and processed in accordance with the process definitions given in Section 3.2.

3.2 Process definition

The product after any suitable preparation shall be subjected to a freezing process and shall comply with the conditions laid down hereafter. The freezing process shall be carried out in appropriate

equipment in such a way that the range of temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18°C (0°F) or colder at the thermal centre after thermal stabilization. The product shall be kept deep frozen so as to maintain the quality during transportation, storage and distribution.

These products shall be processed and packaged so as to minimize dehydration and oxidation.

The recognized practice of repacking quick frozen products under controlled conditions which will maintain the quality of the product, followed by the reapplication of the quick freezing process as defined, is permitted.

3.3 Presentation

3.3.1 Any presentation of the product shall be permitted provided that it:

- (a) meets all requirements of this standard, and
- (b) is adequately described on the label to avoid confusing or misleading the consumer.

3.3.2 Fillets may be presented as boneless, provided that boning has been completed including the removal of pin-bones.

4 Essential composition and quality factors

4.1 Fish

Quick frozen fish fillets shall be prepared from sound fish which are of a quality fit to be sold fresh for human consumption.

4.2 Glazing

If glazed, the water used for glazing or preparing glazing solutions shall be of potable quality or shall be clean sea-water. Potable water is fresh water fit for human consumption and complying with EAS 12. Clean sea-water is sea-water which meets the same microbiological standards as potable water and is free from objectionable substances.

4.3 Other ingredients

All other ingredients used shall be of food grade quality and conform to all applicable East African standards.

4.4 Decomposition

The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested. This shall apply only to species of *Clupeidae*, *Scombridae*, *Scombrosocidae*, *Pomatomidae* and *Coryphaenidae* families.

4.5 Final product

Products shall meet the requirements of this standard when lots examined in accordance with Section 9 comply with the provisions set out in Section 8. Products shall be examined by the methods given in Section 7.

5 Food additives

Only the use of the following additives is permitted.

Additive	Maximum level in the final product
<u>Moisture/Water Retention Agents</u>	
339(i) Monosodium orthophosphate	10 mg/kg expressed as P ₂ O ₅ , singly or in combination (includes natural phosphate)
340(i) Monopotassium orthophosphate	
450(iii) Tetrasodium diphosphate	
450(v) Tetrapotassium diphosphate	
451(i) Pentasodium triphosphate	
451(ii) Pentapotassium triphosphate	
452(i) Sodium polyphosphate	
452(v) Calcium, polyphosphates	
401 Sodium alginate	GMP
<u>Antioxidants</u>	
301 Sodium ascorbate	GMP
303 Potassium ascorbate	GMP

6 Hygiene and handling

6.1 The final product shall be free from any foreign material that poses a threat to human health.

6.2 When tested by appropriate methods of sampling and examination listed in Clause 2, the product:

- (i) shall be free from micro-organisms capable of development under normal conditions of storage;
- (ii) shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health;
- (iii) shall not contain histamine that exceeds 20 mg/100 g in any sample unit. This applies only to species of *Clupeidae*, *Scombridae*, *Scombrosocidae*, *Pomatomidae* and *Coryphaenidae* families;

6.3 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of CAC/RCP 1 and CAC/RCP 52.

6.4 The material shall also satisfy the limits for heavy metals and microbiological activity prescribed in Table 1 and Table 2.

Table 1 — Microbiological limits for quick frozen fish

S/No.	Type of microorganism	Maximum limit	Method of test
(i)	<i>Pseudomonas</i> species per gram	Absent	ISO 13720
(ii)	<i>Salmonella</i> in 30 g	Absent	ISO 6579
(iii)	<i>E. coli</i> per g	Absent	ISO 7251
(iv)	<i>Shigella</i> per g	Absent	ISO 21567
(v)	<i>Coliforms</i> g (per 100 g)	Absent	ISO 4832
(vi)	<i>Staphylococcus aureus</i> per 10 g	2×10^3 g	ISO 6888
(vii)	Total viable count	10^5 /g	ISO 4833
(viii)	<i>Vibrio cholerae</i>	Absent	ISO/TS 21872
(ix)	<i>Clostridium perfringens</i>	Absent	ISO 7937

Table 2 — Contaminant limits for quick frozen fish

Type of contaminant		Maximum limit (mg/kg)	Method of test
(i)	Arsenic	0.1	EAS 41
(ii)	Copper	0.4	EAS 41
(iii)	Iron	5.0	EAS 41
(iv)	Tin		
	(a) For product packed in tin plate	50.00	EAS 41
	(b) For product packed in other packing containers	250.00	EAS 41
(v)	Mercury	0.5	EAS 41
(vi)	Lead	0.3	EAS 41
(vii)	Cadmium	0.3	EAS 41
(viii)	Methylmercury	0.5	EAS 41
(ix)	Zinc	50.0	EAS 41

7 Labelling

In addition to provisions of EAs 38 the following specific provisions apply:

7.1 Name of the food

7.1.1 The name of the product as declared on the label shall be "... fillets" or "fillets of..." according to the law, custom or practice in the country in which the product is to be distributed.

7.1.2 There shall appear on the label reference to the form of presentation in close proximity to the name of the food in such additional words or phrases that will avoid misleading or confusing the consumer.

7.1.3 The term "quick frozen", shall also appear on the label, except that the term "frozen" may be applied in countries where this term is customarily used for describing the product processed in accordance with subsection 3.2 of this standard.

7.1.4 The label shall state that the product should be maintained under conditions that will maintain the quality during transportation, storage and distribution.

7.1.5 If the product has been glazed with sea-water, a statement to this effect shall be made.

7.2 Net Contents (Glazed Products)

Where the food has been glazed the declaration of net contents of the food shall be exclusive of the glaze.

7.3 Storage Instructions

The label shall include terms to indicate that the product shall be stored at a temperature of -18°C or colder.

7.4 Labelling of Non-retail Containers

Information on the above provisions shall be given either on the container or in accompanying documents, except that the name of the product, lot identification, and the name and address of the manufacturer or packer as well as storage instructions, shall appear on the container.

However, lot identification, and the name and address of the manufacturer or packer may be replaced by an identification mark provided that such a mark is clearly identifiable with the accompanying documents.

8 Sampling, examination and analyses

8.1 Sampling

- (i) Sampling of lots for examination of the product shall be in accordance with CD/K/572:2010. For prepackaged goods the sample unit is the entire container. A sample unit is the primary container or for individually quick frozen products is at least a 1 kg portion of the sample unit.
- (ii) Sampling of lots for examination of net weight shall be carried out in accordance with an appropriate sampling plan meeting the criteria established in CD/K/572:2010.

8.2 Sensory and physical examination

Samples taken for sensory and physical examination shall be assessed by persons trained in such examination and in accordance with procedures elaborated in Sections 8.3 through 8.6, Annex A and in accordance with the *Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories (CAC/GL 31 - 1999)*.

8.3 Determination of net weight

8.3.1 Determination of net weight of products not covered by glaze

The net weight (exclusive of packaging material) of each sample unit representing a lot shall be determined in the frozen state.

8.3.2 Determination of net weight of products covered by glaze

As soon as the package is removed from frozen temperature storage, open immediately and place the contents under a gentle spray of cold water until all ice glaze that can be seen or felt is removed. Remove adhering water by the use of paper towel and weigh the product.

An alternate method is outlined in Annex B.

8.4 Procedure for the detection of parasites for skinless blocks of fish fillets (Type I method)

The entire sample unit is examined non-destructively by placing appropriate portions of the thawed sample unit on a 5 mm thick acryl sheet with 45% translucency and candled with a light source giving 1500 lux 30 cm above the sheet.

8.5 Determination of gelatinous condition

Shall be determined in accordance with Annex D.

8.6 Cooking methods

The following procedures are based on heating the product to an internal temperature of 65 -70°C. The product must not be overcooked. Cooking times vary according to the size of the product and the temperatures used. The exact times and conditions of cooking for the products should be determined by prior experimentation.

Baking Procedure: Wrap the product in aluminum foil and place it evenly on a flat cookie sheet or shallow flat pan.

Steaming Procedure: Wrap the product in aluminum foil and place it on a wire rack suspended over boiling water in a covered container.

Boil-In-Bag Procedure: Place the product into a boilable film-type pouch and seal. Immerse the pouch into boiling water and cook.

Microwave Procedure: Enclose the product in a container suitable for microwave cooking. If plastic bags are used, check to ensure that no odour is imparted from the plastic bags. Cook according to equipment instructions.

8.7 Determination of histamine

Shall be determined in accordance with Annex C.

9 Definition of defects

The sample unit shall be considered defective when it exhibit any of the properties defined below.

9.1 Deep dehydration

Greater than 10% of the surface area of the sample unit or for pack sizes described below, exhibits excessive loss of moisture clearly shown as white or yellow abnormality on the surface, which masks the colour of the flesh and penetrates below the surface, and cannot be easily removed by scraping with a knife or other sharp instrument without unduly affecting the appearance of the product.

Pack Size	Defect Area
a) = 200 g units	= 25 cm ²
b) 201 -500 g units	= 50 cm ²
c) 501 - 5000 g units	= 150 cm ²

9.2 Foreign matter

The presence in the sample unit of any matter, which has not been derived from fish, does not pose a threat to human health, and is readily recognized without magnification or is present at a level determined by any method including magnification that indicates non-compliance with good manufacturing and sanitation practices.

9.3 Parasites

The presence of two or more parasites per kg of the sample unit detected by the method described in 8.4 with a capsular diameter greater than 3 mm or a parasite not encapsulated and greater than 10 mm in length.

9.4 Bones (in packs designated boneless)

More than one bone per kg of product greater or equal to 10 mm in length, or greater or equal to 1 mm in diameter; a bone less than or equal to 5 mm in length, is not considered a defect if its diameter is not more than 2 mm. The foot of a bone (where it has been attached to the vertebra) shall be disregarded if its width is less than or equal to 2 mm, or if it can easily be stripped off with a fingernail.

9.5 Odour and flavour

A sample unit affected by persistent and distinct objectionable odours or flavours characteristic of decomposition, rancidity or feed.

9.6 Flesh abnormalities

A sample unit affected by excessive gelatinous condition of the flesh together with greater than 86% moisture found in any individual fillet or a sample unit with pasty texture resulting from parasitic infestation affecting more than 5% of the sample unit by weight.

10 Lot acceptance

A lot shall be considered as meeting the requirements of this standard when:

CD/K/538:2010

- (i) the total number of defective sample units as classified according to Section 9 does not exceed the acceptance number (c) of the sampling plan in Section 8; and
- (ii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any container; and
- (iii) the Food Additives, Hygiene and Labelling requirements of Sections 4.4, 5, 6.1, 6.2 and 7 are met.



Individually quick frozen fillets



Example of package

Annex A
(normative)

Sensory and physical examination

1. Complete net weight determination, according to defined procedures in Section 8.3 (de-glaze as required).
2. Examine the frozen block for the presence of dehydration by measuring those areas which can only be removed with a knife or other sharp instrument. Measure the total surface area of the sample unit, and calculate the percentage affected.
3. Thaw and individually examine each block in the sample unit for the presence of foreign matter, bone where applicable, odour, and textural defects.
4. In cases where a final decision on odour cannot be made in the thawed uncooked state, a small portion of the disputed material (approximately 200 g) is sectioned from the block and the odour and flavor confirmed without delay by using one of the cooking methods defined in Section 8.8.
5. In cases where a final decision on gelatinous condition cannot be made in the thawed uncooked state, the disputed material is sectioned from the block and the gelatinous condition confirmed by cooking as defined in Section 8.7, or by using procedure in Section 8.6 to determine if greater than 86% moisture is present in any fillet. If cooking evaluation is inconclusive, then procedure in 8.6. would be used to make the exact determination of moisture content.

Annex B
(normative)

Method for the determination of net content of frozen fish blocks covered by glaze

Glazing is not used for Q.F. blocks of white fish. Only Q.F. blocks of herring, mackerel and other brown (fat) fish are glazed, which are destined for further processing (canning, smoking). For such blocks the following procedure may be applicable (tested with block frozen shrimps).

B.1 Principle

The pre-weighed glazed sample is immersed into a water bath by hand till all glaze is removed (as felt by fingers). As soon as the surface becomes rough, the still frozen sample is removed from the water bath and dried by use of a paper towel before estimating the net product content by repeated weighing. By this procedure thaw drip losses and/or re-freezing of adhering moisture can be avoided.

B.2 Equipment

- Balance - sensitive to 1 g
- Water bath, preferably with adjustable temperature
- Circular sieve with a diameter of 20 cm and 1-3 mm mesh apertures (ISO R 565)
- Paper or cloth towels with smooth surface
- A freezed box should be available at the working place

B.3 Preparation of samples and water bath

- The product temperature should be adjusted to $-18/-20^{\circ}\text{C}$ to achieve standard deglazing conditions (especially necessary if a standard deglazing period shall be defined in case of regular shaped products).
- After sampling from the low temperature store remove, if present, external ice crystals or snow from the package with the frozen product.
- The water bath shall contain an amount of fresh potable water equal to about 10 times of the declared weight of the product; the temperature should be adjusted on about 15°C to 35°C .

B.4 Determination of gross weight "A"

After removal of the package, the weight of the glazed product is determined: In case of single fish fillets, single weights are recorded (A 1-A n). The weighed samples are placed intermediately into the freezer box.

B.5 Removal of glaze

The pre-weighed samples/sub-samples are transferred into the water bath and kept immersed by hand. The product may be carefully agitated, till no more glaze can be felt by the finger-tips on the surface of the product: change from slippery to rough. Needed time, depending on size/shape and glaze content of the product, 10 to 60 sec. (and more in case of higher glaze contents or if frozen together).

For block-frozen products in consumer packs (also for single glaze products, which are frozen together during storage) the following (preliminary) procedure may be applicable: The pre-weighed

block or portion is transferred onto a suitable sized sieve and immersed into the water bath. By slight pressure of the fingers separating deglazed portions are removed fractionally. Short immersing is repeated, if glaze residues are still present.

B.6 Determination of net weight "B"

The deglazed sample/sub-sample, after removal of adhering water by use of a towel (without pressure) is immediately weighed. Single net-weights of sub-samples are summed up: B1-n.

B.6 Determination of glaze weight "C"

Grossweight "A" - Net weight "B" = Glazeweight "C"

B.7 Calculation of percentage proportions

% net content of the products "F" = $\frac{"B"}{"A"} \times 100$

% glaze - related to the gross weight of the product "G" = $\frac{"C"}{"A"} \times 100$

% glaze - related to the net weight of the product "H" = $\frac{"C"}{"B"} \times 100$

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Annex C (normative)

Determination of histamine

C.1 Principle

Sample is extracted with 75% (v/v) methanol. Extract is passed through ion exchange column. *o*-Phthaldialdehyde solution is added to eluate to form fluorescent histamine derivatives. Fluorescent intensity of derivatives is measured using fluorometer and histamine is quantified using external standards.

C.2 Apparatus

Rinse all plastic and glass containers with HCl (1 + 3) and H₂O before use.

- (a) **Chromatographic tube** — 200 ×7 id mm polypropylene tube fitted with small plastic stopcocks and ca 45 cm Teflon tubing. Control flow rate at >3 ml/min by adjusting height of column relative to tubing outlet. Alternatively, use 2-way valve in place of tubing.
- (b) **Photofluorometer** — Equipped with medium pressure Hg lamp with excitation at 350 nm and measuring emission at 444 nm.
- (c) **Repipets** — 1 and 5 ml.

C.3 Reagents

- (a) **Ion-exchange resin** — Bio-Rad AG 1-X8, 50–100 mesh or Dowex 1-X8, 50–100 mesh. Convert to -OH form by adding ca 15 ml 2M NaOH/g resin to beaker. Swirl mixture and let stand <30min. Decant liquid and repeat with additional base. Thoroughly wash resin with H₂O, slurry into fluted paper and wash again with H₂O. Prepare resin fresh weekly and store under H₂O. Place glass wool plug in base of tube, C.2(a), and slurry in enough resin to form 8 cm bed. Maintain H₂O level above top of resin bed at all times. Do not regenerate resin in packed column; rather, use batch regeneration in beaker when necessary. Wash column with ca 10 ml H₂O before applying each extract.
- (b) **Phosphoric acid** — 3.57N. Dilute 121.8 ml 85% H₃PO₄ to 1 L. For other concentration H₃PO₄, volume required for 1 L 1.19M acid = 17493/(density H₃PO₄ × percent H₃PO₄). Standardize 5.00 ml by titration with 1.00M NaOH to phenolphthalein end point, and adjust concentration if necessary.
- (c) ***o*-Phthaldialdehyde (OPT) solution** — 0.1% (w/v). Dissolve 100 mg OPT in 100 ml distilled-in-glass methanol. Store in amber bottle in refrigerator. Prepare fresh weekly.
- (d) **Histamine standard solutions** — Store in refrigerator.
 - (1) **Stock solution** — 1 mg/ml as free base. Accurately weigh ca 169.1 mg histamine 2HCl (98%) into 100 ml volumetric flask, and dissolve and dilute to volume with 0.1M HCl. Prepare fresh weekly.
 - (2) **Intermediate solution** — 10 µg/ml. Pipet 1 ml stock solution into 100 ml volumetric flask, and dilute to volume with 0.1M HCl. Prepare fresh weekly.
 - (3) **Working solutions** — 0.5, 1.0, and 1.5 µg/5 ml. Pipet 1, 2, and 3 ml intermediate solution into separate 100 ml volumetric flasks, and dilute each to volume with 0.1M HCl. Prepare fresh daily.

- (e) **Methanol** — 75% (v/v). Place 75 ml MeOH (distilled in glass) into 100 ml volumetric flask or stoppered graduated cylinder. Dilute to volume with H₂O. Swirl flask while adding H₂O.

C.4 Preparation of standard curve

Pipet duplicate 5 ml aliquots of each working standard solution into separate 50 ml glass or polypropylene Erlenmeyers. Pipet in 10 mL 0.1M HCl to each flask and mix. Pipet in 3 ml 1M NaOH and mix. Within 5 min, pipet in 1 ml OPT solution and mix immediately. After exactly 4 min, pipet in 3 ml 3.57NH₃PO₄ and mix immediately. It is important to mix thoroughly after each addition and at least once during OPT reaction. (Run 6– 10 OPT reactions simultaneously by adding reagents to Erlenmeyers in set order.) Prepare blank by substituting 5 ml 0.1M HCl for histamine solution. Within 1.5 h, record fluorescence intensity (*I*) of working standard solutions with H₂O in reference cell, using excitation wavelength of 350 nm and emission wavelength of 444 nm. Plot *I* (corrected for blank) against µg histamine/5 ml aliquot.

C.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H₂O through column, C.2(a), and discard eluate. Pipet 1 ml extract onto column and add 4–5 ml H₂O. Immediately initiate column flow into 50 ml volumetric flask containing 5.00 ml 1.00M HCl. When liquid level is ca 2 mm above resin, add ca 5 ml H₂O and let elute. Follow with H₂O in larger portions until ca 35 ml has eluted. Stop column flow, dilute to volume with H₂O, stopper, and mix. Refrigerate eluate.

Pipet 5 ml eluate into 50 ml Erlenmeyer, and pipet in 10 ml 0.1M HCl. Proceed as in C.4, beginning "Pipet in 3 ml 1M NaOH . . .".

If test sample contains >15 mg histamine/100 g fish, pipet 1 ml sample–OPT mixture into 10 ml beaker containing exactly 2 ml blank–OPT mixture, and mix thoroughly. Read fluorescence of new solution. Dilute and mix aliquots with blank–OPT mixture as needed to obtain measurable reading. This approximation indicates proper dilution of eluate required prior to second OPT reaction needed for reliable quantitation of test sample. Alternatively, use sensitivity range control of fluorometer (if instrument has one) to estimate dilution. Use these approximations to prepare appropriate dilution of aliquot of eluate with 0.1NHCl, and proceed as in C.4, beginning "Pipet in 3 ml 1M NaOH . . .".

C.6 Calculations

Plot of *I* (measured by meter deflection or recorder response and corrected for blank) against µg histamine/5 ml test solution should be straight line passing through origin with slope = $m = [(I_a / 1.5) + I_b + 2I_c] / 3$.

$$\text{mg Histamine/100 g fish} = (10)(F)(1/m)(I_s)$$

$$\mu\text{g Histamine/g fish} = 10 \times (\text{mg histamine/100 g fish})$$

where *I_s*, *I_a*, *I_b*, and *I_c* = fluorescence from test sample, 1.5, 1.0, and 0.5 µg histamine standards, respectively; and *F* = dilution factor = (ml eluate + ml 0.1M HCl)/ml eluate. *F* = 1 for undiluted eluate.

If calibration plot is not linear, use standard curve directly for quantitation. Each subdivision on abscissa should be ≤0.1 µg histamine/5 ml test solution. Read all values from curve to nearest 0.05 µg histamine/5 ml test solution.

$$\text{mg Histamine/100 g fish} = (10)(F)(W)$$

$$\mu\text{g Histamine/g fish} = 10 \times (\text{mg histamine/100 g fish})$$

where *W* = µg histamine/5 ml test solution as determined from standard curve.

Annex D
(normative)**Determination of moisture in meat****D.1 Drying in vacuo at 95–100°C**

Dry test portion containing ca 2 g dry material to constant weight at 95–100°C under pressure ≤ 100 mm Hg (ca 5 h). For feeds with high molasses content, use temperature $\leq 70^\circ\text{C}$ and pressure ≤ 50 mm Hg. Use covered Al dish ≥ 50 mm diameter and 40 mm deep.

D.2 Air drying

D.2.1 With lids removed, dry test sample containing ca 2 g dry material 16–18 h at 100–102°C in air oven (mechanical convection preferred). Use covered Al dish ≥ 50 mm diameter and ≤ 40 mm deep. Cool in desiccator and weigh. Report loss in weight as moisture, g.

D.2.2 With lids removed, dry test sample containing ca 2 g dry material to constant weight (2–4 h depending on product) in mechanical convection oven or in gravity oven with single shelf at ca 125°C. Use covered Al dish ≥ 50 mm diameter and ≤ 40 mm deep. Avoid excessive drying. Cover, cool in desiccator, and weigh. Report loss in weight as moisture, g. (Dried test sample is not satisfactory for subsequent fat determination.)

Report loss on drying (LOD) as estimate of moisture content.

D.3 Calculations

$$\% \text{ (w/w) LOD} = \% \text{ (w/w) moisture} = 100 \times \frac{\text{wt loss on drying, g}}{\text{wt test portion, g}}$$

$$\% \text{ Dry matter} = 100 - \% \text{ LOD}$$

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