



CD/K/558:2010  
ICS 67.120.30

## EAST AFRICAN STANDARD

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### Frozen whole pomfret — Specification



**EAST AFRICAN COMMUNITY**

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## 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](mailto:eac@eachq.org)

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

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## Introduction

Fish is one of the most perishable of all foods and needs proper care from the time it is caught until it is served or processed. Lowering the temperature of fish by a prompt and efficient chilling procedure is fundamental for preservation of fish freshness. The quality of frozen fishery products is influenced by many different considerations. Among the most important are composition of fish, pre-freezing handling and transport, method of freezing employed and the environment to which the frozen product is subjected during storage and handling. Of principal concern here is the temperature and humidity of the cold storage area and the protective packaging or glazing afforded the product.

Pomfret belonging to family *Stromotidae*, is a high prized species of fish.

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

IS 4793:1997(R2005), *Whole Pomfret — Frozen — Specification*

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

Assistance derived from these sources is hereby acknowledged.

Contents

1	Scope .....	1
2	Normative references .....	1
3	Definitions, presentation and processing .....	2
3.1	Definitions .....	2
3.2	Forms of product presentation .....	3
3.3	Gutting .....	3
3.4	Chilling .....	4
3.5	Grades .....	4
4	Essential composition and quality factors .....	4
5	Food additives .....	4
6	Hygiene and handling .....	5
7	Packing and marking .....	6
7.1	Packing .....	6
7.2	Marking .....	6
8	Sampling, examination and analyses .....	6
9	Definition of defects .....	7
10	Lot acceptance .....	9
	Annex A (normative) Processing of frozen whole pomfret .....	13
	Annex B (normative) Determination of histamine .....	14

## Frozen whole pomfret — Specification

### 1 Scope

This standard prescribes the requirements and the methods of sampling and test for whole frozen pomfret of the following species:

<i>Pampus argenteus</i>	Silver pomfret
<i>P. chinensis</i>	White pomfret
<i>Parastromateus niger</i>	black or brown pomfret

### 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/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 Definitions, presentation and processing**

#### **3.1 Definitions**

For the purpose of this standard the following definitions shall apply:

##### **3.1.1 fresh fin fish**

Fish that has not been frozen, dried or otherwise preserved, except by chilling. Fish in rigor shall be deemed to be fresh fish.

**3.1.2****gutted fish**

Fish from which the guts have been removed. Gutting consists of bleeding the fish and removal of the stomach and gut.

**3.1.3****rigor mortis**

The stiffening of the muscles of an animal which results from a series of complex changes that take place in the tissues shortly after death.

**3.1.4****fin fish**

Fresh water and marine vertebrate fish.

**3.1.5****whole fish**

Fish as captured, ungutted.

**3.1.6****chilling**

The process of cooling fish to a temperature approaching that of melting ice.

**3.1.7****clean sea water**

Sea water which meets the same microbiological standards as potable water and is free from objectionable substance.

**3.2 Forms of product presentation**

**3.2.1** Frozen finfish may be presented as unviscerated, eviscerated, fillets or blocks with or without skin, scales or bones, as appropriate to the style of pack.

**3.2.2** Frozen finfish may also be presented as minced fish blocks.

**3.2.3** Any other presentation of the product may be permitted provided that it:

- a) is sufficiently distinctive from the forms of presentation set out in 3.2.1 and 3.2.2; and
- b) meets all other regulatory requirements; and
- c) is adequately described on the label and in accordance with all regulatory labelling requirements.

**3.3 Gutting**

**3.3.1** Gutting shall commence as soon as the fish is landed.

**3.3.2** Where immediate gutting is not practicable, whole fish shall be washed and chilled within 6 hours of catching.

**3.3.3** Gutting shall be complete and carried out with care.

**3.3.4** Fish guts shall be not allowed to contaminate other fish.

**3.3.5** Fish shall be washed with clean sea water or potable water (see EAS 12).

**3.3.6** Whole fish taken for human consumption shall be free from obvious diseased or parasite tissue or any other abnormal condition.

**3.3.7** All fish for filleting shall be free from debris.

**3.3.8** Offal waste shall be kept clear of the fish and disposed at the earliest possible time.

**3.3.9** Eviscerated fish shall be washed and the belly cavity cleaned in potable running water.

### **3.4 Chilling**

**3.4.1** The fish shall be thoroughly chilled. Chilling shall be accomplished by packing in clean, crushed flaked ice (ratio 1:1) or an equivalent technique so that the temperature of the fish shall at no time exceed temperature of melting ice.

**3.4.2** Fish landed (in a chilled condition) shall be maintained at a temperature not exceeding that of melting ice.

**3.4.3** Fresh fish in ice shall be packed in shallow containers.

### **3.5 Grades**

Frozen pomfret shall be of the following three grades:

<b>Grade designation</b>	<b>Mass, g</b>	
	<b>Silver and white pomfret</b>	<b>Brown or black pomfret</b>
Large	Above 500	Above 1000
Medium	251 to 500	701 to 1000
Small	150 to 250	400 to 700

NOTE The mass of a single, frozen pomfret in grams mentioned above is the mass obtained after thawing.

## **4 Essential composition and quality factors**

**4.1** Fishery products shall be free from objectionable matter and parasites.

**4.2** Fishery products shall be free from micro-organisms in amounts harmful to man, and any toxic substances originating from micro-organisms in amounts which may represent a hazard to health.

**4.3** Fisheries products shall comply with the requirements on pesticide residues and food additives (see EAS 103).

**4.4** The final product shall be stored close at a temperature of melting ice.

**4.5** The colour of whole pomfret (frozen), its flesh, shall be characteristic of the respective pomfret species. While the surface discolouration may vary to slight yellow, it shall not be excessively yellow in appearance. The texture of the meat shall be firm.

**4.6** The material shall be prepared and processed as given in Annex A, under hygienic conditions complying with CAC/RCP 52.

**4.7** The frozen pomfret, on thawing, shall be in sound, intact and undamaged conditions. The product shall be free from any foreign matter.

## **5 Food additives**

If used, food additives shall comply with EAS 103.

## 6 Hygiene and handling

6.1 The 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 in accordance with the standards 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 substances including substances derived from micro organisms in amounts which may represent a hazard to health; and
- (iii) shall be free from container integrity defects which may compromise the hermetic seal.

6.3 The products covered by the provisions of this standard shall be prepared and handled in accordance with the appropriate sections of the current edition of CAC/RCP 1 and the sections on the Products of Aquaculture in the International Code of Practice for Fish and Fishery Products CAC/RCP 52.

6.4 The material shall meet the microbiological and heavy metal requirements as given in Table 1.

**Table 1 — Microbiological and heavy metal limits for frozen pomfret fish**

Characteristic	Requirement	Method of test
(1)	(2)	(3)
i) Total bacterial count/g, in the finished product, Max	100 000	ISO 4833
ii) <i>Escherichia coli</i> count/g, Max	10	ISO 7251
iii) Faecal <i>Streptococci</i> count/g, Max	100	Annex H
iv) Coagulase positive <i>Staphylococci</i> /g, Max	100	ISO 6888
v) <i>Salmonella</i> , per 25 g	Absent	ISO 6579
vi) <i>Shigella</i> , per 25 g	Absent	ISO 21567
vii) <i>Vibrio cholerae</i> , per 25 g	Absent	ISO/TS 21872
viii) <i>Listeria monocytogenes</i> , per 25 g	Absent	ISO 11290
ix) Histamine content, mg/100 g, max	20.0	Annex ___
x) Formaldehyde mg/kg, Max	10.0	Annex F
xi) Indole, mg/kg, Max	2.5	Annex G
<b>xii) Heavy metals:</b>		
a) Mercury, mg/kg, Max	0.5	EAS 41
b) Copper, mg/kg, Max	20.0	EAS 41
c) Zinc, mg/kg, Max	50.0	EAS 41
f) Arsenic, mg/kg, Max	0.1	EAS 41
e) Lead, mg/kg, Max	0.3	EAS 41
f) Tin, mg/kg, Max		
(i) For product packed in tin plate	50.0	EAS 41
(ii) For product packed in other packing containers	250.0	EAS 41
g) Cadmium	0.3	EAS 41
h) Methylmercury	0.5	EAS 41

## 7 Packing and marking

### 7.1 Packing

The frozen pomfret shall be packed in suitable containers as agreed to between the purchaser and the processor. In the absence of any such agreement the material shall be packed in containers which shall withstand the stress and strain of transportation and prevent deterioration during frozen storage. A layer of moisture proof paper or suitable plastic material shall be used between the material and the container when frozen pomfrets are packed individually.

### 7.2 Marking

**7.2.1** In addition to provisions of EAS 38 each wrapped frozen material shall be marked or labelled with the following particulars:

- a) Name and type of the material;
- b) Name and address of the processor;
- c) Batch or code number;
- d) Grade;
- e) Net mass;
- f) Date of packing;
- g) The words 'Best before ..... (month and year to be indicated)'; and
- h) Any other requirement as given OIML R87, *Quantity of product in prepackages*.

#### 7.2.2 Certification marking

The product may also be marked with the relevant Standard Mark.

## 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 fish and the contents thereof. In the case of large containers (sample unit sizes of 10 kg or greater) of individually frozen whole or dressed fish or fresh or individually frozen fillets, the individual fish or fillet can be considered the sample unit for the purpose of collecting samples for examination.

## 8.2 Sensory and physical examination

Samples taken for sensoric and physical examination shall be assessed by persons trained in such examination and in accordance with Annex A and CAC/GL 31.

### 8.3 Examination methods

**8.3.1** Complete net weight determination, according to defined procedures (deglaze as required).

**8.3.2** Examine the frozen fish 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 fish or fillet, and determine the percentage affected using the following formula:

$$\frac{\text{area affected}}{\text{total surface area}} \times 100 = \% \text{ affected by dehydration}$$

**8.3.3** Thaw as necessary. The defrosted fish or fillets in the entire unit are examined individually for the presence of foreign matter, undesirable parts, nematodes and copepods, and other parasites with defined tolerances. Parasite examination for nematodes and copepods will be non-destructive, that is the fish are not filleted or the skin removed from fillets to assist in parasite detection. The parasites are removed and the total number of incidents counted to determine sample unit compliance.

**8.3.4** Each entire sample unit of defrosted fillets is examined in its entirety for odour, colour and texture. In the case of a reinspection, where an inspector is unable to make a decision on acceptance or rejection of a unit without evaluating flavour, the portion of the unit requiring confirmation of odour/flavour may be cooked using a boil-in-bag or similar procedure, or by oven heating or microwaving in a closed container, until the protein at the centre of the fish has coagulated. (Depending on the method chosen and the equipment available, cooking times may vary. For example, a 500 g thawed sample unit should require a cooking time of 3-4 minutes at a microwave power of 700 watts; the unit should be turned once during this procedure to ensure even heating.)

Let cool slightly, then assess odour, flavour and texture of cooked unit. Calculate percentage of unacceptable fish in the unit.

**8.3.5** In the case of whole or dressed fish, the entire sample unit is to be examined in its presented form, using the criteria outlined in Clause 8, for the determination of taint, decomposition and unwholesomeness. A thorough examination is to be made of the belly walls for evidence of perforated or broken bellies caused by enzymatic action of the stomach content (autolysis). Should there be evidence of perforated or broken belly walls or other signs of decomposition then the entire unit is further examined for flesh odours by tearing or making a cut across the back of the neck such that the exposed surface flesh can be evaluated for decomposition or taint.

Where no broken or perforated bellies are encountered, a minimum of at least 10% of the declared weight of each unit, or a minimum of 10 fish, whichever is greater, will be further examined for flesh odours by tearing or making a cut across the back of the neck.

**8.3.6** Record defects on the appropriate worksheet.

### 8.3.7 Classification of defectives

A sample unit shall be classified as "defective" when it fails the defects for decomposition, tainted, or unwholesome conditions as described in Clause 9, or when more than 10% by declared weight of the sample unit is affected by any combination of tainted or decomposed conditions.

## 9 Definition of defects

### 9.1 Taint

A unit will be considered tainted when more than 10% of the declared weight is affected by any of the following conditions:

a) **Rancid**

- Odour characterized by the distinct or persistent odour of oxidized oil; or
- Flavour characterized by that of oxidized oil which leaves a distinct bitter aftertaste.

b) **Abnormal** — Distinct and persistent uncharacteristic odours or flavours such as burnt or acrid, metallic, associated with feed or strong iodoform and not defined as rancid or decomposed.

**9.2 Decomposition**

A unit will be considered decomposed when more than 10% of the declared weight is affected by any of the following conditions:

- a) **Odour or flavour** — Persistent, distinct and uncharacteristic odour or flavour including but not limited to the following: ammonia, bilge, faecal, fruity, hydrogen sulphide, musty, putrid, saltfish-like, sour, sour milk-like, vegetable, and yeasty.
- b) **Discolouration** — Fish showing abnormal discolouration of the flesh, such as green or black as associated with decomposition.
- c) **Texture** — Textural breakdown of the flesh associated with decomposition which is characterized by muscle structure which is very tough or dry, or muscle structure which is mushy, or in the case of whole or dressed fish, perforated bellies or broken bellies or belly walls, caused by enzymatic action.

**9.3** A sample unit shall be classified as defective when more than 10% of the declared weight of the sample unit is affected by any combination of tainted or decomposed conditions.

**9.4 Unwholesome**

a) **Critical foreign material** — A lot will be considered defective when any of the following conditions are found:

- the presence of any material which has not been derived from fish and which poses a threat to human health (such as glass, etc.); or
- distinct and persistent odour or flavour of any material which has not been derived from fish and which poses a threat to human health (such as solvents, fuel oil, etc.).

b) **Foreign material** — A unit will be considered defective when the following condition is found:

- the presence of readily detectable material which has not been derived from fish but does not pose a threat to human health (such as insect pieces, sand, etc.).

c) **Other defects** — A unit will be considered defective when any of the following conditions are found:

- 1) **Dehydration (Freezer burn)** — More than 10% of the declared weight of the fish or fillets in the unit are affected by dehydration affecting more than 10% of their surface area.
- 2) **Parasites** — Only nematodes or copepod parasites having capsular diameter of greater than 3 mm or, if not encapsulated, a length of greater than 10 mm will be considered in determining whether the lot is acceptable with respect to parasites. For packs of 1 kg and greater, the presence of 2 or more parasites per kg of sample unit will result in rejection of the sample. For packs of less than 1 kg, the presence of parasites at a rate of infestation greater than an average of 1 parasite per kg of total sample will result in rejection of the sample. For example, a sample consisting of 13 units of 500g each would be rejected if 7 or more parasites were found.

The following parasite occurrences will result in the sample unit being classified as defective:

Pack Size	Reject Parasite Level
1 kg	Use average as described above
5lb	3
10 lb	5
15 lb	7
16.5 lb	8
18.5 lb	9
20 lb	10
50 lb	23

- 3) **Bones (Boneless packs only)** — One bone A 1 mm in diameter or A 10 mm in length per kg fish.
- 4) **Undesireable parts** — Each incidence of viscera.

## 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 Sections 5, 6, and 7 are met.



Frozen pomfret



Frozen silver pomfret

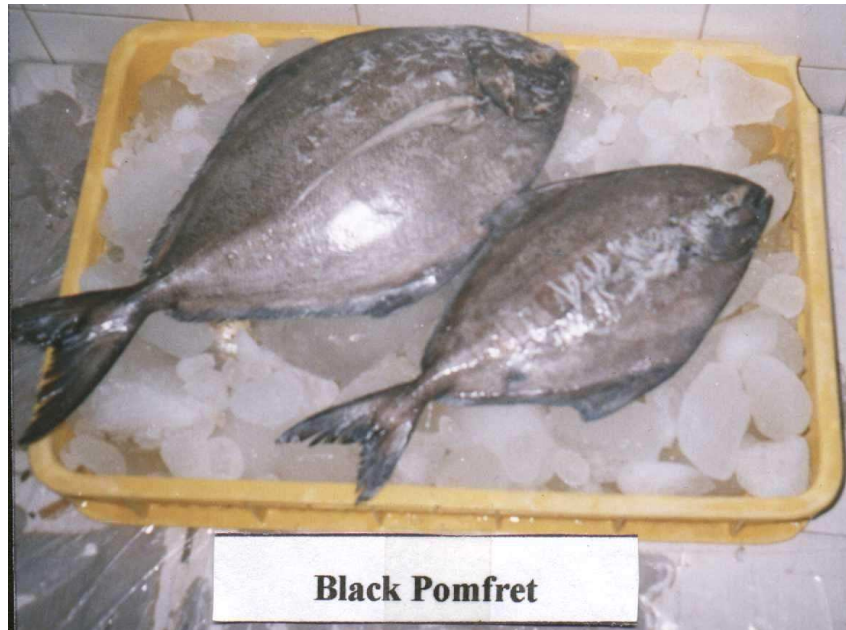


Red mullet



Frozen pomfret

Draft for



**Black Pomfret**

**Frozen pomfret**

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*an Standard*

**Annex A**  
(normative)

**Processing of frozen whole pomfret**

**A.1 Processing**

**A.1.1** The fish shall be properly iced and maintained at a temperature not exceeding 3 °C till it reaches the freezing factory.

**A.1.2** The material shall be washed in clean potable water containing 5 mg/kg to 10 mg/kg chlorine, to remove all adhering impurities and shall be iced immediately in suitable containers.

**A.1.3** The material shall be quick frozen at a temperature of -40 °C or below in the minimum possible time. However, the time taken for freezing the core of the material 2.5 cm thick shall not exceed 3 h.

**A.1.4** The quick frozen material shall be uniformly glazed with chilled water, packed in suitable containers and shifted immediately to the cold storage, the temperature of which shall be -23 °C or still lower.

**A.1.5** The material shall be grouped according to the grade of the fish.

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

## Annex B (normative)

### Determination of histamine

#### B.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.

#### B.2 Apparatus

Rinse all plastic and glass containers with HCl (1 + 3) and H<sub>2</sub>O 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.

#### B.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 H<sub>2</sub>O, slurry into fluted paper and wash again with H<sub>2</sub>O. Prepare resin fresh weekly and store under H<sub>2</sub>O. Place glass wool plug in base of tube, B.2(a), and slurry in enough resin to form 8 cm bed. Maintain H<sub>2</sub>O 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 H<sub>2</sub>O before applying each extract.
- (b) **Phosphoric acid** — 3.57N. Dilute 121.8 ml 85% H<sub>3</sub>PO<sub>4</sub> to 1 L. For other concentration H<sub>3</sub>PO<sub>4</sub>, volume required for 1 L 1.19M acid = 17493/(density H<sub>3</sub>PO<sub>4</sub> × percent H<sub>3</sub>PO<sub>4</sub>). 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 H<sub>2</sub>O. Swirl flask while adding H<sub>2</sub>O.

#### B.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 3.57NH<sub>3</sub>PO<sub>4</sub> 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 H<sub>2</sub>O 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.

#### B.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H<sub>2</sub>O through column, C.2(a), and discard eluate. Pipet 1 ml extract onto column and add 4–5 ml H<sub>2</sub>O. 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 H<sub>2</sub>O and let elute. Follow with H<sub>2</sub>O in larger portions until ca 35 ml has eluted. Stop column flow, dilute to volume with H<sub>2</sub>O, stopper, and mix. Refrigerate eluate.

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

#### B.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<sub>s</sub>*, *I<sub>a</sub>*, *I<sub>b</sub>*, and *I<sub>c</sub>* = 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.

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