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ICS 67.120.30

## EAST AFRICAN STANDARD

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**Salted Atlantic herring and salted sprat — 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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## Introduction

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

CODEX STAN 244:2004, *Standard for Salted Atlantic Herring and Salted Sprat*

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.

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## Salted Atlantic herring and salted sprat — Specification

### 1 Scope

The standard applies to salted Atlantic herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). Fish products produced by use of added natural or artificial enzymatic preparations, acids and/or artificial enzymes are not covered by this standard.

NOTE For the purpose of the standard, fish includes herring and sprats

### 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*

CAC/GL 48, *Model certificate for fish and fishery products*

CAC/GL 53, *Guidelines on the judgement of equivalence of sanitary measures associated with food inspection and certification systems*

EAS 38, *Labelling of prepackaged foods — Specification*

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 prepared from fresh or frozen fish. The fish is salted as whole fish or as headed or nobbed or headed and gutted or gibbed or filleted (skin-on or skin-off) fish. Spices, sugar and other optional ingredients may be added. Countries where the product are to be consumed may allow this product in an uneviscerated state or may require evisceration, either before or after processing, since the margin of error in the control of *Clostridium botulinum* is small even when good practices are followed and the consequences are severe. The product is either intended for direct human consumption or for further processing.

#### 3.2 Process definition

The fish after any suitable preparation shall be subjected to a salting process and shall comply with the conditions laid down hereafter. The salting process including the temperature and time should be sufficiently controlled to prevent the development of *Clostridium botulinum* or fish should be eviscerated prior to brining.

##### 3.2.1 Salting

Salting is the process of mixing fish with the appropriate amount of food grade salt, sugar spices and all optional ingredients and/or of adding the appropriate amount of salt-solution of the appropriate concentration. Salting is performed in watertight containers (barrels etc.).

##### 3.2.2 Types of salted fish

###### 3.2.2.1 Very lightly salted fish

The salt content in the fish muscle is above 1 g/100 g in water phase and below or equal to 4 g/100 g or less in water phase.

###### 3.2.2.2 Lightly salted fish

The salt content in the fish muscle is above 4 g/100 g in water phase and below or equal to 10 g salt/100 g in water phase.

###### 3.2.2.3 Medium salted fish

The salt content in the fish muscle is above 10 g salt/100 g water phase and below or equal to 20 g salt/100 g in water phase.

###### 3.2.2.4 Heavily salted fish

The salt content of the fish muscle is above 20 g salt /100 g in water phase.

##### 3.2.3 Storage temperatures

The products shall be kept frozen or refrigerated at a time/temperature combination which ensures their safety and quality in conformity with Sections 4 and 5. Very lightly salted fish must be kept frozen after processing.

### 3.3 Presentation

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

3.3.1 meets all requirements of this standard, and

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

## 4 Essential composition and quality factors

### 4.1 Fish

Salted Atlantic herring and salted sprats shall be prepared from sound and wholesome fish which are of a quality fit to be sold fresh for human consumption after appropriate preparation. Fish flesh shall not be obviously infested by parasites.

### 4.2 Salt

Salt and all other ingredients used shall be of food grade quality and conform to all applicable Codex standards.

### 4.3 Final product

Products shall meet the requirements of this standard when lots examined in accordance with Section 10 comply with the provisions set out in Section 9. Products shall be examined by the methods given in Section 8.

### 4.4 Decomposition

The products shall not contain more than 10 mg of histamine per 100 g fish flesh based on the average of the sample unit tested.

## 5 Food additives

Only the use of the following additives is permitted.

### Additives

#### Acidity regulators

300 Ascorbic acid

330 Citric acid

#### Antioxidants

200 – 203 Sorbates

#### Preservatives

210 – 213 Benzoates

### Maximum level in the final product

GMP

GMP

200 mg/kg, singly or in combination expressed as sorbic acid

200 mg/kg expressed as benzoic acid

## 6 Hygiene and handling

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

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

- (i) shall be free from micro-organisms capable of development under normal conditions of storage;
- (ii) shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health; and
- (iii) shall not contain histamine that exceeds 10 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 sections of CAC/RCP 1 and CAC/RCP 52.

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

**Table 1 — Microbiological limits for dried fish**

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

**Table 2 — Contaminant limits for dried fish**

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

## 6.5 Parasites

Fish flesh shall not contain living larvae of nematodes. Viability of nematodes shall be examined according to Annex E. If living nematodes are confirmed, products must not be placed on the market for human consumption before they are treated in conformity with the methods laid down in Annex F.

## 7 Labelling

In addition to the provisions of EAS 38, the following specific provisions apply:

### 7.1 Name of the food

**7.1.1** The name of the product shall be ...-salted herring or ...- salted sprat in accordance with the law and custom of the country in which the product is sold, in a manner not to mislead the consumer.

**7.1.2** In addition the label shall include other descriptive terms that will avoid misleading or confusing the consumer.

## 7.2 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 of the manufacturer or packer 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 container of fish and the entire 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 Section 8.3 through 8.8 and Annexes and in accordance with CAC/GL 31.

### 8.3 Determination of net weight

The net weight (excluding packaging material) of each sample unit in the sample lot shall be determined.

Remove the herring from the container (barrel) and put it on an appropriate sieve. Allow to drain for 5 min and remove adhering salt crystals. Weigh the herring and calculate net weight.

### 8.4 Determination of the viability of nematodes

Annex E.

### 8.5 Determination of salt content

#### 8.5.1 Principle

The salt is extracted by water from the preweighed sample. After the precipitation of the proteins, the chloride concentration is determined by titration of an aliquot of the solution with a standardized silver nitrate solution (Mohr method) and calculated as sodium chloride.

#### 8.5.2 Equipment and chemicals

- Brush
- Sharp knife or saw

- Balance, accurate to 0.01 g
- Calibrated volumetric flasks, 250 ml
- Erlenmeyer flasks
- Electric homogenizer
- Magnetic stirrer
- Folded paper filter, quick running
- Pipettes
- Funnel
- Burette
- Potassium hexacyano ferrate (II),  $K_4Fe(CN)_6 \cdot 3H_2O$ , 15% w/v (aq)
- Zinc sulphate,  $ZnSO_4 \cdot 6H_2O$ , 30% w/v (aq)
- Sodium hydroxide, NaOH, 0.1 N, 0.41% w/v (aq)
- Silver nitrate,  $AgNO_3$ , 0.1 N, 1.6987% w/v (aq), standardized
- Potassium chromate,  $K_2CrO_4$  5% w/v (aq)
- Phenolphthalein, 1% in ethanol
- distilled or deionized water

### 8.5.3 Procedure

- (i) Five gram of homogenized subsample is weighted into a 250 ml volumetric flask and vigorously shaken with approximately 100 ml water.
- (ii) Five millilitre of potassium hexacyano-ferrate solution and 5 ml of zinc sulphate solution are added, the flask is shaken.
- (iii) Water is added to the graduation mark.
- (iv) After shaking again and allowing to stand for precipitation, the flask content is filtered through a folded paper filter.
- (v) An aliquot of the clear filtrate is transferred into an Erlenmeyer flask and two drops of phenolphthalein are added. Sodium hydroxide is added dropwise until the aliquot takes on a faint red colour.

The aliquot then diluted with water to approximately 100 ml.

- (vi) After addition of approximately 1 ml potassium chromate solution, the diluted aliquot is titrated under constant stirring, with silver nitrate solution. Endpoint is indicated by a faint, but distinct, change in colour.

This faint reddish-brown colour should persist after brisk shaking.

To recognize the colour change, it is advisable to carry out the titration against a white background.

- (vii) Blank titration of reagents used should be done.
- (viii) Endpoint determination can also be made by using instruments like potentiometer or colorimeter.

### 8.5.4 Calculation of results

In the equation of the calculation of results the following symbols are used:

A= volume of aliquot (ml)

C= concentration of silver nitrate solution in N

V= volume of silver nitrate solution in ml used to reach endpoint and corrected for blank value

W= sample weight (g)

The salt content in the sample is calculated by using the equation:

$$\text{Salt concentration (\%)} = (V \times C \times 58.45 \times 250 \times 100) / (A \times W \times 1000)$$

Results should be reported with one figure after the decimal point.

### 8.5.5 Reference method

As reference method a method should be used which includes the complete ashing of the sample in a muffle furnace at 550°C before chloride determination according to the method described above (leaving out steps (ii) and (iv)).

### 8.5.6 Comments

By using the given equation all chloride determined is calculated as sodium chloride. However it is impossible to estimate sodium by this methodology, because other chlorides of the alkali and earth alkali elements are present which form the counterparts of chlorides. The presence of natural halogens other than chloride in fish and salt is negligible. A step, in which proteins are precipitated (ii), is essential to avoid misleading results.

### 8.6 Determination of water content

- i) Determination of % salt saturation as required by the standard, should be in accordance to AOAC 950.46.B (Air drying (a)) described in Annex D.
- ii) Determination of water content in the whole fish, when needed in the commercial trade of klippfish and wet salted fish, the method of sampling the fish should be carried out according to the "Determination of Water Content in Whole Fish by Cross Section Method" defined in "Annex B".

### 8.7 Determination of histamine

According to the method described in Annex C.

## 9 Definition of defects

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

### 9.1.1 Foreign matter

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

### 9.1.2 Parasites

The presence of readily visible parasites in a sample of the edible portion of the sample unit detected by normal visual inspection of the fish flesh (see Annex III).

### 9.1.3 Odour and flavour/taste

Fish affected by persistent and distinct objectionable odours or flavours indicative of decomposition (such as sour, putrid, fishy, rancid, burning sensation, etc.) or contamination by foreign substances (such as fuel oil, cleaning compounds, etc.).

## 10 Lot acceptance

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

- (i) the total number of defectives as classified according to Section 9 does not exceed the acceptance number (c) of the appropriate sampling plan in Section 8; and
- (ii) the average net weight of all sample units is not less than the declared weight, provided no individual container is less than 95% of the declared weight; and
- iii) the Food Additives, Hygiene and Handling and Labelling requirements of Sections 5, 6 and 7 are met.

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**Annex A**  
(normative)

**Sensory and physical examination**

1. Examine every fish in the sample in its entirety.
2. Examine the product for the form of presentation.
3. Examine the fish for foreign matter, pink conditions, halophilic mould, liver stains, intense bruising, severe burning and texture.
4. Assess odour in accordance with CAC/GL 31.

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## Annex B (normative)

### Determination of water content in whole fish by cross-section method

#### B.1 Principle

The fish is cut in sections as described in method. The sections are cut in smaller bits to a collected sample. The water content of the collected sample is determined by drying. Examinations and experience have shown that the water content of this collected sample is closed to the "true" water content of the fish.

#### B.2 Equipment

- Soft brush
- Basins (steel, glass, porcelain)
- Scissors
- Band saw
- Knife
- Weight, 1 g precision
- Oven, 103-105°C
- Desiccator

#### B.3 Preparation of sample

Salt particles on the surface of the fish are brushed away.

The weight of the fish is determined to 1 g accuracy.

The length of the fish is measured as the distance between the cleft in the tail and a line drawn between the tips of the earbones.

#### B.4 Procedure

- (i) The sampling of the fish is described in the enclosed figure.
  - A) Wet salted fish is sliced in sections by knife
  - B) Salted and dried salted fish is sliced in sections by band saw.
    - 1) A section of 20mm measured from a line drawn between the earbones, dotted line on figure, is cut.
    - 2) The next cut is a 40 mm section.
    - 3) A 2 mm section is cut from the front part of the 40 mm section and collected (see 7. Comments).
    - 4) The next cut is a new cut of a 40 mm section.
    - 5) A 2 mm section is cut from the front part of the 40 mm section and collected.
    - 6) The entire fish is cut in 40 mm sections from which are cut 2 mm sections (see enclosed figure).

- 7) All sections of 2mm, marked II, IV, VI, VIII in the figure, even numbers, are collected to a collected sample.
- (ii) The 2mm sections in the collected sample are cut with scissors in smaller pieces directly in tared basins just after the fish is cut.
  - (iii) The basins containing the sample are weighted.
  - (iv) The basins containing the samples are put in the oven at 103-105°C for drying to constant weight (18 hours over night).
  - (v) The basins are taken from the oven to a desiccator and cooled.
  - (vi) The basins are weighted.

#### **B.5 Calculation of results**

In the equation of the calculation of results the following symbols are used:

W1 = Weight of fish and basins before drying, g.

W2 = Weight of fish and basins after drying, g.

Ws = Weight of tared basins, g

The water content in the fish is calculated by using the equation:

Water content, g/100g =  $100 \times (W1 - W2) / (W1 - Ws)$

(W1 – Ws)

The result is reported to the nearest gram, together with the length and the weight of the analysed fish.

#### **B.6 Control analysis of whole fish.**

The determination of water content in whole fish by cross section method appears to give the closest result compared to water content determined by the drying of the whole fish (ALINORM 03/18, Appendix IX)

#### **B.7 Comments**

Each sampled fish should be packed and sealed in a plastic bag before analysis. The samples should be stored under chilled or refrigerated conditions from the time of sampling to the time of analysis. The analysis must be performed as soon as possible after the fish has been sampled.

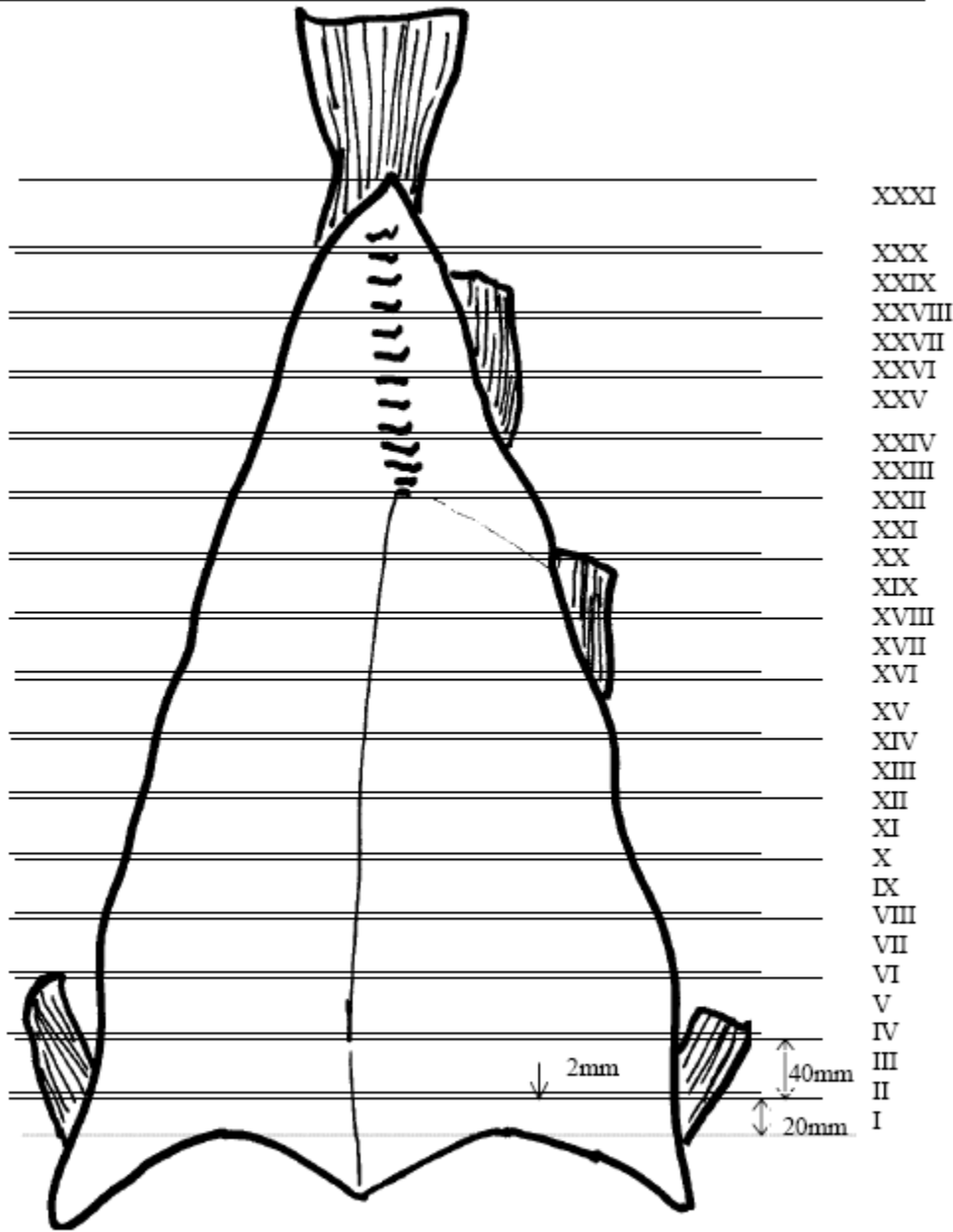
It might be difficult to cut sections of 2 mm when the fish has a water content above 50% but the section must be close to 2 mm.

To minimise the loss of water from the 2mm sections it is important to weight the collected sample immediately after the fish is cut in sections.

Determination should be performed at least in duplicate.

FIGURE

Sampling procedure.



All section labelled by even numbers , II, IV,VI,VIII etc. are collected to constitute one sample.

Standard

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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 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.

#### 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 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, C.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.

#### 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 3.57NH<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.

#### C.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 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<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.

**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  $\leq 100$  mm Hg (ca 5 h). For feeds with high molasses content, use temperature  $\leq 70^\circ\text{C}$  and pressure  $\leq 50$  mm Hg. Use covered Al dish  $\geq 50$  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  $\geq 50$  mm diameter and  $\leq 40$  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  $\geq 50$  mm diameter and  $\leq 40$  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**

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

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

## Annex E (normative)

### Viability test for nematodes

(modified method according to Reference 1)

#### Principle:

Nematodes are isolated from fish fillets by digestion, transferred into 0.5 % Pepsin digestion solution and inspected visually for viability. Digestion conditions correspond to conditions found in the digestive tracts of mammals and guarantee the survival of nematodes.

#### Equipment:

- Stacked sieves (diameter: 14 cm or larger, mesh size: 0.5 mm)
- Magnetic stirrer with thermostated heating plate
- normal laboratory equipment

#### Chemicals:

- Pepsin 2000 FIP-U / g
- Hydrochloric acid

#### Solution:

A: 0.5 % (w/v) Pepsin in 0.063 M HCl

#### Procedure:

Fillets of approximately 200 g are manually shredded and placed in a 2 l beaker containing 1 l Pepsin solution A. The mixture is heated on a magnet stirrer to 37 °C for 1- 2 h under continuous slow stirring. If the flesh is not dissolved, the solution is poured through a sieve, washed with water and the remaining flesh is quantitatively replaced in the beaker. 700 ml digestion solution A is added and the mixture stirred again under gentle heating (max. 37°C) until there are no large pieces of flesh left.

The digestion solution is decanted through a sieve and the content of the sieve rinsed with water.

Nematodes are carefully transferred by means of small forceps into Petri dishes containing fresh Pepsin solution

A. The dishes are placed on a candling dish, and care has to be taken not to exceed 37 °C.

Viable nematodes show visible movements or spontaneous reactions when gently probed with dissecting needles. A single relaxation of coiled nematodes, which sometimes occurs, is not a clear sign of viability. Nematodes must show spontaneous movement.

#### Attention:

When checking for viable nematodes in salted or sugar salted products, reanimation time of nematodes can last up to two hours and more.

#### Remarks:

Several other methods exist for the determination of viability of nematodes (e.g. ref. 2, 3).

The described method has been chosen because it is easy to perform and combines isolation of nematodes and viability test within one step.

**Annex F**  
(normative)

**Viability test for nematodes**

Treatment procedures sufficient to kill living nematodes

- e.g. freezing to - 20° C for not less than 24 h in all parts of the product
- the adequate combination of salt content and storage time (To be elaborated)
- or by other processes with the equivalent effect (To be elaborated)

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**Annex G**  
(normative)

**Determination of the presence of visible parasites**

1. The presence of readily visible parasites in a sample unit that is broken into normal bite-size pieces 20-30 mm of flesh by the thickness of the fillet. Only the normal edible portion is considered even if other material is included with the fillet. Examination should be done in an adequately lighted room (where a newspaper may be read easily), without magnification, for evidence of parasites.
2. Notwithstanding paragraph 1, the verification of the presence of parasites in intermediate entire fishery products in bulk intended for further processing could be carried out at a later stage.

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