



DRAFT EAST AFRICAN STANDARD

Frozen fish fillets — specification

DRAFT EAST AFRICAN STANDARD

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

Web: www.each.int

* © 2014 EAC — All rights of exploitation in any form and by any means reserved worldwide for EAC Partner States' NSBs.

Frozen fish fillets — Specification

1 Scope

This East Africa draft standard specifies requirements and methods of sampling and test for frozen fish fillets intended for human consumption without further processing.

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.

CODEX STAN 192 - CODEX STAN 192-1995, *General standard for food additives*

EAS 39, *Hygiene in the food and drink manufacturing industry — Code of practice*

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

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

EAS 38, *Labelling of pre-packaged foods — Specification*

EAS 103, *Schedule for permitted food additives*

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

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

ISO 21527-1, *Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0.9*

ISO 27085, Animal feeding stuffs -- Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES

ISO 16050, Foodstuffs — Determination of aflatoxin B₁, and the total content of aflatoxin B₁, B₂, G₁ and G₂ in cereals, nuts and derived products — High performance liquid chromatographic method

AOAC 990.04 Mercury (Methyl) in sea food by liquid chromatography-Atomic Absorption Spectroscopy (LC-AAS)

AOAC 977.15 / 974.14 Mercury in fish by Flame Atomic Absorption Spectroscopy (FAA)

3 Terms and definitions

For the purpose of this standard the following terms and definitions shall apply;

3.1

fillet

slice of fish of irregular size and shape removed from the fish flesh by cuts made parallel to the backbone.

3.2

frozen fillets

the fillet that undergone freezing process

3.3

freezing

process of heat removal from fish such that the temperature falls fairly rapidly to just below 0°C, the freezing point of water

4 Requirements

4.1 General requirements

4.1.1 Frozen fish fillets shall be prepared from sound fish which are of a quality fit to be sold fresh for human consumption

4.1.2 Frozen fish fillets shall be prepared from fish that conforms to DEAS 827:2014

4.1.3 Frozen fish fillets shall be checked for foreign matter, colour, texture and odour of the fillet prior freezing

4.1.4 Water used for washing the fish shall comply with EAS 12.

4.1.5 The frozen fish fillets shall have a temperature of -18°C

4.2 Specific requirements

4.2.1 Fillets may be presented as boneless, skin-on, glazed, provided that deboning has been completed including the removal of pin-bones.

4.2.2 When tested as per annex A or any other validated method the level of histamine in species with high levels of histidine such as scromboids shall not exceed 10ppm

5 Hygiene

The product covered by the provisions of this standard shall be prepared and handled in accordance with, EAS 39 and shall comply with microbiological limits given in table 1:

Table 1 — Microbiological limits for frozen fish fillets

S/No	Micro-organisms	Max. limits	Methods of test
i	<i>Salmonella</i> per 25 g	Absent	ISO 6579
ii	<i>E. coli</i> per gram	absent	ISO 7251
iii	<i>listeria monocytogenes</i>	absent	ISO 11290 PART 1 &2
iv	<i>Staphylococcus aureus</i> cfu per gram	100	ISO 6888
v	<i>Clostridium perfringens</i> per gram	Absent	ISO 7937
vi	<i>Vibrio Spp</i> per gram	Absent	ISO/TS 21872
vii	Total viable count per gram	10 ⁵	ISO 4833

6 Contaminant limits

Frozen fish fillets shall comply with the contaminant limits given in table 2.

Table 2 — Contaminant limits for frozen fish fillets

S/No	Type of contaminant	Maximum limit (mg/kg)	Methods of test
i	Arsenic	0.1	ISO 27085
ii	Mercury	0.5	AOAC 977.15/ 974.14
iii	Lead	0.3	ISO 27085
iv	Cadmium	0.3	ISO 27085
v	Methyl mercury	0.5	AOAC 990.04

7 Weight and measures

The weight of the product shall comply with Weight and Measures regulations of Partner States or equivalent legislation.

8 Packaging and labelling

8.1 Packaging

8.1.1 Frozen fish fillets shall be packaged in food grade containers which will safeguard the hygienic, nutritional, and organoleptic qualities of the product.

8.1.2 The containers, including packaging material, shall be made of substances which are safe and suitable for their intended use. They shall not impart any toxic substance or undesirable odour or flavour to the product.

8.2 Labelling

In addition to the requirements in EAS 38, the following specific labelling requirements shall apply and shall be legibly and indelibly marked:

- a) name of the product shall be "... frozen fish fillets
- b) storage and transportation conditions declaring the temperature to be -18C or lower
- c) Name and physical address of processor
- d) Net weight in grams or kilograms
- e) Date of production
- f) Batch or code number
- g) Expiry date
- h) instruction for use
- i) Country of origin

8.3 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 processor or packer as well as storage instructions, shall appear on the container.

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

9. Method of sampling and test

Sampling and tests shall be done as per test methods described in respective tables

Annex A (normative)

Determination of histamine

A.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.

A.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.

A.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 <30 min. 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, A.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 about 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.

A.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.

A.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H₂O through column, A.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 A.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 A.4, beginning "Pipet in 3 ml 1M NaOH . . ."

A.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.

DRAFT EAST AFRICAN STANDARD