EAST AFRICAN STANDARD

Fresh, frozen and canned sardines and sardine-type products — 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

This standard for canned sardines derives defines minimum acceptability of canned sardines for taint, decomposition, unwholesomeness and other requirements, other than weight, and describes methods for determining that acceptability.

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

KS CODEX STAN 94:1981, Specification for canned sardines and sardine-type products

IS 14890:2001, Sardines — Fresh, frozen and canned — Specification

Codex Alimentarius website: http://www.codexalimentarius.net/mrsls/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


Assistance derived from these sources is hereby acknowledged.
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Fresh, frozen and canned sardines and sardine-type products — Specification

1 Scope

This standard applies to fresh, frozen and canned sardines and sardine-type products packed in water or oil or other suitable packing medium. It does not apply to specialty products where fish content constitute less than 50% m/m of the net contents of the can.

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/RCP 52[CD/K/521:2010], Code of practice 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 12, Drinking (potable water) — Specification
EAS 35, Edible salt — 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.
CD/K/519:2010

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 6887-2, Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products

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 16050, Foodstuffs — Determination of aflatoxin B\textsubscript{1}, and the total content of aflatoxin B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1} and G\textsubscript{2} in cereals, nuts and derived products — High performance liquid chromatographic method

ISO 16654, Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Escherichia coli O157

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

3 Description

3.1 Product definition

Canned sardines or sardine type products are prepared from fresh or frozen fish of the following species:

- Sardina pilchardus (Walbaum)
- Sardinops melanostictus, S. neopilchardus, S. ocellatus, S. sagax, S. caeruleus, S. albella
- Sardinella aurita, S. brasiliensis, S. maderensis, S. longiceps, S. gibbosa, S. fimbriata
3.1.2 Head and gills shall be completely removed; scales and/or tail may be removed. The fish may be eviscerated. If eviscerated, it shall be practically free from visceral parts other than roe, milt or kidney. If ungutted, it shall be practically free from undigested feed or used feed.

3.2 Process definition

The products are packed in hermetically sealed containers and shall have received a processing treatment sufficient to ensure commercial sterility.

3.3 Presentation

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

(i) contains at least two fish in each can; and
(ii) meets all requirements of this standard; and
(iii) is adequately described on the label to avoid confusing or misleading the consumer;
(iv) contain only one fish species.

3.4 Grades

Fresh sardines and frozen sardines shall be of the following three grades:

<table>
<thead>
<tr>
<th>Grade designation</th>
<th>Count (No. per kg) with head on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>Below 15</td>
</tr>
<tr>
<td>Medium</td>
<td>15 to 25</td>
</tr>
<tr>
<td>Small</td>
<td>26 and more</td>
</tr>
</tbody>
</table>

3.5 Packing media

The product shall be presented in one of the following packing media with or without permitted optional ingredients:

a) Own juice — Fish packaged without added liquid.
b) **Potable water** — In conformity with the requirements of the Fish Inspection Regulations for water used in registered establishments.

c) **Spring water or mineral water** — Potable water from an underground source but not obtained from a public community water supply and which meets the requirements of EAS 13.

d) **Vegetable broth** — The liquid arising from the cooking of sound wholesome vegetables in water and which may be prepared from one or more types of vegetables. Vegetable broth may also be prepared from hydrolysed vegetable protein, but a broth so prepared requires that it’s components be declared in a list of ingredients.

e) **Olive oils** — In conformity with CODEX STAN 33, *Standard for olive oils and olive pomace oils*

f) **Other vegetable oils** — Clear, refined, deodorized, edible vegetable oil in conformity with the relevant East African Standards.

g) **Sauces** — A thickened liquid made from acceptable food ingredients giving a characterizing flavour and odour to the product.

h) **Marinades** — A thin liquid made from acceptable food ingredients, usually containing a sweetener, an acid solution or an alcoholic solution, with or without spices, herbs, seasonings, vegetables and other condiments.

3.6 **Optional ingredients**

a) Salt.

b) Natural starches.

c) Other optional ingredients provided that all ingredients are suitable for human consumption, are free from abnormal taste, flavour or odour and are permitted in EAS 103. Examples of such ingredients are spices, herbs, vegetable seasonings, vinegar and wine and vegetables and fruits for decorative and flavouring purposes only.

4 **Essential composition and quality factors**

4.1 **Requirements for fresh sardines**

4.1.1 The fish, its skin and flesh shall have characteristic colour, free from any discoloration. The gills should be bright red in colour and free from discoloured mucous. The eyes shall be bright red in colour. The meat and stomach portion should be firm and shall not leave a mark when lightly pressed with finger.

4.1.2 The material shall also conform with the requirements given in Table 1.

4.2 **Requirements for frozen sardines**

4.2.1 Fresh sardines used for freezing shall conform to the requirements given in 5.

4.2.2 The material shall be clean, wholesome and free from defects.

4.2.3 The fish may either be whole or gutted. The entrails shall be removed. Eviscerated fish shall be washed thoroughly with clean water to remove blood.

4.2.4 The frozen sardine, on thawing, shall be in sound, intact and undamaged conditions and free from defects.

4.2.5 The product shall be free from any foreign matter.

4.2.6 The material shall conform to the requirements prescribed in Table 1.
Table 1 — Requirements for sardines, fresh and frozen

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Requirement</th>
<th>Method of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Total bacterial count/g, Max</td>
<td>100 000</td>
<td>ISO 4833</td>
</tr>
<tr>
<td>i) <em>Escherichia coli</em> count/g, Max</td>
<td>20</td>
<td>ISO 7251</td>
</tr>
<tr>
<td>ii) Faecal <em>Streptococci</em> count/g, Max</td>
<td>100</td>
<td>Annex C</td>
</tr>
<tr>
<td>iv) Coagulase positive <em>Staphylococci</em> count/g, Max</td>
<td>100</td>
<td>ISO 6888</td>
</tr>
<tr>
<td>V) <em>Salmonella</em>, per 25 g</td>
<td>Absent</td>
<td>ISO 6579</td>
</tr>
<tr>
<td>vi) <em>Shigella</em>, per 25 g</td>
<td>Absent</td>
<td>ISO 21567</td>
</tr>
<tr>
<td>vii) <em>Vibrio cholerae</em>, per 25 g</td>
<td>Absent</td>
<td>ISO/TS 21872</td>
</tr>
<tr>
<td>viii) <em>Listeria monocytogenes</em>, per 25 g</td>
<td>Absent</td>
<td>ISO 11290</td>
</tr>
<tr>
<td>ix) Formaldehyde mg/kg, Max</td>
<td>10.0</td>
<td>Annex D</td>
</tr>
<tr>
<td>x) Indole, mg/kg, Max</td>
<td>2.5</td>
<td>Annex E</td>
</tr>
<tr>
<td>xi) Heavy metals:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Mercury, mg/kg, Max</td>
<td>0.5</td>
<td>EAS 41</td>
</tr>
<tr>
<td>b) Copper, mg/kg, Max</td>
<td>20.0</td>
<td>EAS 41</td>
</tr>
<tr>
<td>c) Zinc, mg/kg, Max</td>
<td>50.0</td>
<td>EAS 41</td>
</tr>
<tr>
<td>f) Arsenic, mg/kg, Max</td>
<td>0.1</td>
<td>EAS 41</td>
</tr>
<tr>
<td>e) Lead, mg/kg, Max</td>
<td>0.3</td>
<td>EAS 41</td>
</tr>
<tr>
<td>f) Tin, mg/kg, Max</td>
<td>50.0</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(i) For product packed in tin plate</td>
<td>50.0</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(ii) For product packed in other packing containers</td>
<td>250.0</td>
<td>EAS 41</td>
</tr>
<tr>
<td>g) Cadmium</td>
<td>0.3</td>
<td>EAS 41</td>
</tr>
<tr>
<td>h) Methylmercury</td>
<td>0.5</td>
<td>EAS 41</td>
</tr>
</tbody>
</table>

4.2.7 No sample of fresh and frozen sardines shall contain histamine content exceeding 20 mg/kg when tested by the method given in Annex C.

4.3 Requirements for canned sardines

4.3.1 Raw material

The products shall be prepared from sound fresh or frozen fish of the species listed in 3.1 which are of a quality fit to be sold fresh for human consumption and shall conform to the requirements given in 4.1 or 4.2 respectively.

Heads and gills shall be completely removed; scales and/or tail may be removed. The fish may be eviscerated. If eviscerated, it shall be practically free from visceral parts other than roe, milt or kidney. If ungutted, it shall be practically free from undigested feed or used feed.

4.3.2 Other ingredients

The packing medium and all other ingredients used shall be of food grade quality and conform to all applicable Codex standards. One or more of the following ingredients which are of food grade quality shall be used depending on type of presentation: edible oil, common salt, tomato ketchup, tomato puree, spices and condiments.

4.3.3 The can shall not show any visible external defects like denting, panelling, swelling or rusting.

The contents of the can on opening shall not display any appreciable disintegration. Pieces from which portions have separated out would be treated as disintegrated units. The percentage of detached portion of fish calculated on the basis of the drained mass shall not exceed 5 percent by mass based on the average of 5 cans.
4.3.4 Decomposition

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

4.3.4 The product shall have the odour, flavour and colour characteristic of the species.

4.3.5 The product shall be free from foreign materials, filth and from grittiness.

4.3.6 The can shall give a negative pressure when punctured. If round cans are used, the vacuum shall be not less than 100 mm of Hg, when measured at 27 ± 2 °C with a vacuum gauge of the piercing type or as electric vacuum recorder.

4.3.7 The drained mass of the contents in each can shall be not less than 65 % of the net water capacity of the can as tested by the method given in 8.4. A tolerance of ±5 % is permitted provided average content of fish on the basis of 5 cans lot shall not be less than 60 % of the net mass.

4.3.8 The percentage of sodium chloride in the final product shall be 3.5 percent in the case of brine treated cans when tested by the method given in Annex D.

The acidity of brine as citric acid anhydrous shall be between 0.06 to 0.20 percent (m/v) when tested by the method given in Annex E.

4.3.9 The canned sardines shall also conform to the requirements prescribed in Table 2.

### Table 2 Requirements for Sardines, Canned

<table>
<thead>
<tr>
<th>Type of contaminant</th>
<th>Maximum limit (mg/kg)</th>
<th>Method of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Microbiological requirements</td>
<td>Shall be commercially sterile</td>
<td>See 8.6</td>
</tr>
<tr>
<td>(i) Arsenic</td>
<td>0.1</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(ii) Copper</td>
<td>0.4</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(iii) Iron</td>
<td>5.0</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(iv) Tin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) For product packed in tin plate</td>
<td>50.00</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(b) For product packed in other packing containers</td>
<td>250.00</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(v) Mercury</td>
<td>0.5</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(vi) Lead</td>
<td>0.3</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(vii) Cadmium</td>
<td>0.3</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(viii) Methylmercury</td>
<td>0.5</td>
<td>EAS 41</td>
</tr>
<tr>
<td>(ix) Zinc</td>
<td>50.0</td>
<td>EAS 41</td>
</tr>
</tbody>
</table>

4.3.10 Final product

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

5 Food additives for canned sardines

Only the use of the following additives is permitted.
### Additive

<table>
<thead>
<tr>
<th>Additive</th>
<th>Maximum limit in the final product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thickening or Gelling Agents (for use in packing media only)</strong></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>Alginic acid</td>
</tr>
<tr>
<td>401</td>
<td>Sodium alginate</td>
</tr>
<tr>
<td>402</td>
<td>Potassium alginate</td>
</tr>
<tr>
<td>404</td>
<td>Calcium alginate</td>
</tr>
<tr>
<td>406</td>
<td>Agar</td>
</tr>
<tr>
<td>407</td>
<td>Carrageenan and its Na, K, and NH₄ salts (including furcellaran)</td>
</tr>
<tr>
<td>407a</td>
<td>Processed <em>Euchema</em> Seaweed (PES)</td>
</tr>
<tr>
<td>410</td>
<td>Carob bean gum</td>
</tr>
<tr>
<td>412</td>
<td>Guar gum</td>
</tr>
<tr>
<td>413</td>
<td>Tragacanth gum</td>
</tr>
<tr>
<td>415</td>
<td>Xanthan gum</td>
</tr>
<tr>
<td>440</td>
<td>Pectins</td>
</tr>
<tr>
<td>466</td>
<td>Sodium carboxymethylcellulose</td>
</tr>
<tr>
<td><strong>Modified Starches</strong></td>
<td></td>
</tr>
<tr>
<td>1401</td>
<td>Acid treated starches</td>
</tr>
<tr>
<td>1402</td>
<td>Alkaline treated starches</td>
</tr>
<tr>
<td>1404</td>
<td>Oxidized starches</td>
</tr>
<tr>
<td>1410</td>
<td>Monostarch phosphate</td>
</tr>
<tr>
<td>1412</td>
<td>Distarch phosphate esterified with sodium trimetaphosphate; esterified with phosphorus oxychloride</td>
</tr>
<tr>
<td>1413</td>
<td>Phosphated distarch phosphate</td>
</tr>
<tr>
<td>1414</td>
<td>Acetylated distarch phosphate</td>
</tr>
<tr>
<td>1420</td>
<td>Starch acetate</td>
</tr>
<tr>
<td>1422</td>
<td>Acetylated distarch adipate</td>
</tr>
<tr>
<td>1440</td>
<td>Hydroxypropyl starch</td>
</tr>
<tr>
<td>1442</td>
<td>Hydroxypropyl starch phosphate</td>
</tr>
<tr>
<td><strong>Acidity Regulators</strong></td>
<td></td>
</tr>
<tr>
<td>260</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>270</td>
<td>Lactic acid (L-, D-, and DL-)</td>
</tr>
<tr>
<td>330</td>
<td>Citric acid</td>
</tr>
<tr>
<td><strong>Natural Flavours</strong></td>
<td></td>
</tr>
<tr>
<td>Spice oils</td>
<td></td>
</tr>
<tr>
<td>Spice extracts</td>
<td></td>
</tr>
<tr>
<td>Smoke flavours (Natural smoke solutions and extracts)</td>
<td></td>
</tr>
</tbody>
</table>

### 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 as prescribed by the Codex Alimentarius Commission, the product:

(i) shall be free from micro-organisms capable of development under normal conditions of storage;

(ii) no sample unit shall contain histamine that exceeds 20 mg per 100 g;
(iii) shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health in accordance with standards established by the Codex Alimentarius Commission;

(iv) shall be free from container integrity defects which may compromise the hermetic seal.

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 the following relevant Codes:

(i) CAC/RCP 10, Recommended International Code of Practice for Canned Fish;

(ii) CAC/RCP 23, Recommended International Code of Hygienic Practice for Low-Acid and Acidified Low-Acid Canned Foods;

7 Packing and labelling

7.1 Packing

7.1.1 Sardine (fresh and frozen) shall be packed in suitable container as agreed between the purchaser and the processor. In the absence of any such agreement the material shall be packed in containers which may withstand the stress and strain of transportation and prevent deterioration during transportation and frozen storage. A layer of food grade polyethylene shall be used between the material and the container when individually frozen sardines are packed.

7.1.2 Canned sardine shall be packed in suitable containers, free from rust and hermetically sealed. Cans shall be lacquered, the lacquer used shall be non-toxic and shall be of such quality that it does not impart any foreign taste and smell to the contents of the cans and does not peel off during processing and storage of the product. The lacquer shall not be soluble in oil or brine.

7.2 Labelling

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

7.2.1 Name of the food

The name of the product with indication of fresh or frozen or canned shall be:

7.2.1.1 (i) “Sardines” (to be reserved exclusively for Sardina pilchardus (Walbaum)); or

(ii) “X sardines” where “X” is the name of a country, a geographic area, the species, or the common name of the species, or any combination of these elements in accordance with the law and custom of the country in which the product is sold, and in a manner not to mislead the consumer.

7.2.1.2 The name of the packing medium shall form part of the name of the food for canned sardines.

7.2.1.3 If the fish has been smoked or smoke flavoured, this information shall appear on the label in close proximity to the name.

7.2.1.4 Name and address of the processor;

7.2.1.5 Batch or code number;

7.2.1.6 Grade, in case of fresh and frozen sardines;

7.2.1.7 Net mass;
7.2.1.8 List of ingredients in descending order;

7.2.1.9 Date of packing;

7.2.1.10 The words ‘Best before . . . . . . . . . . . ’ (month and year to be indicated)

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

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 can of sardines 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 Annex A and CAC/GL 31.

8.3 Determination of net weight

Net contents of all canned sample units shall be determined by the following procedure:

(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. The resultant figure will be the net content.

8.4 Determination of drained weight

The drained weight of all sample units shall be determined by the following procedure:

(i) Maintain the container at a temperature between 20 °C and 30 °C for a minimum of 12 hours prior to examination.

(ii) Open and tilt the container to distribute the contents on a pre-weighed circular sieve which consists of wire mesh with square openings of 2.8 mm x 2.8 mm.
(iii) Incline the sieve at an angle of approximately 17-20° and allow the fish to drain for two minutes, measured from the time the product is poured into the sieve.

(iv) Weigh the sieve containing the drained fish.

(v) The weight of drained fish is obtained by subtracting the weight of the sieve from the weight of the sieve and drained product.

8.5 Procedure for packs in sauces (washed drained weight)

(i) Maintain the container at a temperature between 20°C and 30°C for a minimum of 12 hours prior to examination.

(ii) Open and tilt the container and wash the covering sauce and then the full contents with hot tap water (approx. 40°C), using a wash bottle (e.g. plastic) on the tared circular sieve.

(iii) Wash the contents of the sieve with hot water until free of adhering sauce; where necessary separate optional ingredients (spices, vegetables, fruits) with pincers. Incline the sieve at an angle of approximately 17-20° and allow the fish to drain two minutes, measured from the time the washing procedure has finished.

(iv) Remove adhering water from the bottom of the sieve by use of paper towel. Weigh the sieve containing the washed drained fish.

(v) The washed drained weight is obtained by subtracting the weight of the sieve from the weight of the sieve and drained product.

8.6 Determination of histamine

See Annex C.

8.7 Commercial sterility test

With a sterile pipette, transfer aseptically 1 ml of the liquid portion from the can to the thioglycollate broth and incubate the tube at 37 °C for 48 hours. If there is growth in the tubes after 48 hours, the cans are not commercially sterile. In doubtful cases the contents of the tube may be reinoculated and tested for a period of 48 hours. No cans shall show non-sterile conditions.

9 Definition of defectives

9.1 Taint

A unit will be considered tainted when any of the following conditions are found:

a) **Rancid** — The contents in the container show the following defects:
   1) Odour characterized by the distinct or persistent odour of oxidized oil; or
   2) 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, or associated with feed, and not defined as rancid or decomposed; or
   Flavour or odour resulting from the improper addition or mixing of ingredients.

9.2 Decomposition

A unit will be considered decomposed when any of the following conditions are found:
a) **Odour or flavour** — Persistent, distinct and uncharacteristic odour or flavour including but not limited to the following: fruity, vegetable, musty, yeasty, sour, faecal, ammonia, hydrogen sulphide, bilge-like, putrid.

b) **Discolouration** — Discolouration uncharacteristic of the species and type of pack, such as flushed pink (with a somewhat raw appearance), dark brown, yellowish to orange colours or definite red along the backbone.

c) **Texture** — Breakdown of muscle structure characterized by:

1) muscle structure which is very tough, dry, mealy or chalky; or

2) muscle structure which is very soft, mushy or pasty.

### 9.3 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 packing media) 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 packing media) 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 any material which has not been derived from fish (and packing media) 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) **Struvite crystals** (magnesium ammonium phosphate crystals) — Any struvite crystal greater than 5 mm in length.

2) **Sulphide blackening** (smut) — Staining affecting greater than 5% of the fish in the sample unit.

3) **Undesirable parts** — Any combination of sardines from which the heads and gills have not been removed which exceeds 5% of the number of fish in the sample unit.

### 10 Lot acceptance

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

(i) not any single instance of critical foreign matter occurs; or

(ii) the total number of sample units found defective for taint, decomposition or unwholesomeness, individually or in combination, does not exceed the acceptance number for the sample size designated in the sampling plans in CD-K-572:2010; or

(iii) the total number of sample units found defective for decomposition does not exceed the acceptance number (c) shown in parentheses for the sample size designated in the sampling plans in CD-K-572:2010; or

(iv) the average net weight and the average drained weight of all sample units examined is not less than the declared weight and provided there is no unreasonable shortage in any individual container;
(v) the Food Additives, Hygiene and Labelling requirements of Clauses 5, 6, and 7 are met.

(vi) the total number of sample units found defective for standards of identity (style of presentation) and size designation or count range (if a size designation or count range is declared), does not exceed the acceptance number for the sample size designated in the sampling plans.

Fresh sardines
Dried sardines

Frozen sardines in sales unit
Fresh sardines in market stall

Frozen sardines in sales unit
Annex A
(normative)

Sensory and physical examination

1. Complete external can examination for the presence of container integrity defects or can ends which may be distorted outwards.

2. Open can and complete weight determination according to defined procedures in 8.3, 8.4 and 8.5.

3. Carefully remove product and examine for discolouration, foreign matter and struvite crystals. The presence of a hard bone is an indicator of underprocessing and will require an evaluation for sterility.

Annex B
(normative)

Preparation and processing of sardine, fresh, frozen and canned

B.1 Fresh sardine

B.1.1 Preparation

B.1.1.1 The material shall be washed in clean potable water mg/kg to remove all adhering impurities and shall be iced immediately in suitable containers. The top, bottom and sides shall be covered with a layer of crushed ice.

B.1.1.2 The material shall be grouped according to the grade of the fish (see 3.4).

B.1.1.3 The temperature of the fish in the container shall not exceed 5 °C.

B.2 Frozen sardine

B.2.1 Processing

B.2.1.1 Clean, wholesome and fresh sardine (see B.1.1.1) which do not show any signs of spoilage shall be used.

B.2.1.2 To prevent belly bursting, the fish may be given a dip treatment in 15 percent brine for 30 min before freezing.

B.2.1.3 The material shall be properly arranged and quick frozen at a temperature not exceeding -40 °C in the minimum possible time quick-frozen material shall be stored in cold storage at a temperature of -23 °C or below throughout.

B.2.1.4 The material shall be packed according to grade.

B.3 Canned sardine

B.3.1 Processing

B.3.1.1 Processing shall be at such a temperature and for such length of time as will ensure thorough cooking and commercial sterility. The water used for cooling cans shall be maintained in clean condition and chlorinated to maintain a minimum free residual chlorine concentration of one ppm.

B.3.1.2 Fish curry, if used shall be prepared as agreed to between the purchaser and the producer, care being taken that during preparation all the fish juices or other ingredients are retained in full.
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 H2O before use.

(a) Chromatographic tube — 200 X 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 m.

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 m 2M NaOH/g resin to beaker. Swirl mixture and let stand <30min. Decant liquid and repeat with additional base. Thoroughly wash resin with H2O, slurry into fluted paper and wash again with H2O. Prepare resin fresh weekly and store under H2O. Place glass wool plug in base of tube, C.2(a), and slurry in enough resin to form 8 cm bed. Maintain H2O level above top of resin bed at all times. Do not regenerate resin in packed column; rather, use batch regeneration in beaker when necessary. Wash column with ca 10 ml H2O before applying each extract.

(b) Phosphoric acid — 3.57N. Dilute 121.8 ml 85% H3PO4 to 1 L. For other concentration H3PO4, volume required for 1 L1.19M acid = 17493/(density H3PO4 percent H3PO4). Standardize 5.00 ml by titration with 1.00M NaOH to phenolphthalein end point, and adjust concentration if necessary.

(c) o-Phthaldialdehyde (OPT) solution — 0.1% (w/v). Dissolve 100 mg OPT in 100 ml distilled-in-glass methanol. Store in amber bottle in refrigerator. Prepare fresh weekly.

(d) Histamine standard solutions — Store in refrigerator.

(1) Stock solution — 1 mg/ml as free base. Accurately weigh ca 169.1 mg histamine 2HCl (98%) into 100 ml volumetric flask, and dissolve and dilute to volume with 0.1M HCl. Prepare fresh weekly.

(2) Intermediate solution — 10 µg/ml. Pipet 1 ml stock solution into 100 ml volumetric flask, and dilute to volume with 0.1M HCl. Prepare fresh weekly.

(3) Working solutions — 0.5, 1.0, and 1.5 µg/5 ml. Pipet 1, 2, and 3 ml intermediate solution into separate 100 ml volumetric flasks, and dilute each to volume with 0.1M HCl. Prepare fresh daily.
(e) **Methanol** — 75% (v/v). Place 75 ml MeOH (distilled in glass) into 100 ml volumetric flask or stoppered graduated cylinder. Dilute to volume with H2O. Swirl flask while adding H2O.

### 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 3.57NH3PO4 and mix immediately. It is important to mix thoroughly after each addition and at least once during OPT reaction. (Run 6–10 OPT reactions simultaneously by adding reagents to Erlenmeyers in set order.) Prepare blank by substituting 5 ml 0.1M HCl for histamine solution. Within 1.5 h, record fluorescence intensity (I) of working standard solutions with H2O in reference cell, using excitation wavelength of 350 nm and emission wavelength of 444 nm. Plot I (corrected for blank) against µg histamine/5 ml aliquot.

### C.5 Determination

Extract prepared sample with 75% (v/v) methanol. Pass 4–5 ml H2O through column, C.2(a), and discard eluate. Pipet 1 ml extract onto column and add 4–5 ml H2O. Immediately initiate column flow into 50 ml volumetric flask containing 5.00 ml 1.00M HCl. When liquid level is ca 2 mm above resin, add ca 5 ml H2O and let elute. Follow with H2O in larger portions until ca 35 ml has eluted. Stop column flow, dilute to volume with H2O, stopper, and mix. Refrigerate eluate.

Pipet 5 ml eluate into 50 ml Erlenmeyer, and pipet in 10 ml 0.1M HCl. Proceed as in 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 $m = [(l_a/1.5) + l_b + 2l_c]/3$.

$$
\text{mg Histamine/100 g fish} = (10)(F)(1/m)(l_a)
$$

$$
\text{µg Histamine/g fish} = 10 \times (\text{mg histamine/100 g fish})
$$

where $l_a$, $l_b$, and $l_c$ = fluorescence from test sample, 1.5, 1.0, and 0.5 µg histamine standards, respectively; and $F = \text{dilution factor} = (\text{ml eluate} + \text{ml 0.1M HCl})/\text{ml eluate}$. $F = 1$ 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.

$$
\text{mg Histamine/100 g fish} = (10)(F)(W)
$$

$$
\text{µg Histamine/g fish} = 10 \times (\text{mg histamine/100 g fish})
$$

where $W = \text{µg histamine/5 ml test solution as determined from standard curve}$. 

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Annex D
(normative)

Determination of sodium chloride

D.1 Reagents

D.1.1 Standard Silver Solution — 0.1 N, standardized against 0.1 N sodium chloride solution.

D.1.2 Dilute Nitric Acid — 1:4.

D.1.3 Ferric ammonium indicator solution — A saturated solution of ferric alum \([\text{Fe (NH}_4\text{) (SO}_4\text{)}_{2.12\text{H}_2\text{O}}]\).

D.1.4 Standard Potassium Thiocyanate Solution — 0.1N

D.2 Procedure

D.2.1 Wash the emptied can thoroughly with water and wash the residue on the sieve at least thrice with cold water. Collect the drained liquid and all the washings together in a 1 000 ml graduated flask and make up the volume. Centrifuge the made-up liquid for at least 5 min at 1 000 rev/min.

D.2.2 Take a suitable aliquot of the clear supernatant solution prepared as in D.2.1, add a known volume of the standard silver nitrate solution in slight excess and then add 20 ml of dilute nitric acid. Boil gently on a hotplate or a sand-bath until all solids except silver chloride dissolve (usually 15 min). Cool, add 50 ml of water and 5ml of the ferric alum indicator solution and titrate with the standard ammonium thiocyanate solution until permanent light brown colour appears.

D.3 Calculation

D.3.1 Sodium chloride, per cent by weight

\[
\frac{5.85(V_1N_1 - V_2N_2)}{W}
\]

where,

\(V_1\) = volume of the standard silver nitrate solution;

\(V_2\) = volume of the standard potassium thiocyanate;

\(N_1\) = normality of the standard silver nitrate solution;

\(N_2\) = normality of the standard potassium thiocyanate; and

\(W\) = weight, in g, of the dried product taken for the test.
Annex E
(normative)

Determination of acidity in brine

E.1 Reagents

E.1.1 Standard sodium hydroxide solution
0.1N.

E.1.2 Phenolphthalein indicator solution
Dissolve one gram of phenolphthalein in 100 ml of 95 percent (m/v) alcohol.

E.2 Procedure

E.2.1 Take a suitable aliquot of the brine solution (see D.2.1), add about 200 ml of water and titrate against the standard sodium hydroxide solution using phenolphthalein indicator solution. Calculate the percentage acidity of the brine in terms of citric acid from the relationship: 1ml of 0.1 N sodium hydroxide solution is equivalent to 0.0064 g of citric acid (anhydrous).