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EAST AFRICAN STANDARD

Crackers from marine and freshwater fish, crustaceans and molluscan shellfish — 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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Introduction

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

CODEX STAN 222:2001, *Standard for Crackers from Marine and Freshwater Fish, Crustaceans and Molluscan Shellfish*

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.mrldatabase.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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Crackers from marine and freshwater fish, crustaceans and molluscan shellfish — Specification

1 Scope

This East African Standard shall apply to crackers prepared from marine and freshwater fish, crustacean and molluscan shellfish. It does not include ready-to-eat fried as well as artificially flavoured fish, crustacean and molluscan shellfish crackers.

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

The product is a traditional food made from fresh fish or frozen minced flesh of either marine (including both the red meat and white meat species) or freshwater fish, crustacean (including prawns and shrimps) and molluscan shellfish (including squids, cuttlefish, oysters, clams, mussels and cockles) as described in section 4.1 and other ingredients as described in section 4.2.

3.2 Process definition

3.2.1 The product shall be prepared by mixing all the ingredients, forming, cooking, cooling, slicing and drying.

3.2.2 The product shall be packed in a suitable packaging material which is moisture proof and gas impermeable. It shall be processed and packaged so as to minimize oxidation.

3.3 Handling practice

Fresh marine and freshwater fish, crustacean and molluscan shellfish shall be preserved immediately after harvesting by chilling or icing to bring its temperature down to 0°C (32°F) as quickly as possible as specified in the Recommended International Code of Practice for Fresh Fish (CAC/RCP 9-1976) and kept at an adequate temperature to prevent spoilage and bacterial growth prior to processing.

4 Essential composition and quality factors

4.1 Raw material

Fresh marine and freshwater fish, crustacean and molluscan shellfish shall mean freshly caught, chilled or frozen marine and freshwater fish, crustacean and molluscan shellfish. Frozen minced flesh shall mean freshly caught, chilled or frozen marine and freshwater fish, crustacean and molluscan shellfish which has been appropriately processed. The marine and freshwater fish, crustacean and molluscan shellfish shall have a characteristic fresh appearance, colour and odour.

4.2 Other ingredients

Other ingredients shall be of food grade quality and conform to all applicable Codex Standards.

4.3 Optional ingredients

The product may contain sugar as well as suitable spices.

4.4 Final product

4.4.1 The product shall display a uniform size, shape, colour, thickness and texture.

4.4.2 The product shall comply with the requirements prescribed in Table 1.

Table 1 — Requirements for crackers from marine and freshwater fish, crustacean and molluscan shellfish

Characteristics	Grade	Fish	Crustacean and Molluscan Shellfish
Crude protein (N x 6.25), percent w/w min.	I	12	8
	II	8	5
	III	5	2
Moisture content, percent w/w	I))
	II)	8 to 14
	III))

5 Food additives

Only the use of the following additives is permitted.

Additive

Maximum level in the final product

Sequestrants

452 Polyphosphates

5 mg/kg expressed as P₂O₅, singly or in combination (includes natural phosphate)

Flavour enhancers

621 Monosodium glutamate

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:
- shall be free from micro-organisms capable of development under normal conditions of storage;
 - shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health;
 - shall not contain histamine that exceeds 20 mg/100 g in any sample unit.
- 6.3 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate clauses of CAC/RCP 1, CAC/RCP 9 and CAC/RCP 52.
- 6.4 The material shall also satisfy the limits for heavy metals and microbiological activity prescribed in Table 2 and Table 3.

Table 2 — Microbiological limits for crackers from marine and freshwater fish, crustacean and molluscan shellfish

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 3 — Contaminant limits for crackers from marine and freshwater fish, crustacean and molluscan shellfish

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

The name of the product from marine and freshwater fish shall be "Fish Crackers" and those from crustacean and molluscan shellfish shall depict the common name of the species, like "Prawn Crackers" or "Squid Crackers".

7.2 Grades

When declared by grade, the package shall declare the grade as prescribed in Table 1.

7.3 Additional requirements

The package shall bear clear directions for keeping the product from the time it is purchased from the retailer to the time of its use and directions for cooking.

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

8.1.1 The sampling and tolerance plans in CD-K-572:2010 shall be used to determine the acceptability of the lot. The sampling plans dictate the minimum sample size to be taken. If necessary, in the opinion of the inspector, more than the minimum sample size specified may be taken.

8.1.2 Sampling of lots for the sensory examination of the product shall be in accordance with CD-K-572:2010 except that a lower acceptance number for decomposition shall be used as indicated in the sampling tables.

The tables specify the minimum number of sample units to be used for the following types of inspections:

- a) Level I — Sensory examinations of all products subject to inspection other than lots which are subject to reinspection.
- b) Level II — Sensory examinations of all products which are under reinspection.

8.1.3 The sample unit shall consist of a container of crackers and the contents thereof.

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 CAC/GL 31.

8.3 Determination of crude protein

Shall be determined in accordance with Annex B.

8.4 Determination of moisture

Shall be determined in accordance with Annex D.

8.5 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 Foreign matter

The presence in the sample unit of any matter which has not been derived from materials specified in section 4.1, 4.2, 4.3, does not pose a threat to human health and is readily recognized without magnification that indicates non-compliance with good manufacturing and sanitation practices.

9.2 Odour and flavour

Unfried crackers affected by persistent and distinct objectionable odours and fried crackers affected by persistent and distinct objectionable flavours indicative of decomposition (such as putrid), or contamination by foreign substances (such as fuel oil and cleaning compound).

9.3 Bones

Crackers with more than one bone greater than 3 mm in diameter and 5mm in length that affects more than 25% of the sample unit.

9.4 Discolouration

Pronounced black, whitish or yellowish discolouration indicative of mould or fungal growth on the surface of crackers that affects more than 10% of the sample unit.

10 Lot acceptance

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

- (i) not any single instance of critical foreign matter occurs; or
- (ii) the total number of sample units found defective for taint, decomposition or unwholesomeness, individually or in combination, does not exceed the acceptance number for the sample size designated in the sampling plans in CD-K-572:2010; or
- (iii) the total number of sample units found defective for decomposition does not exceed the acceptance number (c) shown in parentheses for the sample size designated in the sampling plans in CD-K-572:2010; or
- (iv) the Food Additives, Hygiene and Labelling requirements of Clauses 5, 6, and 7 are met.

Annex A
(normative)

Sensory and physical examination

The sample used for sensory evaluation should not be same as that used for other examination.

1. Examine the sample unit for foreign matter, bones and discolouration.
2. Assess the odour in the uncooked sample in accordance with CAC/GL 31).
3. Assess the flavour in cooked sample in accordance with CAC/GL 31).
4. The sample shall be deep-fried in fresh cooking oil at 190 °C for 20-60 seconds as appropriate to the thickness of the crackers.

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Annex B (normative)

Determination of crude protein

(Not applicable to material containing N–N or N–O linkages.)

B.1 Reagents

- (a) **Sulfuric acid** — Specific gravity 1.84, N-free.
- (b) **Copper catalyst** — Prepare using 15.0 g K_2SO_4 and 0.45 g $CuSO_4$. As boiling aid, 0.1 g pumice may be added.
- (c) **Potassium sulphate** — N-free.
- (d) **Sodium hydroxide–sodium thiosulfate solution** — Dissolve 60 g NaOH and 5 g $Na_2S_2O_3 \cdot 5H_2O$ in H_2O and dilute to 100 mL or add 25 mL 25% $Na_2S_2O_3 \cdot 5H_2O$ to 100 mL 50% NaOH solution.
- (e) **Boric acid solution** — Saturated solution.
- (f) **Indicator solution** — (1) Methyl red–methylene blue.— Mix 2 parts 0.2% alcoholic methyl red solution with one part 0.2% alcoholic methylene blue solution; or (2) Methyl red–bromocresol green solution — Mix one part 0.2% alcoholic methyl red solution with 5 parts 0.2% alcoholic bromocresol green solution.
- (g) **Hydrochloric acid** — 0.02M. Prepared as in Table B.1.

Table B.1 — Volumes of concentrated HCl required to prepare solutions of different molarities

Approximate molarity	mL HCl to be diluted to 10 L
0.01	8.6
0.02	17.2
0.10	86.0
0.50	430.1
1.0	860.1

B.2 Apparatus

- (a) **Digestion rack** — With either gas or electric heaters which will supply enough heat to 30 ml flask to cause 15 ml H_2O at 25°C to come to rolling boil in ≥ 2 but < 3 min.
- (b) **Distillation apparatus** — One-piece or Parnas–Wagner distillation apparatus recommended by Committee on Microchemical Apparatus, ACS.
- (c) **Digestion flasks** — Use 30 ml regular Kjeldahl or Soltys-type flasks. For small samples, 10 ml Kjeldahl flasks may be used.

B.3 Determination

Weigh test portion requiring 3–10 ml 0.01 or 0.02M HCl and transfer to 30 ml digestion flask. If test portion weight is < 10 mg, use microchemical balance (maximum weight 100 mg dry organic matter).

Use charging tube for dry solids, porcelain boat for sticky solids or nonvolatile liquids, and capillary or capsule for volatile liquids. Add 1.9 ± 0.1 g K_2SO_4 , 40 ± 10 mg HgO , and 2.0 ± 0.1 ml H_2SO_4 . If test portion weight is >15 mg, add additional 0.1 ml H_2SO_4 for each 10 mg dry organic matter >15 mg. Make certain that acid has specific gravity ≥ 1.84 if material contains nitriles. (10 ml flasks and $\frac{1}{4}$ quantities of reagents may be used for test portions <7 mg.) Add boiling chips which pass No. 10 sieve. If boiling time for digestion rack heaters is 2–2.5 min, digest 1 h after all H_2O is distilled and acid comes to true boil; if boiling time is 2.5–3 min, digest 1.5 h. (Digest 0.5 h if sample is known to contain no refractory ring N.)

Cool, add minimum volume of H_2O to dissolve solids, cool, and place thin film of petroleum jelly on rim of flask. Transfer digest and boiling chips to distillation apparatus and rinse flask 5 or 6 times with 1–2 ml portions H_2O . Place 125 ml Phillips beaker or Erlenmeyer containing 5 ml saturated H_3BO_3 solution and 2–4 drops indicator under condenser with tip extending below surface of solution. Add 8–10 mL $NaOH-Na_2S_2O_3$ solution to still, collect ca 15 ml distillate, and dilute to ca 50 ml. (Use 2.5 ml H_3BO_3 and 1–2 drops indicator, and dilute to ca 25 ml if 0.01M HCl is to be used.) Titrate to end point. Make blank determination and calculate.

$$\text{Percent N} = \frac{(\text{ml of HCl} - \text{ml blank}) \times \text{molarity} \times 14.007 \times 100}{\text{mg of sample}}$$

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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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