



EAST AFRICAN STANDARD

Quick frozen finfish, eviscerated or uneviscerated — Specification



EAST AFRICAN COMMUNITY

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Introduction

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

CODEX STAN 36:1981(Rev. 1:1995), *Standard for quick frozen finfish, eviscerated or uneviscerated*

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.mrlatabase.com>

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

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

Assistance derived from these sources is hereby acknowledged.

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Quick frozen finfish, eviscerated or uneviscerated — Specification

1 Scope

This East African Standard applies to frozen finfish uneviscerated and eviscerated. It does not apply to fish frozen in brine intended for further processing.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CD/K/572:2010, *Fish and fisheries products — Methods of sampling*

CAC/RCP 52[CD/K/521:2010], *Code of practice for fish and fishery products*

EAS 35, *Edible salt — Specification*

EAS 12, *Drinking (potable water) — Specification*

EAS 38, *Labelling of prepackaged foods — Specification*

EAS 41, *Fruits, vegetables and derived products — Sampling and methods of test*

EAS 103, *Schedule for permitted food additives*

EAS 123, *Distilled water — Specification*

ISO 4831, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 degrees C*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7937, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique*

ISO 13720, *Meat and meat products — Enumeration of Pseudomonas spp.*

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

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

ISO 21567, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.*

ISO/TS 21872-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 1: Detection of Vibrio parahaemolyticus and Vibrio cholerae*

ISO/TS 21872-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 2: Detection of species other than Vibrio parahaemolyticus and Vibrio cholerae*

ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method*

ISO 11290-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 2: Enumeration method*

3 Description

3.1 Product definition

Frozen finfish suitable for human consumption, with or without the head, from which the viscera or other organs may have been completely or partially removed.

3.2 Process definition

The product, after any suitable preparation, shall be subjected to a freezing process and shall comply with the conditions laid down hereafter. The freezing process shall be carried out in appropriate equipment in such a way that the range of temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18°C or colder at the thermal centre after thermal stabilization. The product shall be kept deep frozen so as to maintain the quality during transportation, storage and distribution.

Industrial repacking of quick frozen products under controlled conditions which maintain the quality of the products followed by the reapplication of the quick freezing process is permitted.

Quick frozen finfish, shall be processed and packaged so as to minimize dehydration and oxidation.

3.3 Presentation

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

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

4 Essential composition and quality factors

4.1 Fish

Quick frozen finfish shall be prepared from sound fish which are of a quality fit to be sold fresh for human consumption.

4.2 Glazing

If glazed, the water used for glazing or preparing glazing solutions shall be of potable quality or shall be clean sea-water. Potable water is fresh-water fit for human consumption complying with EAS 12. Clean sea-water is sea-water which meets the same microbiological standards as potable water and is free from objectionable substances.

4.3 Other ingredients

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

4.4 Decomposition

The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested. This shall apply only to species of *Clupeidae*, *Scombridae*, *Scombresocidae*, *Pomatomidae* and *Coryphaenidae* families.

4.5 Final product

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

5 Food additives

Only the use of the following additives is permitted.

Additive	Maximum level in the final product
<u>Antioxidants</u>	
300 Ascorbic acid	GMP
301 Sodium ascorbate	
303 Potassium ascorbate	

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 outlined in the standards listed in Clause 2:

- (i) shall be free from micro-organisms capable of development under normal conditions of storage; and
- (ii) shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health;
- (iii) shall be free from container integrity defects which may compromise the hermetic seal; and
- (iv) shall not contain histamine that exceeds 20 mg/100 g. This applies only to species of *Clupeidae*, *Scombridae*, *Scombrosocidae*, *Pomatomidae* and *Coryphaenidae* families.

6.3 The product covered by the provisions of this standard shall be prepared and handled in accordance with the appropriate sections of CAC/RCP 1 and CAC/RCP 52.

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

Table 1 — Microbiological and heavy metal limits for canned tuna and bonito

Type of contaminant		Maximum limit (mg/kg)	Method of test
(i)	Microbiological requirements	Shall be commercially sterile	See J.5.1; CD/K/551:2010
(i)	Arsenic	1.0	EAS 41
(iii)	Copper	0.4	EAS 41
(iv)	Tin	250.0	EAS 41
(v)	Mercury	0.5	EAS 41
(vi)	Lead	0.3	EAS 41
(vii)	Cadmium	0.3	EAS 41
(viii)	Zinc	50.0	EAS 41
(ix)	Histamine content, mg/100 crab meat, max	20.0	Annex B

7 Labelling

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

7.1 Name of the food

7.1.1 In addition to the common or usual name of the species, the label, in the case of eviscerated fish, shall include terms indicating that the fish has been eviscerated and whether presented as "head-on" or "headless".

7.1.2 If the product has been glazed with sea-water, a statement to this effect shall be made.

7.1.3 The term "quick frozen", shall also appear on the label, except that the term "frozen" may be applied in countries where this term is customarily used for describing the product processed in accordance with subsection 3.2 of this standard.

7.1.4 The label shall state that the product should be maintained under conditions that will maintain the quality during transportation, storage and distribution.

7.2 Net contents (glazed products)

Where the food has been glazed the declaration of net contents of the food shall be exclusive of the glaze.

7.3 Storage instructions

The label shall include terms to indicate that the product shall be stored at a temperature of -18°C or colder.

7.4 Labelling of non-retail containers

Information specified above shall be given either on the container or in accompanying documents, except that the name of the food, lot identification, and the name and address, as well as storage instructions shall always appear on the container.

However, lot identification, and the name and address may be replaced by an identification mark, provided that such a mark is clearly identifiable with the accompanying documents.

8 Sampling, examination and analyses

8.1 Sampling

- (i) Sampling of lots for examination of the final product as prescribed in Section 3.3 shall be in accordance with the FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods (1969) (AQL-6.5) (Ref. CAC/RM 42-1969).
- (ii) Sampling of lots for examination of net weight and drained weight shall be carried out in accordance with an appropriate sampling plan meeting the criteria established by the CAC.

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 the *Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories (CAC/GL 31 - 1999)*.

8.3 Determination of net weight

Net weight of all sample units shall be determined by the following procedures:

- (i) Weigh the unopened container.
- (ii) Open the container and remove the contents.
- (iii) Weigh the empty container, including the end and any wrapping material, after removing excess liquid and adhering flesh.
- (iv) Subtract the weight of the empty container and any wrapping material from the weight of the unopened container. The resultant figure is the net content.

8.4 Determination of drained weight

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

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

CD/K/531:2010

- (ii) Open the container and distribute the contents on a pre-weighed circular sieve having a wire mesh with square openings of 2.8 mm x 2.8 mm.
- (iii) Remove all wrapping material and incline the sieve at an angle of approximately 17-20° and allow the fish to drain two minutes, measured from the time the product is poured onto the sieve.
- (iv) Weigh the sieve containing the drained fish.
- (v) Determine the weight of drained fish by subtracting the mass of the sieve from the mass of the sieve with drained product.

8.5 Thawing

8.6 Determination of Gelatinous Conditions

According to the AOAC Methods- "Moisture in Meat and Meat Products, Preparation of Sample Procedure"; 883.18 and "Moisture in Meat" (Method A); 950.46; AOAC 1990.

8.7 Cooking methods

The following procedures are based on heating the product to an internal temperature of 65-70°C. The product must not be overcooked. Cooking times vary according to the size of the product and the temperatures used. The exact times and conditions of cooking for the product should be determined by prior experimentation.

Baking Procedure: Wrap the product in aluminum foil and place it evenly on a flat cookie sheet or shallow flat pan.

Steaming Procedure: Wrap the product in aluminum foil and place it on a wire rack suspended over boiling water in a covered container.

Boil-In-Bag Procedure: Place the product into a boilable film-type pouch and seal. Immerse the pouch into boiling water and cook.

Microwave Procedure: Enclose the product in a container suitable for microwave cooking. If plastic bags are used, check to ensure that no odour is imparted from the plastic bags. Cook according to equipment specifications.

8.8 Determination of histamine

See Annex B.

9 Definition of defects

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

9.1 Deep dehydration

Greater than 10% of the surface area of the block or greater than 10% of the weight of fish in the sample unit exhibits excessive loss of moisture clearly shown as white or yellow abnormality on the surface which masks the colour of the flesh and penetrates below the surface, and cannot be easily removed by scraping with a knife or other sharp instrument without unduly affecting the appearance of the fish.

9.2 Foreign matter

The presence in the sample unit of any matter which has not been derived from fish (excluding packaging material), does not pose a threat to human health, and is readily recognized without

magnification or is present at a level determined by any method including magnification, that indicates non-compliance with good manufacturing and sanitation practices.

9.3 Odour/flavour

A sample unit affected by persistent and distinct objectionable odours or flavours indicative of decomposition or of feed.

9.4 Texture

- (i) Textural breakdown of the flesh, indicative of decomposition characterized by muscle structure which is mushy or paste-like, or by separation of flesh from the bones.
- (ii) **Flesh abnormalities** — A sample unit affected by excessive gelatinous condition of the flesh together with greater than 86% moisture found in any individual fish or sample unit with pasty texture resulting from parasitic infestation affecting more than 5% of the sample unit by weight.

9.5 Belly burst

The presence of ruptured bellies in uneviscerated fish, indicative of decomposition.

9.5 Objectionable matter

A sample unit affected by struvite crystals — any struvite crystal greater than 5 mm in length.

10 Lot acceptance

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

- (i) the total number of defectives as classified according to Section 9 does not exceed the acceptance number (c) of the appropriate sampling plan in the Sampling Plans for Prepackaged Foods (AQL-6.5) (CAC/RM 42-1977);
- (ii) the total number of sample units not meeting the form of presentation defined in Section 3.3 does not exceed the acceptance number (c) of the appropriate sampling plan in the Sampling Plans for Prepackaged Foods (AQL-6.5) (CAC/RM 42-1977);
- (iii) the average net weight and the average drained weight where appropriate of all sample units examined is not less than the declared weight, and provided there is no unreasonable shortage in any individual container.
- (iv) the Food Additives, Hygiene and Labelling requirements of Sections 5, 6.1, 6.2 and 7 are met.



Eviscerated fish



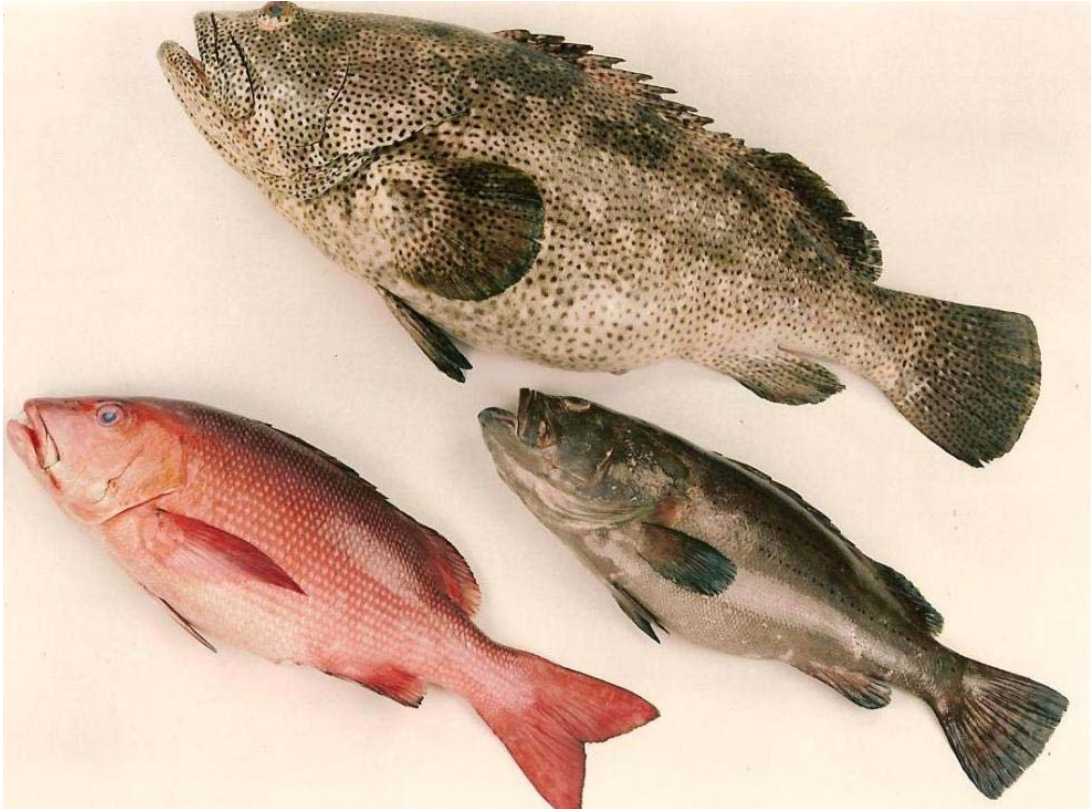
Eviscerated fish



Fish —Eviscerated

Draft for comment

ward



Whole round fish



Gutted head-on

Draft for comments



Frozen whole fish

Draft for comments only

Standard

Draft for



Reef Cod, Pomfort, Seer Fish and Mackerel



Frozen catfish

Draft for



frozen seabass, seabream and trout



Fresh Water Frozen Fish



Whole croaker, scaled, gutted



Frozen whole tilapia



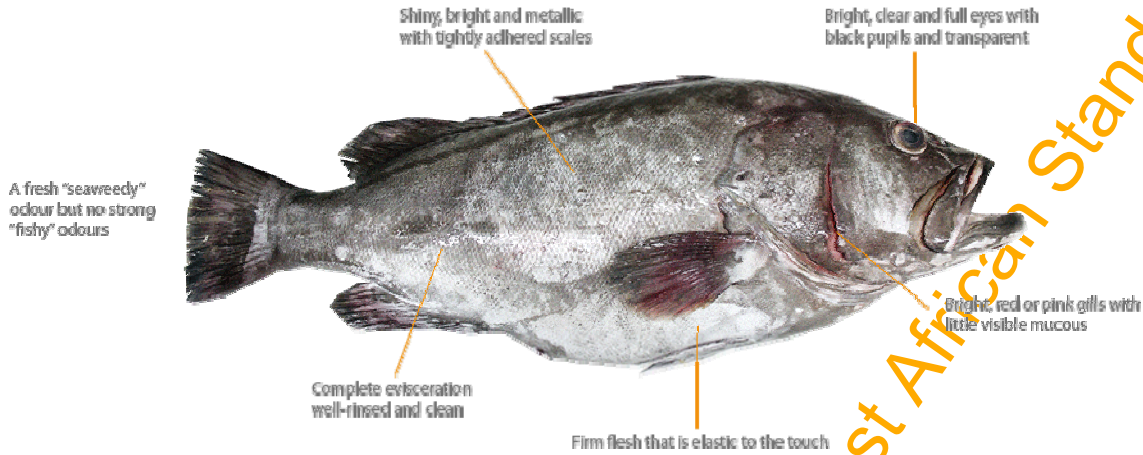
Frozen whole tilapia



Frozen whole sardines

Draft for

Standard



Frozen whole pangasius

Draft for comments only — Not

Annex A
(normative)

Sensory and physical examination

1. Complete net weight determination, according to defined procedures in Section 8.3 (de-glaze as required).
2. Examine the frozen sample unit for the presence of deep dehydration by measuring those areas or counting instances which can only be removed with a knife or other sharp instrument. Measure the total surface area of the sample unit, and calculate the percentage affected.
3. Thaw and individually examine each fish in the sample unit for the presence of foreign matter.
4. Examine each fish using the criteria outlined in Section 9. Flesh odours are examined by tearing or making a cut across the back of the neck such that the exposed surface of the flesh can be evaluated.
5. In cases where a final decision regarding the odour or texture cannot be made in the thawed uncooked state, a small portion of the flesh (approximately 200 g) is sectioned from the product and the odour, flavour or texture confirmed without delay by using one of the cooking methods defined in Section 8.5.
6. In cases where a final decision on gelatinous condition cannot be made in the thawed uncooked state, the disputed material is sectioned from the product and gelatinous condition confirmed by cooking as defined in Section 8.6 or by using the procedure in Section 8.5 to determine if greater than 86% moisture is present in any fish. If a cooking evaluation is inconclusive, then the procedure in 8.5 would be used to make the exact determination of moisture content.

Annex B (normative)

Determination of histamine

B.1 Introduction

B.1.1 Principle — Histamine is extracted with methanol and derivatized with o-phthalaldehyde (OPT) to generate the fluorescent product. This method is used to determine the histamine content in raw, pre-cooked, and canned tuna.

B.1.2 Interference — All methods of histamine determination are overwhelmed with interfering substances which have to be removed in order to accurately measure the histamine present. The two naturally occurring substances that cause the most interference are histamine and spermidine since they also react with OPT to form fluorescent products. However, spermidine, the major contaminant in extracts can be separated from histamine on cellulose phosphate cation-exchange columns. There is also variability due to the pH and temperature sensitivity of the o-phthalaldehyde-histamine fluorophor. Because of the ubiquity of interfering fluorophors, all reagents used must be of the highest obtainable purity. Exposure of any of the materials involved to rubber or silicones may produce erratic results. It is recommended that polyethylene labware be used in place of glass, due to an observed loss of fluorescence. All labware should be acid-washed and rinsed in distilled water prior to use. New solution must be prepared after four to seven days, due to an observed increase in blank readings.

B.1.3 Summary of method — The histamine-containing material are homogenized and extracted with methanol. The extract can then be passed through an anion exchange column to remove any remaining interfering substances. The elutant is reacted with the OPT reagent and allowed to stand for 4 minutes. The mixture is acidified with H_3PO_4 and the corresponding fluorescence is read on a calibrated instrument.

B.2 Material required

TD-360 Min-Fluorometer with U.V. optical configuration of (P/N 36000-010) 10 mm x 10 mm Methacrylate fluorescence cuvettes (P/N 7000-959).

B.2.1 Labware — All re-usable labware (glass, polyethylene, Teflon etc.) should be cleaned by soaking in laboratory grade detergent and water for 4 h, rinsed with tap water, deionized water, and methanol. It is recommended that polyethylene ware be used due to absorbency observed when using glass.

B.2.1.1 Assorted Class A calibrated pipettes

B.2.1.2 Graduated cylinder — 100 ml.

B.2.1.3 Assorted Volumetric Flasks — For preparing dilution standards.

B.2.2 Chromatographic Columns (Kontes M.K 422250).

B.3 Reagents and standards

B.3.1 Ion Exchange Resin — Sigma 1 x 8-200, chloride form 100-200 mesh: or BioRad AG1- x 8, 50-100 mesh, chloride form, Cat. No. 140-1431, or equivalent.

B.3.2 Ion Sodium Hydroxide — Dissolve 40 g NaOH in 1 Litre of distilled water.

B.3.3 2.0N Sodium Hydroxide — Dissolve 80 g NaOH in 1 Litre of distilled water.

B.3.4 Histamine Dihydrochloride — MCB X 0440 or J.T. Baker 1-N330.

B.3.5 1.0N Hydrochloric Acid — Add 83 ml concentrated HCL to about 500 ml distilled water. Cool and bring to 1-litre volume with distilled water.

B.3.6 0.1N Hydrochloric Acid — Add 100-mL 1N HCl to about 500-mL distilled water. Cool and bring to 1-Litre volume with distilled water.

B3.7 Methanol Reagent Grade

B3.8 0.1 % o-phthalaldehydol (OPT reagent) — Phthalic dicarboxaldehyde (Aldrich, Milwaukee, WI), or o-phthaldialdehyde (Sigma, St. Louis, MO) $C_6H_4(CHO)_2$. F.W, 134.13. Dissolve 0.10 g OPT in 100-mL methanol. Store in an amber bottle and refrigerate when not in use. Prepare fresh weekly.

B.3.9 3.57N Phosphoric Acid — Add 121.8 ml of 85 % H_3PO_4 to about 500-mL distilled water. Bring to 1- litre volume with distilled water.

B.3.10 Histamine Standard Solution A, 1 mg Hm/ml — Weigh 0.1656 g of histamine dihydrochloride into 100-ml volumetric flask. Dissolve in, and dilute to volume with 0.1N HCl.

B.3.11 Histamine Standard Solution B, 10 µg Hm/ml — Dilute 1.0 ml Solution A to 100 ml with 0.1N HCL.

B.3.12 Histamine Standard Solution A1 (This is our control solution) — Dilute 1.0 ml Solution A to 100 ml with methanol.

B.3.13 Histamine Standard Solution C, 0.1 mg Hm/ml — Dilute 1.0 ml Solution B to 100 ml with 0.1N HCl.

B.3.14 Histamine Standard Solution D, 0.2 M 0.2 M Hm/ml — Dilute 2.0 ml Solution B to 100 ml with 0.1 N HCl.

B.3.15 Histamine Standard Solution E, 0.3 mg Hm/ml — Dilute 3.0 ml Solution B to 100 ml with 0.1N HCl.

NOTE Prepare Solution A and B monthly. Prepare Solutions C, D, E, and A1 weekly. Refrigerate solutions when not in use.

B.4 Preparation

B.4.1 Resin preparation

B.4.1.1 Place 20 g of ion exchange resin in a beaker.

B.4.1.2 Add 2 N sodium hydroxide to the resin in a ratio of 15 ml per gram of resin.

B.4.1.3 Mix well and allow the resin to settle for a minimum of 15 minutes, but no more than 30 minutes. Decant liquid and repeat with additional 2 N sodium hydroxide.

B.4.1.4 Wash resin thoroughly with distilled water to remove traces of the sodium hydroxide until pH is less than or equal to 8.5.

B.4.1.5 Slurry resin with distilled water and transfer to a funnel containing a fluted filter paper. Thoroughly wash with distilled water.

B.4.1.6 Transfer resin to a suitable container and make sure the distilled water level is above the resin level at all times.

B.4.2 Column preparation

B.4.2.1 Slurry sufficient prepared resin into each column to form a bed 8 cm in height. Maintain a liquid level above the top of the resin at all times.

B.4.2.2 Refill columns with fresh resin at least twice per week.

B.5 Instrument set-up

B.5.1 Check that light source and filter holder are installed in your TD-360 Mini-Fluorometer. Turn on the instrument and allow to warm-up. For additional assistance, refer to your TD-360 Operating Manual.

B.5.2 Blank with a reagent blank — Calibrate instrument with the prepared histamine standard Solution E. Enter standard value of 3 000 mg/lm. Remember later to divide all reading by 10 000 to get mg Hm/ml of sample.

B.5.3 Analyze Histamine Standard Solutions C and D like you would a sample. You now have a standard curve for your samples.

B.6 Procedure

B.6.1 Sample preparation

B.6.1.1 Blend fish in a warring blender with an equal weight of deionized water to produce a 1:1 slurry.

B.6.1.2 Transfer 10.0 g of the slurry to a 150-ml beaker. Add 40.0 ml of methanol and mix thoroughly.

B.6.1.3 Using Whatman No.1 filter paper, or equivalent, filter the mixture into a suitable container. If the filtrate is to be saved for later analysis, refrigerate in a closed container.

NOTE Evaporation of methanol from the filtrate can cause erroneous results.

B.6.2 Histamine Elusion

B.6.2.1 Pass 15-20-ml distilled water through the exchange column and discard.

B.6.2.2 Place a 50-ml volumetric flask containing 5 ml in HCl at the column outlet.

B.6.2.3 Pipette 1.0 ml of filtrate (methanol extract) onto the resin bed with 5.10 ml distilled water.

B.6.2.4 Immediately initiate column flow. Flow should be maintained at a rate grater than 3 ml/min.

B.6.2.5 When liquid level is slightly above the resin, add about 5-ml distilled water and allow it to flow through the resin. Repeat with distilled water in larger increments until total water through column is about 40 ml.

B.6.2.6 Discontinue Column Flow

B.6.2.7 Remove volumetric flask and bring to 50-ml volume with distilled water. Store column effluent in the refrigerator if necessary to postpone determination for more than 2 h.

B.6.3 Controls and blanks

B.6.3.1 At the beginning of a set of analysis, and again at the end, pass 1 ml of Solution A1 through one of the columns and proceed through the procedure as though it were a fish extract. Fluorescence readings should be very similar to Solution D reading. If readings are not within 20 per cent of Solution D, all analysis performed at the same time are suspect and should be repeated.

B.6.4 Histamine determination

B.6.4.1 Into separate 25-ml glass stoppered flask, pipette 5.0 ml of 0.1 HCl (Blank); Solutions C, D and E: and each diluted column effluent.

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B.6.4.2 Add 10 ml 0.1N HCl to each flask.

B.6.4.3 Add 3 ml in NaOH. Mix thoroughly.

B.6.4.4 Within 5 minutes, add 1 ml OPT solution and mix thoroughly.

B.6.4.5 After exactly 4 minutes, add 3 ml 3.57 N H₃PO₄ and mix immediately.

B.6.4.6 Let solutions stand for 15-20 minutes and then determine the fluorescence intensities on the TD-360 Min-fluorometer. If a sample reading is greater than that of Solution E, dilute 25 ml of the column effluent to 100 ml with 0.1N HCl and proceed from B.6.4.1.

CAUTION! Fish with high salt content may cause problems with the resin necessitating more frequent changing of columns.

B.6.4.7 If sample dilution was necessary in B.6.4.6, multiply the obtained result by 4.

B.6.4.8 After all readings are obtained, divide all results by 10, 1 000 to get histamine concentration in mg Hm/ml

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Annex C
(normative)

Determination of moisture in meat

C.1 Drying in vacuo at 95–100°C

Dry test portion containing ca 2 g dry material to constant weight at 95–100°C under pressure ≤100 mm Hg (ca 5 h). For feeds with high molasses content, use temperature ≤70°C and pressure ≤50 mm Hg. Use covered Al dish ≥50 mm diameter and 40 mm deep.

C.2 Air drying

C.2.1 With lids removed, dry test sample containing ca 2 g dry material 16–18 h at 100–102°C in air oven (mechanical convection preferred). Use covered Al dish ≥50 mm diameter and ≤40 mm deep. Cool in desiccator and weigh. Report loss in weight as moisture, g.

C.2.2 With lids removed, dry test sample containing ca 2 g dry material to constant weight (2–4 h depending on product) in mechanical convection oven or in gravity oven with single shelf at ca 125°C. Use covered Al dish ≥50 mm diameter and ≤40 mm deep. Avoid excessive drying. Cover, cool in desiccator, and weigh. Report loss in weight as moisture, g. (Dried test sample is not satisfactory for subsequent fat determination.)

Report loss on drying (LOD) as estimate of moisture content.

C.3 Calculations

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

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

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