



CD/K/542:2010  
ICS 67.120.30

## **EAST AFRICAN STANDARD**

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**Live and raw bivalve molluscs — Specification**

**EAST AFRICAN COMMUNITY**

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

## 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 292:2008, *Standard for Live and Raw Bivalve Molluscs*

AOAC Official Method 963.18:1963, *Net Contents of Frozen Seafoods — Drained Weight Procedure*

AOAC Official Method 963.26:1963, *Net Contents of Frozen Food Containers*

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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## Live and raw bivalve molluscs — Specification

### 1 Scope

This standard applies to live bivalve molluscs and to raw bivalve molluscs that have been shucked and/or frozen, and/or processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs. Raw bivalve molluscs are marketed either in a frozen or chilled state. Both live and raw bivalve molluscs may be intended for direct consumption or further processing.

The standard does not apply to scallops when the final product is the adductor muscle only. Part I below applies to live bivalve molluscs while Part II applies to raw bivalve molluscs.

### 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*

## **Part 1: Live bivalve molluscs**

### **3 Live bivalve molluscs**

#### **3.1 Product definition**

Live bivalve molluscs are products that are alive immediately prior to consumption. Presentation includes the shell.

### 3.2 Process definition

Live bivalve molluscs are harvested alive from a harvesting area either approved for direct human consumption or classified to permit harvesting for an approved method of purification, e.g. relaying or depuration, prior to human consumption. Both relaying and depuration must be subject to appropriate controls implemented by the official agency having jurisdiction.

### 3.3 Presentation

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

- meets all requirements of this standard; and
- is adequately described on the label to avoid confusing or misleading the consumer.

The bivalve molluscs may be packed by weight, count, count per unit of weight, volume or per package.

### 3.4 Essential composition and quality factors

#### 3.4.1 Bivalve Molluscs

Live bivalve molluscs should possess organoleptic characteristics associated with freshness, as well as an adequate response to percussion (i.e. the shellfish will close by themselves when tapped) and freedom from extraneous matter, as determined by specialists familiar with the species concerned.

#### 3.4.2 Final Product

Live bivalve molluscs shall meet the requirements of this standard when lots examined in accordance with Section 3.11 comply with the provisions set out in Section 3.10. Live bivalve molluscs shall be examined by the methods given in Section 3.9.

### 3.5 Food additives

Food additives are not permitted in live bivalve molluscs.

### 3.6 Contaminants

**3.6.1** The products covered by this Standard shall comply with the Maximum Levels of the Codex General Standard for Contamination and Toxins in Foods (CODEX/STAN 193) and the maximum residue limits for pesticides and/or veterinary drugs established by the CAC.

**3.6.2** The following provisions apply to the edible parts of live bivalve mollusc (the whole part or any part intended to be eaten separately)

Name of biotoxin groups	Maximum level /kg of mollusc flesh
Saxitoxin (STX) group	≤0.8 milligrams (2HCL) of saxitoxin equivalent
Okadaic acid (OA) group	≤0.16 milligrams of okadaic equivalent
Domoic acid (DA) group	≤20 milligrams domoic acid
Brevetoxin (BTX) group	≤200 mouse units or equivalent
Azaspiracid (AZP) group	≤0.16 milligrams

### 3.7 Hygiene and handling

**3.7.1** It is recommended that the products covered by provisions of this standard be prepared and handled in accordance with the appropriate sections of CAC/RCP 1, CAC/RCP 52 and other relevant Codex texts such as Codes of Hygienic Practice and Codes of Practice.

**3.7.2** The products should comply with any microbiological criteria established in accordance with CAC/GL 21.

**3.7.3** Growing area monitoring programs, irrespective of the type of indicator bacteria used, must ensure that live bivalve molluscs destined for direct human consumption meet the *E.coli* limit as identified below when tested in accordance with an MPN method specified in ISO 16649-3 or equivalent.

**3.7.4** In analysis involving five (5) 100g samples of the edible parts (the whole part or any part intended to be eaten separately), none may contain more than 700 *E. coli* and not more than one (1) of five (5) samples may contain between 230 and 700 *E.coli*, or equivalent as decided by the competent authority having jurisdiction

Microorganism = *Escherichia coli*      n=5      c=1      m=230      M=700      3 Class Plan

where 'n' = the number of sample units, 'c' = the number of sample units that may exceed the limit 'm', and 'M' is the limit which no sample unit may exceed.

**3.7.5** In analysis involving five (5) 25g samples of the edible parts (the whole part or any part intended to be eaten separately), no sample may indicate the presence of *Salmonella* when tested using a method validated against the reference method ISO 6579.

Microorganism = *Salmonella*      n=5      c=0      m=0/25g      2 Class Plan

where n = number of samples that must conform to the criteria; c = the maximum allowable number of defective sample units; m = a microbiological limit which separates good quality from defective quality.

**3.7.6** Where the microbiological criteria are not met, actions should be taken as deemed appropriate by the competent authority. In following up, consideration should be given to detention, recall and further processing in a manner to eliminate the hazard from implicated lots. In addition, assessment of the status of harvesting areas and/or establishment controls should be undertaken.

## 3.8 Labelling

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

### 3.8.1 The Name of the Food

The name of the food to be declared on the label shall be the common or usual name of the species of bivalve molluscs in accordance with the law and custom of the country in which the food is sold and in a manner not to mislead the consumer.

**3.8.1.1** There shall appear on the label, reference to the presentation provided for in 3.3 (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.

**3.8.1.2** 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.

### 3.8.2 Content declaration

Live bivalve molluscs shall be labelled by weight, count, count per unit weight, or volume as appropriate to the product.

### 3.8.3 Storage Instructions

The label shall specify the conditions for storage and/or temperature that will maintain the product safety/viability during transportation, storage and distribution.

### 3.8.4 Labelling of non-retail containers

Labelling for live bivalve molluscs shall contain the following information:

- (i) Identification of the product by common and/or scientific names as determined by the competent authority. The country where the product is sold can determine if the scientific name must be indicated on the label.
- (ii) Information that might be needed in the event of a food safety problem, including lot identification which could be lot code or date and location of harvest, information about harvest area, date of harvesting, purification or relaying as appropriate, as well as identification of the dispatch centre or other establishment from which they were shipped.
- (iii) Durability or shelf life.

Date of minimum durability may be replaced by the statement "Bivalves must be alive when sold".

## 3.9 Sampling, examination and analyzes

### 3.9.1 Sampling

- (i) Each sample shall contain a sufficient number of bivalve molluscs to ensure that the sample is representative.
- (ii) The portion of the bivalve mollusc analysed should be the edible part. This is generally the whole tissue. Where whole-tissue analysis is not possible or practical, the most contaminated tissue (e.g. the digestive gland) may be dissected and analysed and the results converted to an edible tissue basis. The conversion factor should be supported by adequate data.

### 3.9.2 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 3.8.3 through 3.8.5, and CAC/GL 31.

### 3.9.3 Determination of Count per Unit Weight or Volume

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

### 3.9.4 Method of Analysis of *Escherichia coli* in bivalve molluscs

The ISO/TS 16649-3, *Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli – Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide* or other validated methods in accordance with the protocol set out in the ISO 16140 or other internationally accepted similar protocol.

### 3.9.5 Method of Analysis of *Salmonella* in bivalve molluscs

The methods to be employed for *Salmonella* should be ISO 6579, or other validated methods that provide equivalent sensitivity, reproducibility and reliability.

**3.9.6 Determination of Biotoxins**

<b>Provision</b>	<b>Methodology</b>	<b>Principle</b>	<b>Type</b>
Saxitoxin group	AOAC Official Method 2005.06 (Paralytic Shellfish Poisoning Toxins in Shellfish) four matrices and 12 toxins	LC-FL	II

**3.10 Definition of defectives**

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

**3.10.1 Foreign Matter**

The presence in the sample unit of any matter which has not been derived from bivalve molluscs, 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.

**3.10.2 Dead or damaged product**

The presence of dead or damaged product. Dead product is characterised by no response to percussion (i.e. shellfish will close by themselves when tapped). Damaged product includes product that is damaged to the extent that it can no longer function biologically. A Sample unit shall be considered defective if dead or damaged bivalve molluscs exceed 5% by count.

**3.11 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 3.10 does not exceed the acceptance number (c) of the appropriate sampling plan in CD/K/572:2010;
- (ii) the total number of sample units not meeting the count designation as defined in 3.9.3 does not exceed the acceptance number (c) of the appropriate sampling plan in CD/K/572:2010;
- (iii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any individual container;
- (iv) the Food Additives, Contaminants, Hygiene and Labelling requirements of Sections 3.5, 3.6, 3.7 and 3.8 are met.

## Part 2: Raw bivalve molluscs

### 4 Raw bivalve molluscs

#### 4.1 Product definition

Raw bivalve molluscs processed for direct consumption or for further processing are products that were alive immediately prior to the commencement of processing and comply with Section 3.2 relating to harvesting, purification and relaying. They have been shucked and/or frozen and/or processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs. Raw bivalve molluscs are marketed in a frozen or chilled state.

#### 4.2 Process definition

Raw bivalve molluscs must meet the process definition in 3.2 before they can be processed for direct consumption or further processing.

Bivalve molluscs that have been processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs are ones that have been processed to assure reduction or limitation of the target organisms to the satisfaction of the official agency having jurisdiction.

#### 4.3 Presentation

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

- meets all requirements of this standard; and
- is adequately described on the label to avoid confusing or misleading the consumer.

The bivalve molluscs may be packed by weight, count, count per unit of weight, volume or per package.

#### 4.4 Essential composition and quality factors

##### 4.4.1 Raw Bivalve Molluscs

Raw bivalve molluscs shall be of a quality fit for human consumption.

##### 4.4.2 Ingredients

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

##### 4.4.3 Final Product

Raw bivalve molluscs shall meet the requirements of this standard when lots examined in accordance with Section 4.10 comply with the provisions set out in Section 4.9. Raw bivalve molluscs shall be examined by the methods given in Section 4.8.

#### 4.5 Food additives

Only the use of the following additives is permitted in raw bivalve molluscs.

##### Antioxidants

For chilled shucked molluscs any antioxidant listed in food category 09.1.2 (Fresh Molluscs, crustaceans and echinoderms) of the General Standard for Food Additives (CODEX STAN 192).

For raw frozen molluscs any antioxidant listed in food category 09.2.1 (Frozen fish, fish fillets, and fish products, including molluscs, crustaceans, and echinoderms) of the General Standard for Food Additives (CODEX STAN 192).

### 4.6 Contaminants

Raw bivalve molluscs should meet the requirements of 3.6.

### 4.7 Hygiene and handling

Raw bivalve molluscs should meet the requirements of 3.7.

### 4.8 Labelling

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

#### 4.8.1 The Name of the Food

The name of the food to be declared on the label shall be the common or usual name of the species of bivalve molluscs in accordance with the law and custom of the country in which the food is sold and in a manner not to mislead the consumer.

**4.8.1.1** There shall appear on the label, reference to the presentation provided for in Section II-2.3- 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.

**4.8.1.2** 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.

#### 4.8.2 Content Declaration

Raw bivalve molluscs shall be labelled by weight, count, count per unit weight, or volume as appropriate to the product.

#### 4.8.3 Storage Instructions

The label shall specify the conditions for storage and/or temperature that will maintain the food safety and characteristics of the product during transportation, storage and distribution including date of minimum durability and for date of shucking.

#### 4.8.4 Labelling of Non-retail Containers

Refer to I-6.4 Labelling of Non-retail Containers.

**4.8.4.1** Every package containing bivalve molluscs that have been processed to reduce or limit target organisms must be provided with a label certifying that all molluscs have been processed to reduce the target organism to levels acceptable to the official agency having jurisdiction.

**4.8.4.2** Safety claims for bivalve molluscs processed to reduce or limit target organisms should be specific to the target organisms that have been reduced or limited as described in the Code of Practice.

### 4.9 Sampling, examination and analyses

#### 4.9.1 Sampling

Sampling of lots for examination of net weight shall be carried out in accordance with an appropriate sampling plan meeting the criteria established by the CAC.

#### 4.9.2 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 4.8.3 through 4.8.7 and CAC/GL 31.

#### 4.9.3 Determination of Net Weight and Drained Weight

The net weight and drained weight of all sample units shall be determined by the procedures described or mentioned in sections 4.8.3.1 through 4.8.3.5.

##### 4.9.3.1 Determination of Net Weight

- (i) Weigh the unopened container;
- (ii) Open the container and remove the contents;
- (iii) Weigh the empty container, (including the end) after removing excess liquid and adhering meat;
- (iv) Subtract the weight of the empty container from the weight of the unopened container.
- (v) The resultant figure will be the total net content.

##### 4.9.3.2 Determination of Net Weight of Frozen 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.

##### 4.9.3.3 Determination of Net Weight of Products Covered by Glaze

AOAC official method 963.18, Net Contents of Frozen Seafoods

4.9.3.4 The AOAC official method 963.26 should be used to determine the net weight of products with water added that is inside a "block-frozen" product.

##### 4.9.3.5 Determination of Drained Weight

In the case of shucked bivalve molluscs, the drained weight shall be determined according to AOAC official method 953.11.

#### 4.9.4 Determination of Count per Unit Weight or Volume

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

#### 4.9.5 Sample Preparation

##### 4.9.5.1 Procedures for Thawing

For frozen product, 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 bivalve molluscs, until no hard core or ice crystals are left.

#### 4.9.6 Methods of Analysis of *Escherichia coli*

Refer to 3.9.4 Methods of Analysis of *Escherichia coli*

**4.9.7 Method of Analysis of *Salmonella***

Refer to 3.9.5 Method of Analysis of *Salmonella*

**4.9.8 Determination of Biotoxins**

Refer to 3.9.6 Determination of Biotoxins

**4.10 Definition of defectives**

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

**4.10.1 Deep Dehydration (Frozen Products)**

Greater than 10% of the weight of the bivalve molluscs 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 abnormal colour 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 bivalve molluscs.

**4.10.2 Foreign Matter**

The presence in the sample unit of any matter which has not been derived from bivalve molluscs, 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.

**4.10.3 Odour/Flavour**

Persistent and distinct objectionable odours or flavours indicative of decomposition or rancidity.

**4.10.4 Texture**

Textural breakdown of the flesh, indicative of decomposition, characterized by muscle structure that is mushy or paste-like.

**4.11 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 4.9 does not exceed the acceptance number (c) of the appropriate sampling plan in the General Guidelines on Sampling (CAC/GL 50-2004);
- (ii) the total number of sample units not meeting the count designation as defined in section 4.3 does not exceed the acceptance number (c) of the appropriate sampling plan in the General Guidelines on Sampling (CAC/GL 50);
- (iii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any individual container;
- (iv) the Food Additives, Contaminants, Hygiene and Labelling requirements of Sections 4.5, 4.6, 4.7 and 4.8 are met.

**Annex A**  
(normative)

**Net Contents of Frozen Seafoods — Drained Weight Procedure**

**A.1 Apparatus**

- (a) For packages up to 5 lbs. (2268 g).—Use scale of adequate capacity with sensitivity of 0.01 oz (0.284 g).
- (b) For packages over 5 lbs.—Use scale of adequate capacity with sensitivity of 0.025 oz (0.71 g).

**A.2 Procedures**

Set scale on firm support and level. Adjust 0 load indicator or rest point and check sensitivity.

- (a) **Glazed seafoods** — Remove package from low temperature storage, open immediately, and place contents under gentle spray of cold H<sub>2</sub>O. Agitate carefully so product is not broken. Spray until all ice glaze that can be seen or felt is removed. Transfer product to circular No. 8 sieve, 8 in. (20 cm) diameter for 0.9 kg (2 lb) and 12 in. (30 cm) for >0.9 kg (2 lb). Without shifting product, incline sieve at angle of 17–20°C to facilitate drainage and drain exactly 2 min (stop watch). Immediately transfer product to tared pan (B) and weigh (A). Weight product = A - B.
- (b) **Unglazed frozen foods** — Remove package from low temperature storage, remove frost and ice from outside of package, and weigh immediately (W). Open package; remove contents, including any product particles and frost crystals. Air-dry empty package at room temperature and weigh (E). Weight contents = W - E.

**Annex B**  
(normative)

**Method for the determination of net content of frozen fish blocks covered by glaze**

Glazing is not used for Q.F. blocks of white fish. Only Q.F. blocks of herring, mackerel and other brown (fat) fish are glazed, which are destined for further processing (canning, smoking). For such blocks the following procedure may be applicable (tested with block frozen shrimps).

**B.1 Principle**

The pre-weighed glazed sample is immersed into a water bath by hand till all glaze is removed (as felt by fingers). As soon as the surface becomes rough, the still frozen sample is removed from the water bath and dried by use of a paper towel before estimating the net product content by repeated weighing. By this procedure thaw drip losses and/or re-freezing of adhering moisture can be avoided.

**B.2 Equipment**

- Balance - sensitive to 1 g
- Water bath, preferably with adjustable temperature
- Circular sieve with a diameter of 20 cm and 1-3 mm mesh apertures (ISO R 565)
- Paper or cloth towels with smooth surface
- A freezed box should be available at the working place

**B.3 Preparation of samples and water bath**

- The product temperature should be adjusted to -18/-20°C to achieve standard deglazing conditions (especially necessary if a standard deglazing period shall be defined in case of regular shaped products).
- After sampling from the low temperature store remove, if present, external ice crystals or snow from the package with the frozen product.
- The water bath shall contain an amount of fresh potable water equal to about 10 times of the declared weight of the product; the temperature should be adjusted on about 15°C to 35°C.

**B.4 Determination of gross weight "A"**

After removal of the package, the weight of the glazed product is determined: In case of single fish fillets, single weights are recorded (A 1-A n). The weighed samples are placed intermediately into the freezer box.

**B.5 Removal of glaze**

The pre-weighed samples/sub-samples are transferred into the water bath and kept immersed by hand. The product may be carefully agitated, till no more glaze can be felt by the finger-tips on the surface of the product: change from slippery to rough. Needed time, depending on size/shape and glaze content of the product, 10 to 60 sec. (and more in case of higher glaze contents or if frozen together).

For block-frozen products in consumer packs (also for single glaze products, which are frozen together during storage) the following (preliminary) procedure may be applicable: The pre-weighed

block or portion is transferred onto a suitable sized sieve and immersed into the water bath. By slight pressure of the fingers separating deglazed portions are removed fractionally. Short immersing is repeated, if glaze residues are still present.

#### B.6 Determination of net weight "B"

The deglazed sample/sub-sample, after removal of adhering water by use of a towel (without pressure) is immediately weighed. Single net-weights of sub-samples are summed up: B1-n.

#### B.6 Determination of glaze weight "C"

Grossweight "A" - Net weight "B" = Glazeweight "C"

#### B.7 Calculation of percentage proportions

% net content of the products "F" =  $\frac{"B"}{"A"} \times 100$

% glaze - related to the gross weight of the product "G" =  $\frac{"C"}{"A"} \times 100$

% glaze - related to the net weight of the product "H" =  $\frac{"C"}{"B"} \times 100$

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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**

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

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

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