



CD/K/532:2010  
ICS 67.120.30

## EAST AFRICAN STANDARD

---

Standard for quick frozen shrimps or prawns



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.

© East African Community 2010 — All rights reserved\*

East African Community

P O Box 1096

**Arusha**

Tanzania

Tel: 255 27 2504253/8

Fax: 255-27-2504481/2504255

E-Mail: [eac@eachq.org](mailto:eac@eachq.org)

Web: [www.each.int](http://www.each.int)

## Introduction

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

CODEX STAN 092:1981(Rev. 1:1995), *Standard for quick frozen shrimps or prawns*

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](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](http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm)

Assistance derived from these sources is hereby acknowledged.

Draft for comments only — Not to be cited as East African Standard

**Contents**

1	Scope .....	1
2	Normative references .....	1
3	Description .....	2
4	Essential composition and quality factors .....	3
5	Food additives .....	3
6	Hygiene and handling .....	4
7	Labelling .....	5
8	Sampling, examination and analyses .....	5
9	Definition of defects .....	7
10	Lot acceptance .....	8
	Annex A (normative) Sensory and physical examination .....	11
	Annex B (normative) Determination of histamine .....	12
	Annex C (normative) Determination of moisture in meat .....	16

*Draft for comments only — Not to be cited as East African Standard*

## Quick frozen finfish, eviscerated or uneviscerated — Specification

### 1 Scope

This standard applies to quick frozen raw or partially or fully cooked shrimps or prawns (hereafter referred to as shrimp), peeled or unpeeled.

### 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**

**2.1.1** Quick frozen shrimp is the product obtained from species of the following families:

- (a) *Penaeidae*
- (b) *Pandalidae*
- (c) *Crangonidae*
- (d) *Palaemonidae*

**2.1.2** The pack shall not contain a mixture of genera but may contain a mixture of species of the same genus which have similar sensory properties.

### 3.2 Process definition

The water used for cooking and cooling shall be of potable quality or clean seawater.

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  $-18^{\circ}\text{C}$  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. Quick frozen shrimps shall be processed and packaged so as to minimize dehydration and oxidation.

### 3.3 Presentation

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

3.3.1.1 meets all requirements of this standard; and

3.3.1.2 is adequately described on the label to avoid confusing or misleading the consumer.

3.3.2 The shrimp may be packed by count per unit of weight or per package.

## 4 Essential composition and quality factors

### 4.1 Shrimp

Quick frozen shrimp shall be prepared from sound shrimp 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. Standards of potability shall not be less than those contained in the latest edition of the WHO "International Guidelines for Drinking Water Quality". 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 Codex standards.

### 4.4 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
Acidity Regulators	
330 Citric acid	GMP

450(iii) Tetrasodium diphosphate  
 450(v) Tetrapotassium diphosphate  
 451(i) Pentasodium triphosphate  
 451(ii) Pentapotassium triphosphate

10 mg/kg expressed as P<sub>2</sub>O<sub>5</sub>, singly or in combination (includes natural phosphate)

Antioxidant

300 Ascorbic acid (L-)

GMP

Colours

124 Ponceau 4R

30 mg/kg in heat-treated products only

Preservatives

221 Sodium sulphite  
 223 Sodium metabisulphite  
 224 Potassium metabisulphite  
 225 Potassium sulphite

100 mg/kg in the edible part of the raw product, or 30 mg/kg in the edible part of the cooked product, singly or in combination, expressed as SO<sub>2</sub>

**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; and
- (ii) shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health;
- (iii) shall be free from container integrity defects which may compromise the hermetic seal; and
- (iv) shall not contain histamine that exceeds 20 mg/100 g.

**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.

**Table 1 — Microbiological and heavy metal limits for canned tuna and bonito**

Type of contaminant		Maximum limit (mg/kg)	Method of test
(i)	Microbiological requirements	Shall be commercially sterile	See J.5.1; CD/K/551:2010
(i)	Arsenic	1.0	EAS 41
(iii)	Copper	0.4	EAS 41
(iv)	Tin	250.0	EAS 41
(v)	Mercury	0.5	EAS 41
(vi)	Lead	0.3	EAS 41
(vii)	Cadmium	0.3	EAS 41
(viii)	Zinc	50.0	EAS 41
(ix)	Histamine content, mg/100 crab meat, max	20.0	Annex B

## 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 as declared on the label shall be "shrimps" or "prawns" according to the law, custom or practice in the country in which the product is to be distributed.

**7.1.1** There shall appear on the label, reference to the presentation in close proximity to the name of the product in such descriptive terms that will adequately and fully describe the nature of the presentation of the product to avoid misleading or confusing the consumer.

**7.1.2** In addition to the specified labelling designations above, the usual or common trade names of the variety may be added so long as it is not misleading to the consumer in the country in which the product will be distributed.

**7.1.3** Products shall be designated as cooked, or partially cooked, or raw as appropriate.

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

**7.1.5** 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.

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

### 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 specified above shall be given either on the container or in accompanying documents, except that the name of the food, lot identification, and the name and address, as well as storage instructions shall always appear on the container.

However, lot identification and the name and address 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 package of shrimp and the contents thereof. For package sizes of 2.27 kg (5 lb.) or greater, it is permissible to examine a sub-unit consisting of at least 1 kg of product, if, in the Inspector's opinion, a representative sub-unit can be obtained.

## **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 CAC/GL 31.

### **8.3 Determination of net weight**

#### **7.3.1 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.

#### **7.3.2 Determination of net weight of products covered by glaze**

##### **Procedure**

- (1) Open the package with quick frozen shrimps or prawns immediately after removal from low temperature storage.
  - (i) For the raw product, place the contents in a container into which fresh water at room temperature is introduced from the bottom at a flow of approximately 25 litres per minute.
  - (ii) For the cooked product place the product in a container containing an amount of fresh potable water of 27°C (80° F) equal to 8 times the declared weight of the product. Leave the product in the water until all ice is melted. If the product is block frozen, turn block over several times during thawing. The point at which thawing is complete can be determined by gently probing the block apart.
- (2) Weigh a dry clean sieve with woven wire cloth with nominal size of the square aperture 2.8 mm (ISO Recommendation R565) or alternatively 2.38 mm (US No. 8 Standard Screen).
  - (i) If the quantity of the total contents of the package is 500 g (1.1 lbs) or less, use a sieve with a diameter of 20 cm (8 inches).
  - (ii) If the quantity of the total contents of the package is more than 500 g (1.1 lbs) use a sieve with a diameter of 30 cm (12 inches).
- (3) After all glaze that can be seen or felt has been removed and the shrimps or prawns separate easily, empty the contents of the container on the previously weighed sieve. Incline the sieve at an angle of about 20° and drain for two minutes
- (4) Weigh the sieve containing the drained product. Subtract the mass of the sieve; the resultant figure shall be considered to be the net content of the package.

### **7.4 Determination of count**

When declared on the label, the count of shrimp shall be determined by counting the numbers of shrimp in the container or a representative sample thereof and dividing the count of shrimp by the actual deglazed weight to determine the count per unit weight.

## 7.5 Procedures for thawing

The sample unit is thawed by enclosing it in a film type bag and immersing in water at room temperature (not greater than 35°C). The complete thawing of the product is determined by gently squeezing the bag occasionally so as not to damage the texture of the shrimp, until no hard core or ice crystals are left.

## 7.6 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 temperature used. The exact times and conditions of cooking for the product 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.

## 8.8 Determination of histamine

See Annex B.

## 9 Definition of defects

A sample unit will be considered defective when it exhibits any of the properties defined below.

### 9.1 Deep dehydration

Greater than 10% of the weight of the shrimp in the sample unit or greater than 10% of the surface area of the block 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 shrimp.

### 9.2 Foreign matter

The presence in the sample unit of any matter which has not been derived from shrimp 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 Odour/flavour

Shrimp affected by persistent and distinct objectionable odours or flavours indicative of decomposition or rancidity or of feed.

### 9.4 Discolouration

Distinct blackening or green or yellow discoloration, singly or in combination of more than 10% of the surface area of individual shrimp which affects more than 25% 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 total number of sample units found defective for standards of identity (style of presentation) and size designation or count range (if a size designation or count range is declared), exceeds the acceptance number for the sample size designated in the sampling plans.
- (v) the Food Additives, Hygiene and Labelling requirements of Clauses 5, 6, and 7 are met.



Individually quick frozen shrimps

uard



Frozen river prawns



Bag of Frozen Shrimp

Draft for



Frozen tiger prawn



Fins,  
Furs, &  
Feathers, Inc.

Draft for comments

Standard

**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 shrimp in the sample unit or the surface of the block for the presence of dehydration. Determine the percentage of shrimp or surface area affected.
3. Thaw using the procedure described in Section 8.5 and individually examine each shrimp in the sample unit for the presence of foreign matter and presentation defects. Determine the weight of shrimp affected by presentation defects.
4. Examine product for count declarations in accordance with procedures in Section 8.4.
5. Assess the shrimp for odour and discolouration as required.
6. In cases where a final decision regarding the odour/flavour cannot be made in the thawed state, a small portion of the sample unit (100 to 200 g) is prepared without delay for cooking and the odour/flavour confirmed by using one of the cooking methods defined in Section 8.6.

Draft for comments only — Not to be cited as East African Standard

**Annex B**  
(normative)**Determination of histamine****B.1 Introduction**

**B.1.1 Principle** — Histamine is extracted with methanol and derivatized with o-phthalaldehyde (OPT) to generate the fluorescent product. This method is used to determine the histamine content in raw, pre-cooked, and canned tuna.

**B.1.2 Interference** — All methods of histamine determination are overwhelmed with interfering substances which have to be removed in order to accurately measure the histamine present. The two naturally occurring substances that cause the most interference are histamine and spermidine since they also react with OPT to form fluorescent products. However, spermidine, the major contaminant in extracts can be separated from histamine on cellulose phosphate cation-exchange columns. There is also variability due to the pH and temperature sensitivity of the o-phthalaldehyde-histamine fluorophor. Because of the ubiquity of interfering fluorophors, all reagents used must be of the highest obtainable purity. Exposure of any of the materials involved to rubber or silicones may produce erratic results. It is recommended that polyethylene labware be used in place of glass, due to an observed loss of fluorescence. All labware should be acid-washed and rinsed in distilled water prior to use. New solution must be prepared after four to seven days, due to an observed increase in blank readings.

**B.1.3 Summary of method** — The histamine-containing material are homogenized and extracted with methanol. The extract can then be passed through an anion exchange column to remove any remaining interfering substances. The elutant is reacted with the OPT reagent and allowed to stand for 4 minutes. The mixture is acidified with  $H_3PO_4$  and the corresponding fluorescence is read on a calibrated instrument.

**B.2 Material required**

TD-360 Min-Fluorometer with U.V. optical configuration of (P/N 36000-010) 10 mm x 10 mm Methacrylate fluorescence cuvettes (P/N 7000-959).

**B.2.1 Labware** — All re-usable labware (glass, polyethylene, Teflon etc.) should be cleaned by soaking in laboratory grade detergent and water for 4 h, rinsed with tap water, deionized water, and methanol. It is recommended that polyethylene ware be used due to absorbency observed when using glass.

**B.2.1.1 Assorted Class A calibrated pipettes**

**B.2.1.2 Graduated cylinder** — 100 ml.

**B.2.1.3 Assorted Volumetric Flasks** — For preparing dilution standards.

**B.2.2 Chromatographic Columns (Kontes M.K 422250).**

**B.3 Reagents and standards**

**B.3.1 Ion Exchange Resin** — Sigma 1 x 8-200, chloride form 100-200 mesh: or BioRad AG1- x 8, 50-100 mesh, chloride form, Cat. No. 140-1431, or equivalent.

**B.3.2 Ion Sodium Hydroxide** — Dissolve 40 g NaOH in 1 Litre of distilled water.

**B.3.3 2.0N Sodium Hydroxide** — Dissolve 80 g NaOH in 1 Litre of distilled water.

**B.3.4 Histamine Dihydrochloride** — MCB X 0440 or J.T. Baker 1-N330.

**B.3.5 1.0N Hydrochloric Acid** — Add 83 ml concentrated HCL to about 500 ml distilled water. Cool and bring to 1-litre volume with distilled water.

**B.3.6 0.1N Hydrochloric Acid** — Add 100-mL 1N HCl to about 500-mL distilled water. Cool and bring to 1-Litre volume with distilled water.

### **B3.7 Methanol Reagent Grade**

**B3.8 0.1 % o-phthalaldehydol (OPT reagent)** — Phthalic dicarboxaldehyde (Aldrich, Milwaukee, WI), or o-phthalaldehyde (Sigma, St. Louis, MO)  $C_6H_4(CHO)_2$ . F.W, 134.13. Dissolve 0.10 g OPT in 100-mL methanol. Store in an amber bottle and refrigerate when not in use. Prepare fresh weekly.

**B.3.9 3.57N Phosphoric Acid** — Add 121.8 ml of 85 %  $H_3PO_4$  to about 500-mL distilled water. Bring to 1- litre volume with distilled water.

**B.3.10 Histamine Standard Solution A, 1 mg Hm/ml** — Weigh 0.1656 g of histamine dihydrochloride into 100-ml volumetric flask. Dissolve in, and dilute to volume with 0.1N HCl.

**B.3.11 Histamine Standard Solution B, 10 µg Hm/ml** — Dilute 1.0 ml Solution A to 100 ml with 0.1N HCL.

**B.3.12 Histamine Standard Solution A1 (This is our control solution)** — Dilute 1.0 ml Solution A to 100 ml with methanol.

**B.3.13 Histamine Standard Solution C, 0.1 mg Hm/ml** — Dilute 1.0 ml Solution B to 100 ml with 0.1N HCl.

**B.3.14 Histamine Standard Solution D, 0.2 M 0.2 M Hm/ml** — Dilute 2.0 ml Solution B to 100 ml with 0.1 N HCl.

**B.3.15 Histamine Standard Solution E, 0.3 mg Hm/ml** — Dilute 3.0 ml Solution B to 100 ml with 0.1N HCl.

NOTE Prepare Solution A and B monthly. Prepare Solutions C, D, E, and A1 weekly. Refrigerate solutions when not in use.

## **B.4 Preparation**

### **B.4.1 Resin preparation**

**B.4.1.1** Place 20 g of ion exchange resin in a beaker.

**B.4.1.2** Add 2 N sodium hydroxide to the resin in a ratio of 15 ml per gram of resin.

**B.4.1.3** Mix well and allow the resin to settle for a minimum of 15 minutes, but no more than 30 minutes. Decant liquid and repeat with additional 2 N sodium hydroxide.

**B.4.1.4** Wash resin thoroughly with distilled water to remove traces of the sodium hydroxide until pH is less than or equal to 8.5.

**B.4.1.5** Slurry resin with distilled water and transfer to a funnel containing a fluted filter paper. Thoroughly wash with distilled water.

**B.4.1.6** Transfer resin to a suitable container and make sure the distilled water level is above the resin level at all times.

### **B.4.2 Column preparation**

**B.4.2.1** Slurry sufficient prepared resin into each column to form a bed 8 cm in height. Maintain a liquid level above the top of the resin at all times.

B.4.2.2 Refill columns with fresh resin at least twice per week.

## B.5 Instrument set-up

B.5.1 Check that light source and filter holder are installed in your TD-360 Mini-Fluorometer. Turn on the instrument and allow to warm-up. For additional assistance, refer to your TD-360 Operating Manual.

B.5.2 **Blank with a reagent blank** — Calibrate instrument with the prepared histamine standard Solution E. Enter standard value of 3 000 mg/lm. Remember later to divide all reading by 10 000 to get mg Hm/ml of sample.

B.5.3 Analyze Histamine Standard Solutions C and D like you would a sample. You now have a standard curve for your samples.

## B.6 Procedure

### B.6.1 Sample preparation

B.6.1.1 Blend fish in a warring blender with an equal weight of deionized water to produce a 1:1 slurry.

B.6.1.2 Transfer 10.0 g of the slurry to a 150-ml beaker. Add 40.0 ml of methanol and mix thoroughly.

B.6.1.3 Using Whatman No.1 filter paper, or equivalent, filter the mixture into a suitable container. If the filtrate is to be saved for later analysis, refrigerate in a closed container.

NOTE Evaporation of methanol from the filtrate can cause erroneous results.

### B.6.2 Histamine Elusion

B.6.2.1 Pass 15-20-ml distilled water through the exchange column and discard.

B.6.2.2 Place a 50-ml volumetric flask containing 5 ml in HCl at the column outlet.

B.6.2.3 Pipette 1.0 ml of filtrate (methanol extract) onto the resin bed with 5.10 ml distilled water.

B.6.2.4 Immediately initiate column flow. Flow should be maintained at a rate grater than 3 ml/min.

B.6.2.5 When liquid level is slightly above the resin, add about 5-ml distilled water and allow it to flow through the resin. Repeat with distilled water in larger increments until total water through column is about 40 ml.

#### B.6.2.6 *Discontinue Column Flow*

B.6.2.7 Remove volumetric flask and bring to 50-ml volume with distilled water. Store column effluent in the refrigerator if necessary to postpone determination for more than 2 h.

### B.6.3 Controls and blanks

B.6.3.1 At the beginning of a set of analysis, and again at the end, pass 1 ml of Solution A1 through one of the columns and proceed through the procedure as though it were a fish extract. Fluorescence readings should be very similar to Solution D reading. If readings are not within 20 per cent of Solution D, all analysis performed at the same time are suspect and should be repeated.

### B.6.4 Histamine determination

B.6.4.1 Into separate 25-ml glass stoppered flask, pipette 5.0 ml of 0.1 HCl (Blank); Solutions C, D and E: and each diluted column effluent.

**B.6.4.2** Add 10 ml 0.1N HCl to each flask.

**B.6.4.3** Add 3 ml in NaOH. Mix thoroughly.

**B.6.4.4** Within 5 minutes, add 1 ml OPT solution and mix thoroughly.

**B.6.4.5** After exactly 4 minutes, add 3 ml 3.57 N H<sub>3</sub>PO<sub>4</sub> and mix immediately.

**B.6.4.6** Let solutions stand for 15-20 minutes and then determine the fluorescence intensities on the TD-360 Min-fluorometer. If a sample reading is greater than that of Solution E, dilute 25 ml of the column effluent to 100 ml with 0.1N HCl and proceed from B.6.4.1.

**CAUTION!** Fish with high salt content may cause problems with the resin necessitating more frequent changing of columns.

**B.6.4.7** If sample dilution was necessary in B.6.4.6, multiply the obtained result by 4.

**B.6.4.8** After all readings are obtained, divide all results by 10, 1 000 to get histamine concentration in mg Hm/ml

Draft for comments only — Not to be cited as East African Standard

**Annex C**  
(normative)**Determination of moisture in meat****C.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 ≤70°C and pressure ≤50 mm Hg. Use covered Al dish ≥50 mm diameter and 40 mm deep.

**C.2 Air drying**

**C.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.

**C.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.

**C.3 Calculations**

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

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

*Draft for comments only — Not to be cited as East African Standard*