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EAST AFRICAN STANDARD

Pomfret canned in oil — Specification

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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.

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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".

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East African Community

P O Box 1096

Arusha

Tanzania

Tel: 255 27 2504253/8

Fax: 255-27-2504481/2504255

E-Mail: eac@eachq.org

Web: www.each.int

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Introduction

Pomfret is usually canned in oil. In order to ensure the quality and wholesomeness of the finished product, various physical, chemical, microbiological and hygienic requirements have been prescribed in this standard.

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

IS 2168:1971(R2005), *Specification for Pomfret Canned in Oil*

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

USDA Foreign Agricultural Service website: http://www.mrldatabase.com

USDA Agricultural Marketing Service website: http://www.ams.usda.gov/AMSv1.0/Standards

European Union: http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm

Assistance derived from these sources is hereby acknowledged.

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Pomfret canned in oil — Specification

1 Scope

This East African Standard specifies the requirements and the methods of sampling and test for white (*Stromateus cinerus*), silver *(Pampus argenteus)* and brown *(Parastromateus niger)* pomfrets canned in oil.

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*

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

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

Canned pomfret shall be prepared from sound, wholesome raw material processed using good manufacturing practices.

3.2 Material and processing

3.2.1 The pomfrets, after nobbing, shall be thoroughly washed in potable water.

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3.2.2 The material shall have a good odour and flavour, and retain a good colour characteristic of the species. The flesh shall be firm in texture and free from disintegration.

3.2.3 The pomfrets may be given a suitable brine treatment to ensure that the finished canned fish are firm in texture. Brine solutions shall be kept clean. The final saltiness of the pack shall be so adjusted as to produce a palatable product.

3.2.4 Processing — Processing shall be at such temperature and for such length of time as will ensure thorough cooking and adequate sterilization of the finished product without burning, scorching or over-cooking. The water used for the cooling of cans shall be maintained in clean condition and chlorinated to maintain a minimum residual chlorine concentration of three parts per million.

3.3 Preparation

The pomfrets shall be cut to suitable thickness and pieces in any single can shall be of uniform size and of the same species. One tail piece may be included in each can provided the tail-tip is satisfactorily removed. The pieces shall be filled in the can in such a manner that they remain in the same axis, that is, there shall be no cross-filling.

3.3.1 Packing in cans — The pieces shall be packed in suitable cans and hermetically sealed. If the cans are lacquered, the lacquer used shall be of such quality that it does not impart any foreign unpleasant taste and smell to the contents of the can and does not peel off during processing and storage of the product. The lacquer shall not be soluble in oil or brine to any extent. The cans shall show no evidence of rusting. The cans shall be thoroughly cleaned before filling.

3.3.1.1 The cans may also be lacquered externally subject to agreement between the purchaser and the manufacturer or the vendor.

3.3.1.2 Cooking — The pieces shall be cooked and drained properly.

4 Essential composition and quality factors

4.1 General

4.1.1 Fish — The pomfrets shall be of sound quality. The material to be canned shall be free from heads, tail-tips and entrails. Scales, if present, shall be removed from pomfrets before canning.

4.1.2 Oil — Only refined, pure clear and deodorized edible vegetable oil having characteristic colour shall be used for canning pomfret. The oil shall be free from any foreign matter or mineral oil and objectionable flavour and odour.

4.1.3 Salt — Edible salt preferably conforming to EAS 35 shall be used for canning.

4.2 Requirement for the finished product

4.2.1 The contents of the can on opening shall not display any appreciable disintegration and the oil shall be clear. Pieces from which portions have separated out would be treated as disintegrated units. The percentage of detached portions of fish, calculated on the basis of the drained weight, shall not exceed 5 percent based on the average of 5 cans.

4.2.2 The canned pomfret shall be of pleasant flavour characteristic of well-canned pomfret. It shall be free from scorched, bitter, foreign or other objectionable flavour. It shall have no colour other than the characteristic colour of well-preserved pomfret.

4.2.3 It shall be free from any foreign material and from grittiness.

4.2.4 The material shall be free from any type of poisonous and deleterious substances.

4.2.5 Vacuum — The can shall give a negative pressure when punctured. If round cans are used,

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the vacuum shall be not less than 100 mm.

4.2.6 Drained weight of the contents — The drained weight of the contents in each can shall be not less than 65 percent by weight of the water capacity of the can when tested by the method given in Annex A.

4.2.6.1 The drained liquid shall not contain more than 10 percent by volume of water.

NOTE The drained liquid shall be collected in a measuring cylinder and be kept for separating the oil and water. The volumes of oil and water shall be measured and percentage of water determined.

5 Food additives

5.1 The product shall be free from artificial colouring matter and firming agents.

5.2 No additive other than common salt and species shall be used.

6 Hygienic requirements

6.1 The material shall be prepared, filled and processed under hygienic conditions and only in premises maintained in a thoroughly clean and hygienic manner [see CAC/RCP 52] and duly approved and licensed by the authorities concerned for fish products. The water used for processing of the fish shall be potable water, preferably conforming to EAS 12.

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

6.3 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; and

(ii) shall not contain any other substances including substances derived from micro organisms in amounts which may represent a hazard to health; and

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

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

Table 2 — Microbiological and heavy metal limits for pomfret canned in oil

Type of contaminant		Maximum limit (mg/kg)	Method of test
(i)	Microbiological requirements	Shall be commercially sterile	See J.5.1
(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

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7 Packing and marking

7.1 Packing

Unless agreed otherwise between the purchaser and the vendor, the cans shall be packed in cases sufficiently strong to withstand rough handling by rail, road and sea transport without damage to their contents.

7.2 Marking

The labelling of the cans may be done either by printing or lithographing on the cans themselves or by attaching labels printed on paper, subject to agreement between the purchaser and the vendor.

7.2.1 Each container shall be suitably marked with the following information:

a) Name of the material along with brand name, if any;

b) Name and address of the manufacturer;

c) Net weight of the contents of the can;

d) Drained weight of the contents of the can;

e) Batch or lot number and the date of manufacture;

f) The nature of the canning medium used and its ingredients;

g) Declaration to the effect that no artificial colouring matter has been used; and

h) Licence number, date and authority, if any, under which the manufacturer has been permitted to can the product.

7.2.2 The warranty period may also be mentioned subject to agreement between the purchaser and the vendor.

7.2.3 Each container may also be marked with a Certification Mark.

8 Sampling, examination and analyses

8.1 Sampling

8.1.1 The sampling and tolerance plans in CD-K-572:2010 shall be used to determine the acceptability of the lot. The sampling plans dictate the minimum sample size to be taken. If necessary, in the opinion of the inspector, more than the minimum sample size specified may be taken.

8.1.2 Sampling of lots for the sensory examination of the product shall be in accordance with CD-K-572:2010 except that a lower acceptance number for decomposition shall be used as indicated in the sampling tables.

The tables specify the minimum number of sample units to be used for the following types of inspections:

a) Level I — Sensory examinations of all products subject to inspection other than lots which are subject to reinspection.

b) Level II — Sensory examinations of all products which are under reinspection.

8.1.3 The sample unit shall consist of a can of fish and the entire contents thereof.

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8.2 Sensory and physical examination

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

8.3 Determination of the fill of the container

8.3.1 Apparatus

8.3.1.1 Top pan balance

8.3.1.2 Rotary can opener

8.3.2 Procedure

8.3.2.1 The container selected shall be undamaged in all respects. Carefully open the container and note the level of the contents by means of a pencil mark on the internal surface of the can. Wash, dry and weigh the container.

8.3.2.2 Fill the container with distilled water at 20 °C to the height of the contents. Weigh the container plus the water. Subtract the weight of the container from this weight to give the weight of the equivalent to the volume of the contents.

8.3.2.3 Fill the container with additional water at 20 °C a distance of 4.76 mm below the top level of the container if the container has a double seam. (For other container, fill up to the top of the container). Weigh the container plus the water. Subtract the weight of the container from this weight to give the weight of the equivalent to the full volume of the container.

8.3.3 Calculation

Fill of container, per cent mass of remaining water

$$= \frac{\text{mass of the equivalent full volume of the content}}{\text{mass of water equivalent to full volume of content}} \times 100$$

8.4 Physical examination

8.4.1 Complete external can examination. Open can and complete net weight determination, according to defined policies and procedures for these examinations.

8.4.2 Examine appearance of product in can. Carefully remove fish from can to examination tray. Inspect can contents for presence of foreign material or other undesirable parts, carefully separating fish as necessary.

8.4.3 Examine can interior for presence of foreign material, smut, struvite, and corrosion or other can interior defects.

8.4.4 Observe colour of flesh as an indicator of decomposition.

8.4.5 Assess odour, flavour and texture as required.

8.4.6 Record any defect for that unit on the appropriate worksheet.

9 Definition of defectives

A sample unit will be considered defective when it fails to meet any of the following final product requirements referred to in Clause 4.

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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:

Odour characterized by the distinct or persistent odour of oxidized oil; or

Flavour characterized by that of oxidized oil which leaves a distinct bitter aftertaste.

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

Flavour or odour resulting from the improper addition and/or mixing of ingredients.

9.2 Decomposition

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

a) **Odours and flavours** — Persistent, distinct and uncharacteristic odour or flavour including but not limited to the following: fruity, vegetable, stale, musty, yeasty, sour, faecal, ammonia, hydrogen sulphide, bilge-like and putrid.

b) **Discolouration** — Discolouration associated with decomposition which is uncharacteristic of the species and type of pack, such as flushed pink, dark brown, green or yellowish to orange colours.

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

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

— 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 drained contents.

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3) **Undesirable parts** — Any combination of head parts, heads, tails, scales and viscera exceeding 2% of the drained weight.

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; 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 Sections 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.

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Annex A
(normative)

Determination of drained weight

A.1 Apparatus

A.1.1 Test sieve 200 (Aperture 2.00 mm) — BS Sieve 8 or Tyler Sieve 9 or ASA Sieve 10 (same as ASTM Test Sieve), may also be used.

A.2 Procedure

A.2.1 Carefully weigh the clean and dry sieve and transfer the contents of the can to the sieve. Allow to drain for five minutes and weigh the sieve with the contents. The difference between the two weights gives the drained weight. Calculate the drained weight as percentage of the water capacity of the can. Retain the residue on the sieve as well as the drained liquid.

A.2.2 Determine the water capacity of the can by the procedure given in A.2.2.1 to A.2.2.4.

A.2.2.1 Cut out the lid without removing or altering the height of the double seam.

A.2.2.2 wash, dry and weigh the empty can.

A.2.2.3 Fill the container with distilled water at 20 °C to 4 mm vertical distance below the top level of the container and weigh.

A.2.2.4 Subtract the weight in A.2.2.2 from the weight in A.2.2.3. The difference shall be considered to be the weight of water required to fill the container.

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Annex B
(normative)

Determination of arsenic content — Silver diethyldithocarbamate spectrophotometric method

B.1 Scope and field of application

This Annex specifies a method for the determination of the arsenic content of foods and dried products.

B.2 Basis and alternative method

The method described in this annex is based on the following standard:

ISO 17239, *Fruits, vegetables and derived products — Determination of arsenic content — Method using hydride generation atomic absorption spectrometry*

Where conditions permit, the following method of test may be used:

ISO 6634:1982, *Fruits, vegetables and derived products — Determination of arsenic content — Silver diethyldithiocarbamate spectrophotometric method*

B.3 Principle

Decomposition of a test portion, reduction of arsenic (V) to arsenic (III) with tin (II) chloride and transformation of the arsenic into arsine by the action of nascent hydrogen. Formation of a red coloured complex by the action of the arsine on silver diethyldithiocarbamate and spectrophotometric measurement at a wavelength of 520 nm.

B.4 Reagents

All reagents shall be of recognized analytical purity and shall, in particular, be free from arsenic (with the exception of the standard arsenic solution (B.4.9). The water used shall be distilled water or water of at least equivalent quality.

B.4.1 Sulphuric acid, ρ_{20} = 1.84 g/ml.

B.4.2 Nitric acid, ρ_{20} = 1.38 g/ml.

B.4.3 Perchloric acid, ρ_{20} = 1.67 g/ml.

B.4.4 Platinized zinc, prepared as follows

Place a portion of granulated zinc in a capsule, and pour in a volume of 0.05 g/l platinum chloride solution sufficient to cover the zinc. Leave in contact for 30 min, pour of the liquid, wash with water, leave the platinized zinc to drain on a square of blotting paper folded into several layers, and allow to dry. Store in a dry bottle.

The platinized zinc thus prepared shall be submitted to the preliminary test (see B.6.1.1).

NOTE Non-platinized zinc granules may be used if the product is shown to be suitable by the preliminary test.

B.4.5 Potassium hydroxide, in pellets.

B.4.6 Tin (II) chloride solution, prepared as follows:

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Carry out a cold attach on 20 g of pure granulated tin with 100 ml of concentrated hydrochloric acid (ρ_{20} = 1.19 g/ml.

Store in the presence of metallic tin, protected from air, in a bottle provided with a security device (to avoid overpressures caused by the liberation of hydrogen).

B.4.7 Potassium iodide, 10 g/l solution.

B.4.8 Sodium hydroxide, standard volumetric solution, c (NaOH) = 1 mol/l.

B.4.9 Arsenic, standard solution corresponding to 10 mg of arsenic (V) per litre, prepared as follows.

B.4.9.1 Dissolve, in a 500 ml flask, 264 mg of pure, dry arsenic trioxide in 10 ml of the sodium hydroxide solution (B.4.8), make up the volume to about 100 ml with water, add 15 ml of 1 mol/l hydrochloric acid solution and two drops of bromine; heat to boiling in order to expel the excess bromine, cool, transfer quantitatively to a 200 ml volumetric flask and make up to the mark with water.

1 ml of this solution contains 1 mg of arsenic.

B.4.9.2 By means of a pipette, transfer 10 ml of the solution (B.4.9.1) to a 1 000 ml volumetric flask. Make up to the mark with water.

1 ml of this standard solution contains 10 µg of arsenic (V).

B.4.10 Silver diethyldithiocarbamate, standard solution, prepared as follows.[4)]

B.4.10.1 Dissolve 3.4 g of silver nitrate in 200 ml of water; cool the solution to a temperature close to 10 °C.

B.4.10.2 Dissolve 4.5 g of sodium diethyldithiocarbamate in 200 ml of water; cool this solution to the same temperature as the silver nitrate solution (B.4.10.1).

B.4.10.3 Add slowly, and with constant shaking, the sodium diethyldithiocarbamate solution to the silver nitrate solution; collect the precipitate on a fritted glass crucible of porosity grade P 40, wash with water previously cooled to a temperature close to 10 °C, then dry under reduced pressure at ambient temperature, in the dark.

Dissolve the dried silver diethyldithiocarbamate in cold pyridine. Add cold water to precipitate the product. Filter the precipitate and wash with cold water until all traces of pyridine have been eliminated (this can be verified using a pH indicator paper: the pH of the washings shall not be greater than 6.5). Dry the light yellow crystals of silver diethyldithocarbamate in a desiccator under reduced pressure.

The crystals shall be kept in a cold, dark place in a glass bottle fitted with a ground glass stopper.

B.4.10.4 Dissolve 0.4 g of L-ephedrine in about 200 ml of chloroform, add 0.6 g of the silver diethyldithiocarbamate crystals (B.4.10.3), shake for 15 to 20 min, filter, and make up the volume to 250 ml with chloroform.

This reagent may be stored in a refrigerator in the dark for up to two weeks in a well-stoppered bottle.

B.4.11 Phenolphthalein, 10 g/l ethanolic solution.

B.5 Apparatus

Ordinary laboratory equipment, and in particular

B.5.1 Round-bottomed flasks, of capacity 1 000 ml.

[4)] This reagent is also available commercially.

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B.5.2 **Volumetric flasks**, of capacity 50 and 250 ml.

B.5.3 **Beakers**, of capacity 50 ml.

B.5.4 **Pipettes**, of capacity 1, 2, 5, 10, 20 and 50 ml.

B.5.5 **Burette.**

B.5.6 Apparatus for the determination of arsenic, for example as shown in Figure B.1, comprising:

B.5.6.1 **Conical flask,** of capacity 100 ml, fitted with a screw thread joint system for connection to the column B.5.6.2.

B.5.6.2 **Column,** of height 200 mm and diameter 15 mm, to fit the flask (B.5.6.1) at its base and fitted with a screw thread joint at the top.

B.5.6.3 **Delivery tube,** of internal diameter 4 mm, bent twice, at right angles 80 mm apart, to fit the column (5.6.2) at one end and tapered at the opposite end.

B.5.6.4 **Bubbler,** which may consist of a measuring cylinder of capacity 10 ml graduated in 0.1 ml divisions.

B.5.7 **Spectrophotometer,** suitable for use in the visible region of the spectrum, for measurements of absorbance at 520 nm, fitted with suitable cells of optical path length 10 mm.

B.5.8 Analytical balance

B.6 Procedure

B.6.1 Preliminary tests on reagents [2)]

B.6.1.1 Verification of the reactivity of the zinc and silver diethyldithiocarbamate solution.

B.6.1.1.1 Place, at the bottom of the column (B.5.6.2) of the apparatus (B.5.6) to a height of 6 to 8 cm. Fit the delivery tube (B.5.6.3) to the column (B.5.6.2).

Place the end of the delivery tube (B.5.6.3) in the bubbler (B.5.6.4) into which 4 ml of the silver diethyldithiocarbamate solution (B.4.10) have been placed. Cool the reagent by placing the bubbler in an ice-water bath.

B.6.1.1.2 Place in the conical flask (B.5.6.1) 0.5 ml of the standard arsenic solution (B.4.9) and sufficient water to make the volume up to about 35 ml; add 5 ml of the sulphuric acid (B.4.1), cool, add 2 drops of the tin (II) chloride solution (B.4.6), and then 5 ml of the potassium iodide solution (B.4.7). Leave in contact for 15 min, add 5 g of the platinized zinc (4.4), and plug rapidly by fitting the column (B.4.6.2) and the delivery tube (B.4.6.3).

Place the conical flask in a cold-water bath and put the whole apparatus in a dark place.

B.6.1.1.3 Allow the reaction to proceed for at least 1 h. Remove the deliver tube (B.4.6.3). If necessary, adjust the volume in the bubbler to 4 ml with the silver diethyldithiocarbamate solution (B.4.10).

Mix.

By means of the spectrophotometer (B.5.7), measure the absorbance at 520 nm of the solution in the bubbler, using as the reference solution the silver diethyldithiocarbamate solution (B.4.10).

The absorbance shall be at least 0.12.

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B.6.1.2 Verification of the absence of arsenic in the reagents

B.6.1.2.1 Prepare the column (B.5.6.2), the delivery tube (B.5.6.3) and the bubbler (B.5.6.4) of the apparatus (B.4.6) in the same way as for the test specified in 6.1.1.1.

B.6.1.2.2 Place in the conical flask (B.5.6.1) 35 ml of water and 5 ml of the sulphuric acid (B.4.1) and cool; then add 2 drops of the tin (II) chloride solution (B.4.6) and 5 ml of the potassium iodide solution (B.4.7).

Leave in contact for 15 min, add 5 g of the platinized zinc (B.4.4), plug rapidly by fitting the column (B.4.6.2) and the delivery tube (B.5.6.3) and place the apparatus in a dark place.

Proceed as described in B.6.1.1.3

The absorbance compared with that of the silver diethyldithocarbamate solution as reference solution (B.4.10), shall be less than 0.015.

NOTE The absence of arsenic in the nitric and perchloric acids is verified by the blank test (B.5.4).

B.6.2 Preparation of the test sample

Mix the laboratory sample thoroughly. If necessary, first remove seeds and hard seed-cavity walls and pass the remainder through a mechanical grinder.

Allow frozen or deep-frozen products to thaw beforehand in closed vessel and add the liquid formed during this process to the product before homogenization.

B.6.3 Test portion

B.6.3.1 Liquid products

By means of a pipette, transfer 50 ml of the test sample (B.5.2) to a 1 000 ml flask (B.4.1).

If the liquid contains ethanol, expel it by boiling, and allow to cool.

B.6.3.2 Pasty, solid or dehydrated products

Weigh, to the nearest 0.01 g, a mass of the test sample (B.6.2) corresponding to 50 to 100 g of fresh product, according to the nature of the sample, and place it in a 1 000 ml flask (B.5.1).

NOTE IF the sample is reach in organic matter, decomposition will require more time and the mass of the test portion should, therefore, be minimal.

B.6.4 Blank test

Carry out a blank test following the same procedures as for the determination, but replacing the test portion by 50 ml of water.

NOTE The blank test is not necessary if the absence of arsenic in the reagents used for decomposition has been verified.

B.6.5 Decomposition

Add to the contents of the flask 10 ml of the sulphuric acid (B.4.1) and 20 to 30 ml of the nitric acid (B.4.2) and, if necessary, a few drops of the perchloric acid (B.4.3).

When decomposition is complete, transfer the solution to a 50 ml volumetric flask (B.5.2), rinse the original flask with water, and use the rinsings to make up the volume in the volumetric flask to the mark.

Mix by shaking.

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B.6.6 Titration of the acidity of the solution

Transfer, by means of a pipette, 2ml of the solution obtained in B.6.5 to a beaker (B.5.3), dilute with a few millilitres of water, and titrate against the sodium hydroxide solution (B.4.8) in the presence of two drops of phenolphthalein (B.3.11)

The concentration, *C,* of sulphuric acid in the solution obtained in B.6.5, expressed in grams per 100 ml, is equal to

$$0.049 \times n \times 50$$

where n is the volume, in millilitres, of sodium hydroxide solution (B.4.8) and,

or, expressed in millilitres of sulphuric acid (ρ_{20} = 1.84 g/ml) per 100 ml of solution.

$$C' = \frac{C}{1.84}$$

B.6.7 Colorimetric determination

B.6.7.1 Prepare the column (B.5.6.2), the delivery tube (B.5.6.3) and the bubbler (B.5.6.4) of the apparatus (B.5.6) as indicated in B.6.1.1.1.

B.6.7.2 Place in the conical flask (B.5.6.1) a volume*, V,* of the solution obtained in B.6.5, corresponding to 5 ml of pure sulphuric acid, calculated from the formula

$$V = \frac{5 \times 100}{C'}$$

If necessary, add water to obtain a volume of about 40 ml. Allow to cool.

Add two drops of the tin (**II**) chloride solution (B.4.6) and 5 ml of the potassium iodide solution (B.4.7). Leave in contact for 15 min. Add 5 g of platinized zinc (B.4.4) to the contents of the conical flask (B.5.6.1). Rapidly stopper the flask by fitting the column (B.5.6.2) and the delivery tube (B.5.6.3) and place the apparatus in the dark, with the conical flask placed in cold-water bath.

B.6.7.3 Allow the reaction to proceed for at least 1 h. Remove the delivery tube. Adjust the volume in the bubbler to 4 ml with diethyldithiocarbamate solution (B.4.10).

By means of the spectrophotometer (B.5.7), measure the absorbance at 520 nm of the solution obtained, using the blank test solution (B.6.4), or the silver diethyldithiocarbamate solution (B.4.10), as the reference solution, according to the case.

B.6.8 Calibration curve

Proceed as described in B.6.1.1 using 0.5 - 1 - 1.5 and 2 ml of the standard arsenic solution (B.4.9.2) corresponding to 5 - 10 - 15 and 20 µg or arsenic, and plot a graph having, for example, the mass in micrograms of arsenic as abscissae and the corresponding values of absorbance as ordinates.

B.7 Expression of results

B.**7.1 Method of calculation and formulae**

B.7.1.1 Liquid products

The arsenic content, expressed in milligrams per litre of product as received, is equal to $\frac{m_1}{V}$

where

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m_1 is the mass of arsenic, expressed in micrograms, obtained from calibration graph and corresponding to absorbance measured in B.6.7.3.

V is the volume, in millilitres, of solution taken for the determining in B.6.7.2.

B.7.1.2 Pasty, solid or dehydrate products

The arsenic content, expressed in milligrams per kilogram of product as received, is equal to $\frac{m_1 \times 50}{V \times m_0}$

where

m_0 is the mass, in grams, of the test portion (B.6.3.2)

m_1 is the mass of arsenic, expressed in micrograms, obtained from the calibration graph and corresponding to the absorbance measured in B.6.7.3.

V is the volume, in millilitres, of solution taken for the determination in B.6.7.2.

If it is desired to express the arsenic content in relation to the dry product, take this fact into account in the calculation.

B.7.2 Repeatability

The difference between the results of two determinations, carried out simultaneously or in rapid succession by the same analyst, on the same sample, shall not exceed 10% as a relative value.

B.8 Test report

The test report shall show the method used and the result obtained. It shall also mention the method of expression used and any operating conditions not specified in this standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The test report shall include all the information necessary for the complete identification of the sample.

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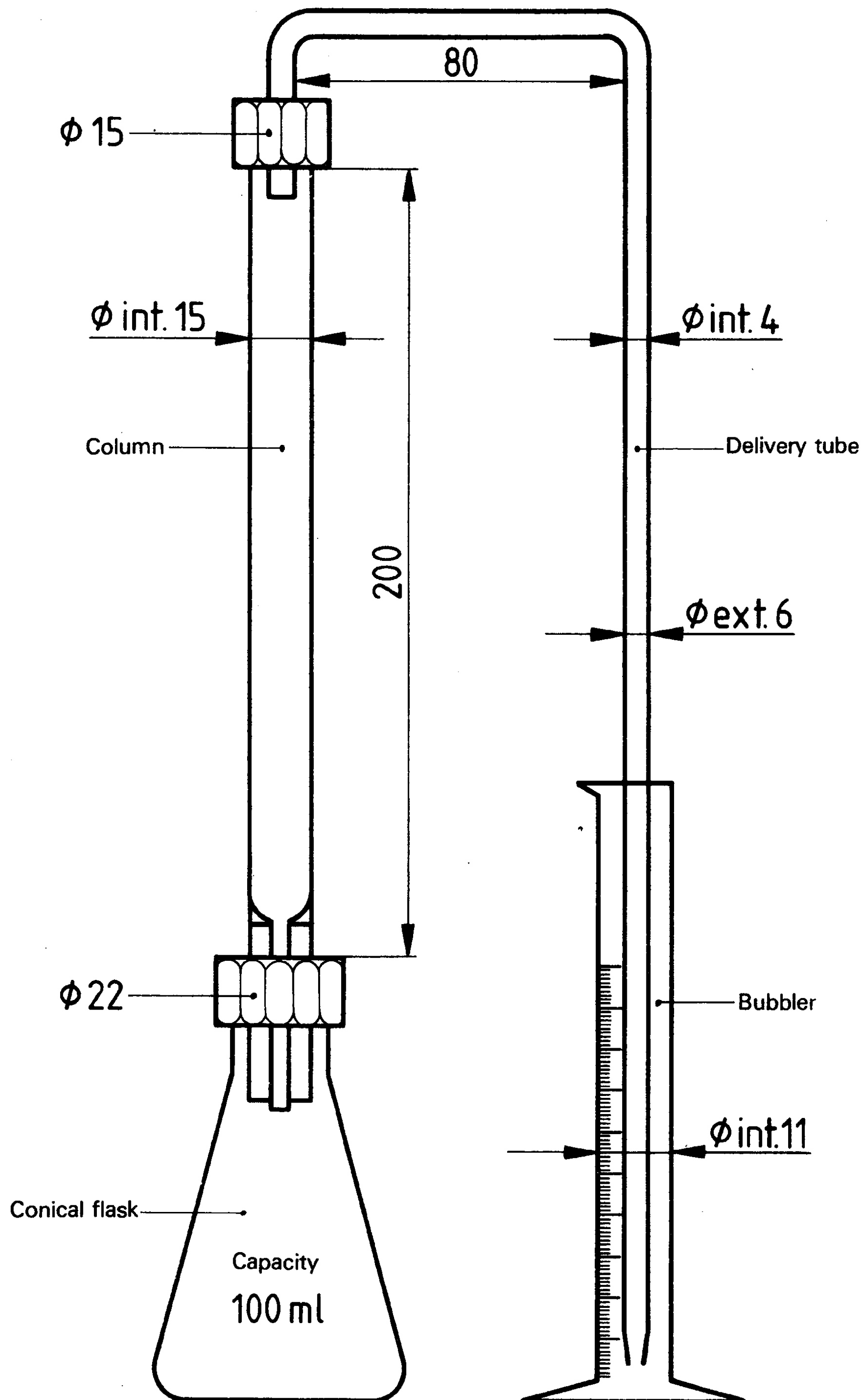

Figure B.1 — Example of apparatus for the determination of arsenic (B.5.6)

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Annex C
(normative)

Determination of lead content

The lead content shall be determined in accordance with:

ISO 6633:1984, *Fruits, vegetables and derived products — Determination of lead content — Flameless atomic absorption spectrometric method*

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Annex D
(normative)

Determination of copper content

NOTE The following method may also be used:
ISO 7952:1994, *Fruits, vegetables and derived products — Determination of copper content — Method using flame atomic absorption spectrometry*

D.1 Scope and field of application

This annex specifies a method for the determination of copper in food products.

NOTE Bismuth and tellurium interfere with this method and the presence of these contaminants will lead to erroneous results.

D.2 Principle

After destruction of organic matter, addition of sodium diethyldithocarbamate to the previously neutralized acid solution. Extraction of the copper complex formed, by means of chloroform or carbon tetrachloride, and measurement of the intensity of the colour of the solution obtained.

D.3 Reagents

All reagents shall be of recognized analytical purity.

The water used shall be water distilled in borosilicate glass or silica apparatus, or of at least equivalent purity.

The reagents to be used for destruct of organic matter shall be provided, and in addition:

D.3.1 Chloroform or carbon tetrachloride, phosgene-free.

D.3.2 Methanol, anhydrous (for this method 99% methanol may be regarded as anhydrous).

D.3.3 Ammonia solution, ρ_{20} 0.88 g/ml.

D.3.4 Solution of ammonium citrate and disodium salt of ethylene-diaminetetraacetic acid (EDTA).

Dissolve 20 g of ammonium citrate and 5 g of disodium slat of DTA in water and dilute to 100 ml.

D.3.5 Sodium diethyldithiocarbamate, 5 g/l solution.

NOTE The solution of the reagent may be accelerated by heating in a water bath at 25 to 30 ^{0}C.

This solution shall have been prepared less than 1 week before use.

D.3.6 Standard copper solution, corresponding to 0.01 g of Cu per litre.

— Dissolve 0.196 g of copper (II) sulphate ($CuSO_4.5H_2O$) in water to which a few drops of sulphuric acid, ρ_{20} 1.84 g/ml, have been added, and dilute to 500 ml with water. Take 10 ml of this solution and dilute it to 100 ml with water.

— 1 ml of this solution contains 10µg of copper.

D.3.7 Thymol blue indicator solution

Dissolve, by warming, 0.1 g of thymol blue in 8.6 ml of 0.1 N sodium hydroxide solution and 10 ml of 96% (V/V) ethanol. Dilute to 250 ml with 20% (V/V) ethanol.

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D.4 Apparatus

In addition to the equipment used for the destruction of organic matter, usual laboratory equipment not otherwise specified and the following items are required:

D.4.1 Separating funnel, short stemmed.

D.4.2 Spectrophotometer or photoelectric absorptiometer, suitable for measurements at a wavelength of 435 nm, fitted with cells of 10 to 20 mm optical path length.

D.5 Procedure

D.5.1 Test portion and destruction of organic matter

Proceed in accordance with any one of the following documents:

— ISO 5515*, Fruits, vegetables and derived products — Decomposition of organic matter prior to analysis — Wet method*

— ISO 5516, *Fruits, vegetables and derived products — Decomposition of organic matter prior to analysis — Ashing method*

NOTE Milling and sieving of the sample, before destruction of organic matter, in equipment made of copper alloys should be avoided.

D.5.2 Formation and extraction of copper complex

If the amount of copper expected in the test portion is less than 50 µg, take the whole of the solution obtained in accordance with D.5.1. This is solution A. If the copper content is expected to be higher, dilute solution A to 100 ml with water and take an aliquot portion.

Cool, and dilute the solution taken with 30 to 40 ml of water; cool again, transfer it into a separating funnel (D.4.1), add 20 ml of citrate-EDTA solution (D.3.4) and subsequently 5 ml of ammonia solution (D.3.3). Add 2 drops of thyme blue indicator solution (D.3.7) and sufficient ammonia solution (D.3.3) for the colour to turn from yellow to blue (pH 8 to 9.6). Cool in running water, loosening the stopper of the separating funnel from time to time. Add 2 ml of sodium diethyldithocarbamate solution (D.3.5) and exactly 10 ml of chloroform or carbon tetrachloride (D.3.1).

Shake for 5 min to establish equilibrium in the composition of the two phases.

Allow the two layers to separate, and then dry the stem of the separating funnel both outside and inside using filter paper or a swab of absorbent cotton wool. Drain the chloroform or carbon tetrachloride layer containing the copper complex into a test tube, keeping the test tube away from light. Leave to stand to permit the water droplets to separate. Filter through a filter paper into another test tube in order to remove the last traces of water.

Add 0.5 ml of methanol (D.3.2) (to avoid turbidity) and keep it in a dark place until the time of measurement.

The colour complex remains stable for 2 h in the dark and for 1 h in the light (protected from direct sunlight).

D.5.3 Blank test

Simultaneously with the determination carry out a blank test as follows:

Take the same amounts of the reagents used for the destruction of organic matter in the sample (sulphuric acid, nitric acid, hydrochloric acid, etc) and proceed in accordance with D.5.1 and D.5.2. Use the chloroform or carbon tetrachloride layer thus obtained for the determination (see D.5.4).

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D.5.4 Determination

Measure the intensity of coloration of the solution of copper complex in chloroform or carbon tetrachloride (see D.5.2) by comparing it with the blank test solution (see D.5.3) in the spectrophotometer or photoelectric absorptiometer (D.4.2) at a wavelength of 435 nm.

Carry out two determinations on the same prepared sample taken for the destruction of organic matter (see D.5.1).

D.5.5 Preparation of the calibration curve

Carry out both the destruction of organic matter and the photometric measurements separately with 1, 2, 3, 4 and 5 ml of the standard copper solution (D.3.6) containing 10, 20, 30, 40 and 50 µg of copper, respectively, treating them in he same manner as the test solution.

Prepare at the same time a blank test solution, in accordance with D.5.3, and proceed in accordance with D.5.4 (see note).

Prepare the calibration curve by plotting the values of absorbance against the corresponding amounts of copper in micrograms.

NOTE If the calibration curve is made simultaneously with the determination, it is not necessary to prepare a separate blank solution.

D.6 Expression of results

D.6.1 Method of calculation and formula

By means of the calibration curve, convert the figure obtained in 5.4 into micrograms of copper. The copper content of the sample, expressed in milligrams per kilogram of the product tested, is equal to

$$\frac{m_1}{1000} \times \frac{100}{V} \times \frac{1000}{m_0} = \frac{m_1 \times 100}{V \times m_0}$$

where

m_0 is the mass, in grams, of the test portion (see D.5.1)

m_1 is the amount of copper, in micrograms, as read on the calibration curve

V is the volume, in millilitres, of solution A taken for the determination (see D.5.2).

Take as the result the arithmetic mean of the two determinations if the requirement concerning repeatability (see D.6.2) is satisfied.

D.6.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0.2 mg of copper per kilogram of product for copper contents up to 5 mg/kg, and shall not exceed 5% of the average value for higher contents.

D.7 Test report

The test report shall give the method used and the result obtained. It shall also mention all operating conditions not specified in this standard, or regarded as optional, and any circumstances, which may have influenced the result.

The report shall give all details required for the complete identification of the sample.

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Annex E
(normative)

Determination of zinc content — Atomic absorption spectrometric method

NOTE This method is based on:

ISO 6636-2:1981, *Fruits, vegetables and derived products — Determination of zinc content — Part 2: Atomic absorption spectrometric method*

Depending on practicable circumstances, the following test methods may also be used:

— ISO 6636-1:1986, *Fruits, vegetables and derived products — Determination of zinc content — Part 1: Polarographic method*

— ISO 6636-3:1983, *Fruit and vegetable products — Determination of zinc content — Part 3: Dithizone spectrometric method*

E.1 Scope and filed of application

This annex specifies atomic absorption spectrometric method for the determination of the zinc content of food products.

E.2 References

The following standards contain provisions which, through reference in this text, constitute provisions of this East African Standard.

ISO 5515, *Fruits, vegetables and derived products — Decomposition of organic matter prior to analysis — Wet method*

ISO 5516, *Fruits, vegetables and derived products — Decomposition of organic matter prior to analysis — Ashing method*

E.3 Principle

Decomposition of organic matter by the dry or wet method and determination of the Zn^{2+} cation by atomic absorption spectrometry.

NOTE In the case of decomposition by the dry method, dissolution of the ash in hydrochloric acid allows transformation of all mineral salts into easily dissociable chlorides.

For some liquid samples (such as wines, clear fruit juices free from pulp), the determination may be carried out directly, without previous decomposition.

E.4 Reagent

All reagents shall be or recognized analytical purity, and shall be especially free of zinc. The water used shall have been distilled twice in an apparatus of borosilicate glass, or shall be water of at least equivalent purity.

E.4.1 Nitric acid, ρ_{20} = 1.38 g/ml.

E.4.2 Sulphuric acid, ρ_{20} = 1.84 g/ml.

E.4.3 Hydrochloric acid, 1 + 1 (by volume) solution.

Mix one volume of concentrated hydrochloric acid (ρ_{20} = 1.19 g/ml.) with one volume of water.

E.4.4 Hydrochloric acid, approximately 3.7 g/l solution.

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In a 1 000 ml one-mark volumetric flask, dilute 8.3 ml of concentrated hydrochloric acid (ρ_{20}=1.19 g/ml.) to the mark with water and mix.

E.4.5 Zinc, standard solution corresponding to 1 g of zinc per litre.

In a conical flask, dissolve 1 g of pure zinc in 10 ml of the hydrochloric acid solution (E.4.3). Transfer quantitatively to a 1 000 ml one-mark volumetric flask, dilute to the mark with water, and mix.

Store the solution in a bottle of borosilicate glass fitted with a ground glass stopper.

E.5 Apparatus

Usual laboratory equipment not otherwise specified, and the following:

E.5.1 Mechanical grinder, the inside and blades of which are coated with polyethylene.

E.5.2 Platinum or quartz dishes, of diameter 70 mm, or kjeldahl flasks, of capacity 250 ml.

E.5.3 One-mark volumetric flasks, of capacity 50 ml.

E.5.4 Volumetric pipettes, of appropriate capacities

E.5.5 Centrifuge

E.5.6 Boiling water bath

E.5.7 Heating device

E.5.8 Electrically heated muffle furnace, capable of being controlled at 525 ± 25 ℃.

E.5.9 Electrically heated oven, capable of being controlled at less than 100 ℃ an d at 525 ± 25 ℃, preferably programmable between 20 and 550 ℃.

E.5.10 Atomic absorption spectrometer, fitted with a burner for air-acetylene mixture fed at a pre-determined flow rate (in general 4 ml/min) corresponding to the specified optimum aspiration rate, suitable for measurements at a wavelength of 213.8nm.

E.5.11 Analytical balance.

E.6 Procedure

E.6.1 Preparation of the test sample

Mix the laboratory sample well. If necessary, first remove seeds and hard seed-cavity walls and pass through the mechanical grinder (E.5.1).

Allow frozen or deep-frozen products to thaw in a closed vessel, and add the liquid formed during this process to the product before homogenization.

E.6.2 Test portion

E.6.2.1 Liquid products

Take 10 ml of the test sample (E.6.1) by means of a pipette (E.5.4). In the case of viscous liquids or liquids containing suspended solid particles, weigh the test portion (see E.6.2.2).

E.6.2.2 Pasty, solid or dried products

Weigh, to the nearest 0.01 g, 5 to 10 g of the test sample (E.6.1), expressed as fresh product, according to the nature of the product.

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E.6.3 Decomposition

Decomposition may be carried out by the dry or wet method.

E.6.3.1 Decomposition by the dry method

Introduce the test portion (E.6.2) into one of the dishes (E.5.2) and place it on the boiling water bath (E.5.6), regulating the temperature of the bath so as to minimize the risk of loss of material by spattering. Evaporate to dryness. Continue the decomposition in the muffle furnace (E.5.8), controlled at 525± 25 °C.

NOTE If possible, it is preferable to avoid evaporation on a boiling water bath and to place the dish directly in the electric oven (5.9), which is temperature programmable from 20 to 525 ± 25 °C, in progressive steps, to avoid spatte ring of the test portion during drying.

Dissolve the ashes in few drops of the nitric acid (E.4.1), evaporate on the boiling water bath (E.5.6), and then transfer to the oven (E.5.9) controlled at 525 ± 25 °C (or controlled at first at temperature less than 100 °C and then at 525 ± 25 °C). Transfer to t he muffle furnace (E.5.8), controlled at 525 ± 25 °C, and leave until white ashes are obtained. Dissolve the ashes in 1 to 2 ml of the hydrochloric acid solution (E.4.3). Transfer the contents of the dish quantitatively to a centrifuge tube (5.5), rinsing the dish with about 20 ml of the hydrochloric acid solution (E.4.4), centrifuge, and transfer the supernatant liquid to a 50 ml volumetric flask (E.5.3). Add a further 10 ml of the hydrochloric acid solution (E.4.4) to the contents of the centrifuge tube, centrifuge, and transfer the supernatant liquid to the same flask. Repeat this procedure using 10 ml of water and make up the volume in the volumetric flask to the mark with water. Mix the solution.

E.6.3.2 Decomposition by the wet method

Introduce the test portion (E.6.2) into a Kjeldahl flask (E.5.2). If the test portion (E.6.2.1) contains ethanol, eliminate it beforehand by boiling, and allow to cool. Add 10 ml of the nitric acid (E.4.1), heat, then carefully add 5 ml of the sulphuric acid (E.4.2).

In some cases it may be useful to effect a preliminary digestion, by leaving the mixture in contact in the flask for a period (overnight for example).

Place the flask containing eh mixture on the heating device (E.5.7) and heat cautiously to avoid excessive frothing.

If necessary, interrupt heating and begin again only when vigorous frothing has ceased.

As soon as possible, bring the liquid to the boil and continue boiling until it begins to turn brown. Then add, drop-by-drop, 1 to 2 ml portions of the nitric acid (E.4.1).

Bring to the boil after every addition, but avoid vigorous heating. A small amount of nitric acid shall always remain the mixture, as indicated by the presence of nitrous vapours.

Cease addition of portions of nitric acid when the solution no longer turns brown on addition of the acid. Continue heating until white fumes appear, indicating a high concentration of sulphuric acid and a reduction in nitric acid. If the solution turns brown again, continue the addition of nitric acid and repeat the operations described above until browning ceases.

Allow the solution to cool. The absence of colour or the presence of a light green or yellow colour indicates that the digestion is complete.

When decomposition is terminated, dilute the sulphuric solution with a few millilitres of water. Transfer the contents of flask quantitatively to a centrifuge tube (E.5.5), rinsing the flask with about 10 ml of water and collecting the rinsing water in the centrifuge tube. Centrifuge and transfer the supernatant liquid to a 50 ml volumetric flask (E.5.3). Add a further 10 ml of water to the contents of the centrifuge tube, centrifuge and transfer the supernatant liquid to the same flask. Repeat this procedure with another 10 ml of water and make up the volume in the volumetric flask to the mark with water. Mix the solution.

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E.6.3.3 Blank test

Carry out a blank test, using the same conditions for decomposition (E.6.3.1 or E.6.3.2 as appropriate), but replacing the test portion (E.6.2) by 10 ml of water.

E.6.4 Determination [1)]

E.6.4.1 Sample decomposed by the dry method

E.6.4.1.1 Preparation of the calibration graph

Dilute the standard zinc solution (E.4.5) with hydrochloric acid solution (E.4.4) to obtain four solutions containing 0.25 - 0.5 - 1 and 1.5 mg of zinc per litre.

Aspirate each of these solutions, in turn, into the flame of the spectrometer (E.5.10), at the rate such that the maximum absorbance is obtained for the solution having a zinc content of 1.5 mg/l. Record the corresponding values of absorbance and draw the calibration graph.

E.6.4.1.2 Spectrometric measurements

Aspirate the test solution obtained (E.6.3.1) and the blank test solution (E.6.3.3) into the flame of the spectrometer (E.5.10) at the same rate as in E.6.4.1.1. Record the corresponding absorbances. [2)].

The absorbance of the blank test solution shall be less than or equal to 0.002.

E.6.4.2 Sample decomposed by the wet method

E.6.4.2.1 Preparation of the calibration graph

Dilute the standard zinc solution (E.4.5) with water to obtain four solutions containing 2.5 - 5 - 10 and 15 mg of zinc per litre.

Into a series of four 50 ml volumetric flasks (E.5.3), place 5 ml of each of these solutions. Add 30 to 35 ml of water, and then 5 ml of the sulphuric acid (E.4.2). Mix, allow to cool and dilute to the mark with water. Mix. These solutions contain respectively 0.25 - 0.5 - 1 and 1.5 mg of zinc per litre.

Aspirate each of these solutions, in turn, into the flame of the spectrometer (E.5.10), at the rate such that the maximum absorbance is obtained for the solution having a zinc content of 1.5 mg/l. Record the corresponding values of absorbance and draw the calibration graph.

E.6.4.2.2 Spectrometer measurements

Aspirate the test solution (E.6.3.2) and the blank test solution (E.6.3.3) into the flame of the spectrometer (E.5.10) at the same rate as in E.6.4.2.1. Record the corresponding absorbances.[3)]

The absorbance of the blank test solution shall be less than or equal to 0.002.

E.7 Expression of results

E.7.1 Method of calculation and formulae

[1)] It is also possible to use solutions obtained using the procedures specified in ISO 5515 or ISO 5516, provided that precautions are taken to avoid the presence of zinc in the reagents.

[2)] If the absorbence of the test solution exceeds that of the most concentrated calibration solution, measure the absorbance of the test solution suitably diluted with the hydrochloric acid solution (4.4).

[3)] If the absorbence of the test solution exceeds that of the most concentrated calibration solution, measure the absorbance of the test solution suitably diluted with the 10 % (V/) sulfuric acid solution.

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E.7.1.1 Liquid products

The zinc content, expressed in milligrams per litre of product, is given by the formula

$$(c_1 - c_2) \times 5$$

where

c_1 is the zinc content of the sample, in milligrams per litre, read from the calibration graph;[4)]

c_2 is the zinc content of the blank test solution, in milligrams per litre, read from the calibration graph.

E.7.1.2 Viscous or inhomogeneous liquids, pasty, solid or dried products

The zinc content, expressed in milligrams per kilogram of product, is given by the formula

$$\frac{(c_1 - c_2) \times 50}{m}$$

where

c_1 is the zinc content of the sample, in milligrams per litre, read from the calibration graph [4)]

c_2 is the zinc content of the blank test solution, in milligrams per litre, read from the calibration graph;

m is the mass, in grams, of the test portion.

If it is desired to express the zinc content relative to the dry product, take the moisture content of the sample into account in the calculation.

E.7.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst, on the sample, shall not exceed 10% (relative).

E.8 Test report

The test report shall show the method used and the result obtained, indicating clearly the method of expression used. It shall also mention all operating details not specified in this standard or regarded as optional, as well as any incidents likely to have affected the results.

The test report shall give all the information necessary for the complete identification of the sample.

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[4)] If the test solution was diluted, use the appropriate dilution factor in the calculation.

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Annex F
(normative)

Determination of tin content

The lead content shall be determined in accordance with:

ISO 2447:1998, *Fruit and vegetable products — Determination of tin content*

ISO 17240:2004, *Fruit and vegetable products — Determination of tin content — Method using flame atomic absorption spectrometry*

F.1 Scope and field of application

This annex specifies a method for the determination of tin in food products.

The method is applicable to products, which may contain, per kilogram, up to:

— 1.25 g of copper;

— 0.6 g of lead;

— 0.6 g of zinc;

— 40 g of phosphorus.

F.2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this East African Standard.

ISO 5515:1979, *Fruits, vegetables and derived products — Decomposition of organic matter prior to analysis — Wet method.*

F.3 Principle

After destruction of the organic matter by means of sulphuric and nitric acids, and conversion of the tin to the stannic form, formation of a complex in a buffered solution of pH 1.0 to 1.2 (the iron (**III**) being masked, if necessary, by reduction with ascorbic acid), the complex being coloured orange with phenylfluorone and the colour compared with those obtained in the same conditions but starting from standard solutions of pure tin.

F.4 Reagents

All reagents used shall be of analytical reagent quality and the water used shall be distilled water or water of at least equivalent purity.

The following reagents are necessary, in addition to those used for the destruction of organic matter.

F.4.1 Sulfuric acid, ρ_{20} = 1.84 g/ml.

F.4.2 Dilute sulfuric acid, 1.25 mol/l.

F.4.3 Ascorbic acid, 50 g/l solution.

F.4.4 Nitric acid, ρ_{20} = 1.42 g/ml.

F.4.5 Hydrochloric acid, ρ_{20} = 1.19 g/ml.

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F.4.6 Methanol.

F.4.7 Ethanol, 95 % (*V*/*V*).

F.4.8 Poly(vinyl alcohol), 16 g/l solution.

Dissolve 1.6 g of poly(vinyl alcohol) in a little water with gentle warming and agitation. Dilute to 100 ml after cooling.

F.4.9 Buffer solution, containing 450 g of sodium acetate (CH_3COONa) and 240 ml of acetic acid (CH_3COOH) per litre.

F.4.10 Tin, standard volumetric solution I, containing 500 µg/ml in a sulfuric acid medium, approximately 3 mol/l.

Dissolve, with heating, 0.5 g of pure tin in a mixture of 50 ml of sulfuric acid (F.4.1), 5 ml of nitric acid (F.4.4) and 25 ml of water. After complete solution, oxidize the tin to the stannic form by boiling until white fumes appear.

Cool the solution and pour it into a 1000 ml volumetric flask containing 116 ml of sulfuric acid (F.4.1) and 100 ml of water. Cool and dilute to 1000 ml with water.

NOTE Alternatively, the tin standard solution I (F.4.10) may be prepared by dilution of a suitable proprietary standard solution.

F.4.11 Tin, standard volumetric solution II, containing 10 µg/ml in a sulfuric acid medium, approximately 0.25 mol/l.

Transfer 20 ml of the tin standard volumetric solution I (F.4.10) to a 1 000 ml volumetric flask. Add 10 ml of sulfuric acid (F.4.1) and dilute to 1 000 ml with water.

F.4.12 Phenylfluorone reagent (2,6,7-trihydroxy-9-phenyl-3-isoxathone).

Dissolve 0.1 g of phenylfluorone in 10 ml of methanol (F.4.6) and 1 ml of hydrochloric acid (F.4.5) in a 500 ml volumetric flask. Dilute to the mark with ethanol (F.4.7).

The reagent shall be stored in a brown bottle in the dark. It is recommended that it should not be stored for longer than 1 week.

F.5 Apparatus

F.5.1 One-mark volumetric flasks, capacity 50 and 200 ml.

F.5.2 Pipettes for delivery 1, 2, 3, 4, 5, 10 and 20 ml.

F.5.3 Spectrophotometer, or photocolorimeter, with green filter, fitted with a cell of 10 mm light path, enabling measurements to be made at wavelengths from 500 to 530 nm.

F.5.4 Analytical balance

F.6 Procedure

F.6.1 Preparation of test portion

Weigh a mass of about 10 g, to the nearest 0.01 g.

F.6.2 Destruction of organic matter

Add 5 ml of sulphuric acid (F.4.1) to the resulting solution, cool and pour it into the 200 ml volumetric flask (F.5.1), and dilute to the mark with water (solution A).

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F.6.3 Colorimetry

F.6.3.1 By means of a pipette (F.5.2) transfer to a 50 ml volumetric flask (F.5.1) an appropriate volume of solution A, i.e.:

— 20 ml, if the tin content of the sample is below 50 mg/kg;

— 10 ml, if the tin content of the sample is between 50 and 125 mg/kg, diluting to 20 ml with sulphuric acid solution (F.4.2);

— 5 ml, if the tin content of the sample is above 125 mg/kg, diluting to 20 ml with sulphuric acid solution (F.4.2).

F.6.3.2 Then add in succession:

— 10 ml of buffer solution (F.4.5)

— 1 ml of ascorbic acid solution (F.4.3) [1)]

— 5 ml of polyvinyl alcohol solution (F.4.4)

— 5 ml of phenylfluorone reagent (F.4.8).

Swirl the flask, avoiding foam formation from the polyvinyl alcohol. Leave to stand for 5 min.

Dilute to the mark with water and leave to stand for 30 min, and then carry out the measurement at a wavelength of 505 nm in the spectrophotometer or photocolorimeter (F.5.3).

F.6.3.3 carry out two determinations on the same prepared sample taken for the destruction of organic matter (see F.6.1)

F.6.4 Preparation of calibration curve

F.6.4.1 Into a series of six 50 ml volumetric flasks (F.5.1), each containing 20 ml of sulfuric acid solution (F.4.2), add the following volumes of standard volumetric tin solution (F.4.7)

— 0 ml, equivalent to 0 µg of tin

— 1 ml, equivalent to 10 µg of tin

— 2 ml, equivalent to 20 µg of tin

— 3 ml, equivalent to 30 µg of tin

— 4 ml, equivalent to 40 µg of tin

— 5 ml, equivalent to 50 µg of tin

F.6.4.2 Then proceed as indicated in F.6.3.2.

F.6.4.3 Prepare the calibration curve, showing the difference of optical density as a function of the number of micrograms of tin.

F.7 Expression of results

F.7.1 Method of calculation and formula

— By means of the calibration curve, convert the figure obtained in F.6.3.2 into micrograms of tin.

— The tin content, expressed in milligrams per kilogram of product, is

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$$= \frac{m_1}{1000} \times \frac{200}{V} \times \frac{1000}{m_0} = \frac{m_1 \times 200}{V \times m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass in micrograms, of tin, read from the calibration curve;

V is the volume, in millilitres, of solution A taken for colorimetric measurement F.6.3.1).

— Take as the result the arithmetic mean of the determinations, if the requirement concerning repeatability (see F.7.2) is satisfied.

F.7.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 5 % of the arithmetic mean of the two results.

Reject both results if the difference exceeds 5 % of the arithmetic mean and carry out two new single determinations.

F.8 Rest report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, together with reference to this Standard;

— all operating details not specified in this Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result(s) obtained;

— if the repeatability has been checked, the final quoted result obtained.

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Annex G
(normative)

Determination of cadmium content

The lead content shall be determined in accordance with:

ISO 6561-1:2005, *Fruits, vegetables and derived products — Determination of cadmium content — Part 1: Method using graphite furnace atomic absorption spectrometry*

ISO 6561-2:2005, *Fruits, vegetables and derived products — Determination of cadmium content — Part 2: Method using flame atomic absorption spectrometry*

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Annex H
(normative)

Determination of mercury content

The lead content shall be determined in accordance with:

ISO 6637:1984, *Fruits, vegetables and derived products — Determination of mercury content — Flameless atomic absorption method*

H.1 Scope and field of application

This annex specifies a method for the determination of the mercury content of food products.

H.2 Principle

Decomposition of organic matter in a sulfuric-nitric acid medium. Reduction of mercury (II) to metallic mercury by the action of tin (II) chloride. Entrainment of the mercury vapour by a current of air and determination by flameless atomic absorption in an enclosed apparatus.

H.3 Reagents

All the reagents shall be of recognized analytical quality and shall, with the exception of the standard mercury chloride solutions (H.3.6 and H.3.7), be free from mercury. The water used shall be distilled water free from mercury, or water of equivalent purity.

H.3.1 Sulfuric acid (ρ_{20} = 1.84 g/ml).

H.3.2 Nitric acid (ρ_{20} = 1.38 g/ml).

H.3.2 Nitric acid, 5 % *(V/V)* solution

H.3.4 Tin (II) chloride, 100 g/l solution.

H.3.5 Urea, 400 g/l solution.

H.3.6 Mercury (II) chloride, standard solution corresponding to 1 g of mercury per litre.

In a 500 ml one-mark volumetric flask, dissolve 0.6768 g of mercury (II) chloride in the nitric acid solution (3.3) and dilute to the mark with the same nitric acid solution.

1 ml of this standard solution contains 1 mg of mercury.

H.3.7 Mercury (II) chloride, standard solution corresponding to 100 µg of mercury per litre.

At the time of use, prepare a 1/10 000 *(V/V)* dilution of the standard mercury (II) chloride solution (H.3.6) in the nitric acid solution (H.3.3).

1 ml of this standard solution contains 0.1 µg of mercury.

H.4 Apparatus

The glassware used shall be washed beforehand with hot concentrated nitric acid and rinsed with water.

Usual laboratory equipment, and

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H.4.1 Mechanical grinder, the internal lining and blades of which are of polytetrafluoroethylene (PTFE).

H.4.2 Decomposition apparatus (see Figure H.1).

The apparatus is made of borosilicate glass and comprises four elements joined by conical ground joints, as follows:

a) Element (A) is a modified Soxhlet extractor of capacity 200 ml, fitted with a stop-cock and a side tube linking this element directly to the round-bottomed flask (D).

b) Element (B) is a dropping funnel of capacity 75 ml, fitted to the second neck of the round-bottomed flask (D).

c) Element (C) is a condenser 35 cm long, fitted to the top of the Soxhlet extractor (A).

d) Element (D) is a round-bottomed flask, of capacity 500 ml, fitted below the Soxhlet extractor (A), and in the second neck (30 mm from the first neck) of which is fitted the dropping funnel (B).

When the stopcock of the Soxhlet extractor (A) is open, the apparatus is in the reflux position; when it is closed, the Soxhlet extractor (A) enables the condensed water and acid vapours to be retained.

H.4.3 Flameless atomic absorption apparatus (mercury analyzer system)

The apparatus comprises a spectrometric system and determination flasks. The arrangement of the apparatus is shown in Figure H.2.

H.4.3.1 The spectrometric system comprises a mercury vapour lamp, the beam of which passes through an absorption cell. The variation in energy transmitted through the cell is measured by a phototube sensitive to ultraviolet radiation. A filter placed in front of the phototube isolates radiation of wavelength 253.7 nm. The equipment also has a read-out device.

The absorption cell shall have an internal diameter of 25 mm and a length of 115 mm and shall have silica windows.

H.4.3.2 The determination flask is equipped with a bubbler and is connected to a closed circuit, in which the metallic mercury is released and entrained by circulation of air thus ensuring, by recycling, uniform distribution of the mercury in the circulating air. The absorption cell (see H.4.3.1) is interposed in this circuit.

H.4.3.3 Any other atomic absorption apparatus having the required sensitivity may also be used.

H.4.4 Pipettes and burettes, of appropriate capacities.

H.4.5 One-mark volumetric flasks, of capacity 100 ml.

H.4.6 Analytical balance.

H.5 Procedure

H.5.1 Preparation of test sample

Mix the laboratory sample well. If necessary, first remove stones and hard seed-cavity walls, and pass the laboratory sample through the mechanical grinder (H.4.1).

Allow frozen or deep-frozen products to thaw in a closed vessel and add the liquid formed during this process to the product before mixing.

H.5.2 Test portion

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H.5.2.1 Liquid products

Transfer, by means of a pipette (H.4.4), 10 ml of the test sample (H.5.1) to the round-bottomed flask (D) of the decomposition apparatus (H.4.2).

NOTE It is also possible to take the test portion by mass by weighing, to the nearest 0.01 g, 10 g of the test sample.

H.5.2.2 Doughy, solid or dehydrated products

Transfer a mass of the test sample (H.5.1) corresponding to about 5 g of product, weighed to the nearest 0.01 g, to the round-bottomed flask (D) of the decomposition apparatus (H.4.2) and add 5 to 10 ml of water.

H.5.3 Decomposition

H.5.3.1 Decomposition of the test portion

H.5.3.1.1 Place a few glass beads in the flask (D), and connect the flask to the rest of the decomposition apparatus (H.4.2). By means of the dropping funnel (B), add, drop-by-drop, 5 ml of the nitric acid (H.3.2). Start a fast flow of water through the condenser (C) and turn the stop-cock of the soxhlet extractor (A) to the reflux position; place a metal sheet with a hole of diameter approximately 5 cm below the flask, and heat with a small flame.

Allow the reaction to proceed very gently so as to avoid any loss of mercury through the transfer of particles supplied by the nitrous vapours in the condenser. Continue decomposition under reflux for about 30 min until the liquid has a uniform appearance. If the mixture turns brown, add several drops of nitric acid (H.3.2) through the flopping funnel (B) until the colour is discharged. Allow to cool.

H.5.3.1.2 Carefully add 10 ml of a mixture of equal parts of nitric acid (H.3.2) and the sulfuric acid (H.3.1). Heat with a small flame and add nitric acid (H.3.2) drop by drop if the digest turns brown. Continue heating until fibrous matter has apparently been destroyed. Close the stop-cock of the Soxhlet extractor (A) to trap the water and acids and continue heating. The decomposition liquid will become more concentrated. If the liquid turns brown, add several drops of nitric acid (H.3.2) in just sufficient quantity to discharge the colour. Continue heating until the nitrous vapours are eliminated and there is a concentration of white fumes above the decomposition liquid.

NOTE Waxes and fats cannot be completely destroyed by hot acids.

H.5.3.1.3 Control the heating so that the white fumes do not rise more than half way up the condenser (C). The liquid shall be colourless or pale yellow. Allow to cool. Carefully entrain the water and acids collected in the Soxhlet extractor (A) into the flask (D) by opening the stop-cock. Add 5 ml of the urea solution (H.3.5) through the side neck, and boil under reflux for 30 min. Allow to cool.

H.5.3.1.4 Disconnect the apparatus and transfer the contents of the flask (D) into a volumetric flask (H.4.5). Ensure that no undigested waxes or fats are transferred. Rinse the condenser (C) and the Soxhlet extractor (A) twice with 15 to 20 ml of the nitric acid solution (H.3.3), collecting the rinsings in the flask (D) and transferring them to the volumetric flask. Carefully rinse the device twice with 10 to 20 ml of water and add the rinsings to the solution contained in the volumetric flask. Dilute to the mark with water.

H.5.3.2 Blank test

Proceed as described in H.5.3.1, but replace the test portion by 10 ml of water.

H.5.4 Determination

H.5.4.1 Transfer the decomposition liquid into a determination flask (H.4.3.2). Reduce the mercury (II) to metallic mercury by adding 5 ml of the tin (II) chloride solution (H.3.4). Immediately connect the air bubbling device and start the device, which provides the air circulation.

H.5.4.2 Measure the absorbance at 253.7 nm using the apparatus described in H.4.3.

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H.5.4.3 Proceed in the same manner using the liquid derived from the blank test (H.5.3.2), and subtract the absorbance obtained from that of the liquid derived from the test portion.

H.5.5 Preparation of the calibration graph

Into a series of six volumetric flasks (H.4.5), place 0 - 1 - 2 - 3 - 4 and 5 ml of the dilute standard mercury (II) chloride solution (H.3.7), corresponding to 0- 0.1 - 0.2 - 0.3 - 0.4 and 0.5 µg of mercury. Introduce into each flask, in the following order, about 80 ml of water, 5ml of sulfuric acid (H.3.1). Mix, leave to cool and make up to the mark with water. Transfer quantitatively into six determination flasks (H.4.3.2). Proceed as described in H.5.4.1 and H.5.4.2.

Plot a graph having, for example, the mercury contents, in micrograms, of the calibration solutions as abscissae and the corresponding values of absorbance as ordinates.

H.5.6 Number of determinations

Carry out two determinations on the same test sample (H.5.1).

H.6 Expression of results

H.6.1 Method of calculation and formulae

H.6.1.1 Test portions taken by volume

The mercury content, expressed in micrograms per litre of product as received, is equal to $\frac{1000m}{V}$

where

m is the mass, in micrograms, of mercury in the test portion, read from the calibration graph (H.5.5).

V is the volume, in millilitres, of the test portion, i.e. 10 ml.

H.6.1.2 Test portions taken by mass

The mercury content, expressed in micrograms per kilogram of product as received, is equal to $\frac{1000m}{m_1}$

where

m is the mass, in µg, of mercury in the test portion, read from the calibration graph (H.5.5).

m_0 is the mass, in grams, of the test portion.

H.6.1.3 Result

Take as the result the arithmetic mean of the values obtained in the two determinations (H.5.6), provided that the requirement for repeatability (see H.6.2) is fulfilled.

H.6.2 Repeatability

The difference between the values obtained in the two determinations (H.5.6), carried out simultaneously or in rapid succession by the same analyst on the same test sample, shall not exceed 10% of the mean.

H.6.3 Other method of expression of results

If it is wished to express the mercury content on the dry basis, modify the formulae accordingly.

H.7 Test report

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The test report shall show the method used and the result obtained. It shall also mention all operating details not specified in this standard, or regarded as optional, together with any incidents likely to have influenced the results.

The test report shall give all the information necessary for the complete identification of the sample.

Dimensions in millimetres

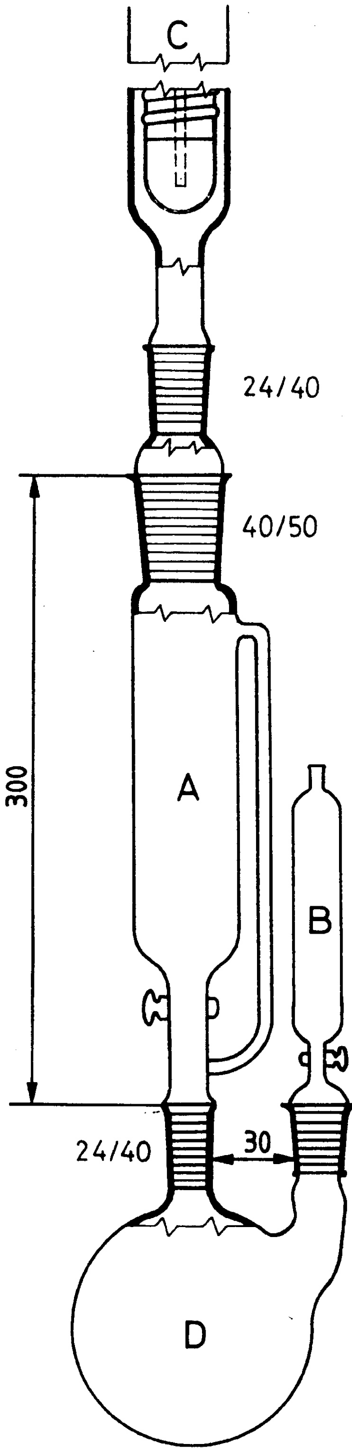

Figure H.1 — Decomposition apparatus

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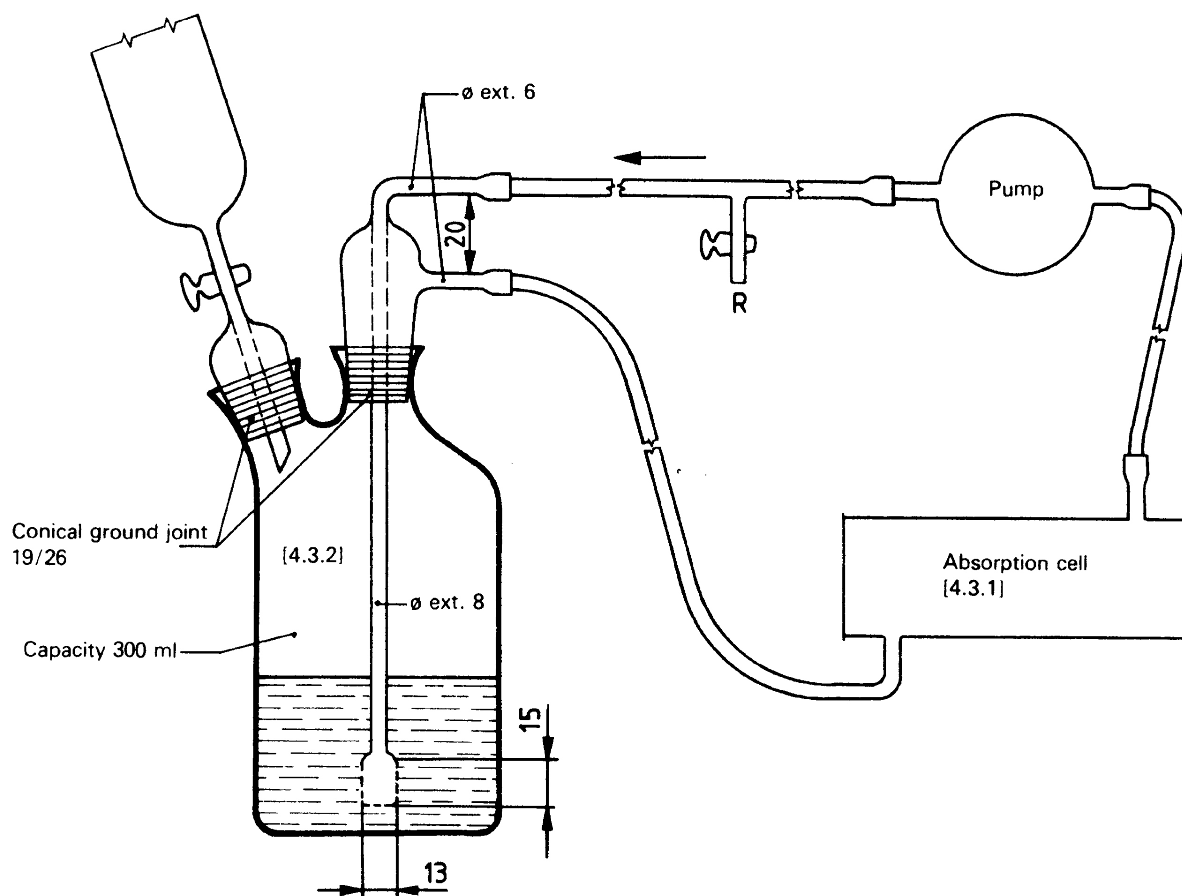

Figure H.2 — Atomic absorption apparatus

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Annex J
(normative)

Test for microbiological activity

J.1 General

J.1.1 Incubation at 37℃ — Half of the cans selected as in K.2.5 shall be incubated at 37℃ for not less than 14 days and subjected to bacteriological examination.

J.1.2 Incubation at 55℃ — The remaining half of the cans shall be incubated at 55℃ for not less than 4 days and subjected to bacteriological examination.

J.2 Apparatus

J.2.1 Glassware — All the glass apparatus used in the microbiological examination shall be sterile,

J.2.2 Accessories — Can opener of the piercing type, sampling petri-dishes, pipettes, scissors, cotton wool — all sterile. Suitable detergent for washing the cans, and rectified spirit.

J.3 Media

J.3.1 Sodium Thioglycollate broth — It has the following composition:

Dextrose	5 g
Yeast extract	5 g
Peptone	15 g
Sodium chloride	2.5 g
1 Cystine	0.75 g
Sodium thioglycollate	0.4 g
Agar-agar	0.75 g
Resazurin	0.001 g
Distilled water	1 000 ml

J.3.1.1 Preparation — Dissolve all the ingredients except resazurin in the distilled water by heating on water-bath, adjust pH to 7.3 filter, add the resazurin, distribute in 10 ml quantities in test tubes and sterilize at 1 kg/cm^3 steam pressure for 30 minutes. Warm the tubes to 80℃ for 30 minutes and immediately cool them prior to use.

J.3.2 Tryptone glucose agar with 0.5 percent sodium chloride — It has the following composition:

Beef extract	3 g
Tryptone	5 g
Dextrose	1 g
Agar-agar	15 g
Sodium chloride	5 g
Distilled water	1 000 ml

J.3.2.1 Preparation — Melt the agar with the other ingredients by steaming, Adjust pH to 7.3, filter through cotton wool, distribute in convenient quantities and sterilize at 1 kg/cm^2 steam pressure for 30 minutes. The medium is melted by heating on water-bath and cooled to 40 ℃ just before use.

J.3.3 Sulphite polymyxin sulphadiazine agar (SPS Agar) — It has the following composition:

Bacto tryptone	15 g
Bacto yeast extract	10 g
Bacto agar	15 g
Ferric citrate	0.5 g
Distilled water	1 000 ml

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J.3.3.1 Preparation — Dissolve the ingredients by steaming. Adjust the pH to 7.0 ± 0.1, filter and distribute in flasks in convenient quantities. Sterilize at 121 °0 for 15 minutes. The medium is melte d and cooled to 40 °C just before use. To each litre of the cooled medium add:

a) 5.0 ml of freshly prepared 10 percent solution of sodium sulphite ($Na_2SO_3.7H_2O$)

b) 10.0 ml of 0.1 percent solution of polymyxin B sulphate, only sterile distilled water to be used for making solutions.

J.3.4 Normal saline — 0.9 percent (*w*/*v*) of sodium chloride (analytical reagent). Distribute in 90 ml quantities and sterilize at 1 kg/cm^3 steam pressure.

J.4 Procedure

J.4.1 Preparation of the cans for test — Allow the incubated cans to cool down to room temperature. Olean the top surface of the cans with a detergent like soap water, wash them well and dry with cotton wool. Sterilize the dried surface by sprinkling rectified spirit and flaming.

J.4.2 Opening of the cans

J.4.2.1 Unswelled cans — Cut open the sterilized side of the can with a sterile can opener.

J.4.2.2 Swelled cans — Place a sterilized glass funnel over the sterilized side of the can. Introduce a sharp and sterilized metal rod through the tail end of the funnel and pierce the can. After the pressure is released cut open the can with a sterile can opener.

J.5 Procedure

J.5.1 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.

J.5.2 Total aerobic plate count — Transfer aseptically 10 g of the solid portion from the centre of the can into a sterile petri dish. Prepare a homogenate of the material with 90 ml of the normal saline under asceptic conditions. Transfer 1 ml each of the homogenate to two sterile petri dishes. Add nearly 10 ml of the melted and cooled agar, mix well and incubate the dishes at 37 °C for 48 hours, after the agar has solidified. Count the number of colonies and compute the number of organisms per gram. Not less than 90 percent of the samples shall be sterile, no sample shall have bacterial count above 100/g.

J.5.3 Examination for *Clostridia* — One millilitre of the liquid portion (J.5.1) is transferred to sodium thioglycollate broth (J.3.1) using sterile pipette and incubated at room temperature for 2 days. Positive tubes may be tested for clostridia using SPS Agar (J.3.3) as given in J.5.3.1.

J.5.3.1 One millilitre of the inoculam from sodium thioglycollate broth is mixed with approximately 10 ml of SPS agar, allowed to solidify in the petri dish and the plate is incubated at room temperature for 2 to 4 days, under anaeorbic conditions using spray's dish or keeping the plate in an enclosed atmosphere consisting of 80 percent nitrogen and 20 percent carbon dioxide. Black colonies show the presence of *clostridium* spp.

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Annex K
(normative)

Sampling of pomfret canned in oil

K.1 General requirements for sampling

K.1.1 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

K.1.2 Samples may be tested at a laboratory agreed to between the purchaser and the vendor.

K.2 Scale of sampling

K.2.1 Lot — In any consignment, all the cases containing cans of the same size and from the same batch of manufacture shall be grouped together to constitute a lot.

K.2.1.1 Samples shall be tested for each lot for ascertaining conformity of the material to the requirements of this standard.

K.2.2 The number of cases to be selected from each lot shall be in accordance with col 1 and 2 of Table K.1.

TABLE K.1 — Selection of packing cases

Number of cartons in the lot	Sample Size
(1)	(2)
Up to 8	2
9 to 25	4
26 to 40	5
41 to 65	6
66 to 110	7
111 to 180	8
181 to 300	9
301 to 500	10

K.2.3 The cases shall be selected at random. In order to ensure randomness of selection, random number tables shall be used. In case such tables are not available, the following procedure may be adopted:

> Starting from any case count them as 1, 2, *3, ... r* and so on in a systematic manner. Every *r*th case thus counted shall be withdrawn, r being the integral part of *N/n,* where *N* is the total number of cases in the lot, and *n* the number of cases to be selected, till the requisite number is obtained.

K.2.4 From each of the cases selected as in K.2.2, draw at random one can for testing the physical and chemical requirements.

K.2.5 In addition to the cans selected as in K.2.4, 8 cans shall be selected at random as far as possible from all the cases chosen (see K.2.2), for testing for microbiological activity.

K.3 Number of tests

K.3.1 Each of the cans selected as in K.2.4 for testing the physical and chemical requirements shall

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be tested for vacuum.

K.3.2 After testing for vacuum the contents of all the cans shall be taken and mixed together to form a composite sample. The composite sample so formed shall be tested for heavy metals.

K.3.3 Tests for microbiological activity

K.3.3.1 Incubation at 37 °C — All the cans selected (see K.2.5) for testing the microbiological activity, shall be incubated at 37 °C for not less than 14 days and subjected to microbiological examination (see Annex J).

K.4 Criteria for conformity

K.4.1 The lot shall be considered as conforming to the requirements of this specification if the criteria mentioned under K.4.1 to K.4.4 are all satisfied.

K.4.2 For Physical Requirements — The lot shall be declared as conforming to the physical requirements of vacuum if all the individual samples tested (see K.3.1) satisfy the requirements specified in 2.7.5.

K.4.3 For Chemical reagents — For declaring the conformity of the lot to the chemical requirements regarding heavy metals, the test results on the composite sample (*see* K.3.2) shall satisfy the relevant requirements specified in 2.7.7.

K.4.4 For Microbiological activity — For declaring the conformity of the lot to microbiological activity the test results (*see* K.3.3.1) shall satisfy the requirements specified in 2.7.7.

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