

EAST AFRICAN STANDARD

Canned shellfish — Specification — General



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

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

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

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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Canned shellfish — Specification — General

1 Scope

This East African Standard applies to canned shellfish in hermetically sealed containers. It is intended to be used for the inspection of canned shellfish products for which specific product standards have not been elaborated.

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

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

Shellfish is a culinary and fisheries term for exoskeleton-bearing aquatic invertebrates used as food, including various species of molluscs, crustaceans, and echinoderms. Although most kinds of shellfish are harvested from saltwater environments, some kinds are found only in freshwater. In addition a few species of land crabs such as *Cardisoma guanhumi*, are eaten, for example in the Caribbean.

Familiar marine molluscs enjoyed as a food source by humans include many species of clams, mussels, oysters, winkles, and scallops. Some crustaceans commonly eaten are shrimp, prawn,

lobster, crayfish, and crabs. Echinoderms are not as frequently harvested for food as molluscs and crustaceans, but sea urchin roe is quite popular in many parts of the world. Most shellfish eat a diet composed primarily of phytoplankton and zooplankton. Shellfish are among the most common food allergens.

The term *shellfish* is used both broadly and specifically. In common parlance, as in having "shellfish" for dinner, it can refer to anything from clams and oysters to lobster and shrimp. For regulatory purposes it is often narrowly defined as *filter-feeding molluscs* such as clams, mussels, and oyster to the exclusion of crustaceans and all else.

Although the term is primarily applied to marine species, edible freshwater invertebrates such as crayfish and river mussels are also sometimes grouped under the umbrella of "shellfish". Although their shells may differ, all shellfish are invertebrates. As non-mammalian animals that spend their entire lives in water they are "fish" in the logical sense; however the denotation *finfish* (or *finnickle fish*) has been developed to distinguish fish as animals *defined by having vertebrae* from shellfish in modern terminology.

The word "shellfish" is both singular and plural; the rarely used "shellfishes" is sometimes employed to distinguish among various types of shellfish.

Archaeological finds has shown that humans have been making use of shellfish as a food item for thousands of years. In the present, shellfish dishes are a feature of almost all the cuisines of the world, providing an important source of protein in many cuisines around the world, especially in the countries with coastal areas.

3.2 Forms of product presentation

This product may be prepared from shellfish, cooked or not, smoked or unsmoked, whole (unshelled or with the half-shell removed) or shucked meats, which have been culled, washed, trimmed if necessary and packed in a container with brine, own juice and/or other suitable food grade packing media.

3.3 Other presentations

Any other presentation of the product may be permitted provided that it:

- a) is sufficiently distinctive from the forms of presentation set out above;
- b) meets all other regulatory requirements; and
- c) is adequately described on the label and in accordance with all regulatory labelling requirements.

4 Essential composition and quality factors

4.1 Shellfish

Canned shellfish shall be prepared from sound shellfish of the species designated in 3.1 which are alive immediately prior to the commencement of processing and of a quality suitable for human consumption.

4.2 Other ingredients

The packing medium and all other ingredients used shall be of food grade quality and conform to all applicable East African Standards.

4.3 Final product

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

5 Food additives

Only the use of the following additives is permitted.

Additive		Maximum Level in the final product
<u>Acidity Regulators</u>		
330	Citric acid	GMP 10 mg/kg expressed as P ₂ O ₅ , singly or in combination (includes natural phosphate)
338	Orthophosphoric acid	
450	Disodium diphosphate	
<u>Sequestrant</u>		
385	Calcium disodium EDTA	250 mg/kg
<u>Flavour Enhancer</u>		
621	Monosodium glutamate	GMP

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 prescribed by the Codex Alimentarius Commission (CAC), the product:

- (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 in accordance with standards established by the CAC; and
- (iii) shall be free from container integrity defects which may compromise the hermetic seal.

6.3 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of CAC/RCP 1 and 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 The name of the product shall be that recognized in common usage in trade, and in accordance with any requirements of the applicable Codex Alimentarius Recommended International Standard.

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

8 Sampling, examination and analyses

8.1 Sampling

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

8.1.2 Sampling of lots for the sensory examination of the product shall be in accordance with CD-K-572:2010 except that a lower acceptance number for decomposition shall be used as indicated in the sampling tables. The tables specify the minimum number of sample units to be used for the following types of inspections:

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

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

8.2 Sensory and physical examination

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

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 meat.
- (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.
- (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 meat to drain two minutes, measured from the time the product is poured onto the sieve.
- (iv) Weigh the sieve containing the drained shellfish.
- (v) Determine the weight of drained shellfish by subtracting the mass of the sieve from the mass of the sieve with drained product.

8.5 Examination

8.5.1 Complete external can examination. Open can and perform net and/or drained weight determination, according to defined policies and procedures for these examinations.

8.5.2 Carefully remove product from can to examination tray. Examine can interior for presence of foreign material, smut, struvite, and corrosion or other can interior defects.

8.5.3 Inspect liquid and shellfish for presence of foreign material, smut, or struvite.

8.5.4 Observe colour of flesh and presence of discolouration in shellfish.

8.5.5 Assess odour. Assess flavour and texture as required.

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

8.5.7 Classification of defectives

A sample unit which contains defects as described in Clause 9 is classified as a "defective".

9 Definition of defects

9.1 Taint

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

- a) **Rancid** — 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 iodine, burnt or acrid, ash-like, 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, musty, yeasty, sour, faecal, ammonia, hydrogen sulphide, and putrid.
- b) **Discolouration** — Discolouration associated with decomposition which is uncharacteristic of the species and type of pack. Depending on the species, abnormal colours may include blue, black, green, grey, or yellowish to orange colours.
- c) **Texture** — Breakdown of muscle structure due to decomposition as characterized by:
 - very tough, rubbery, or dry conditions; or

— very soft, mushy, or pasty conditions.

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 shellfish (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 shellfish (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 shellfish (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 of the meat in excess of 5% of the drained contents.

10 Lot acceptance

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

- (i) not any single instance of critical foreign matter occurs; or
- (ii) the total number of sample units found defective for taint, decomposition or unwholesomeness, individually or in combination, does not exceed the acceptance number for the sample size designated in the sampling plans in CD-K-572:2010; or
- (iii) the total number of sample units found defective for decomposition does not exceed the acceptance number (c) shown in parentheses for the sample size designated in the sampling plans in CD-K-572:2010; or
- (iv) the average net weight and the average drained weight of all sample units examined is not less than the declared weight and provided there is no unreasonable shortage in any individual container;
- (v) the Food Additives, Hygiene and Labelling requirements of 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.

Annex A
(normative)

Sensory and physical examination

1. Complete external can examination for the presence of container integrity defects or can ends which may be distorted outwards.
2. Open can and complete weight determination according to defined procedures in 8.3 and 8.4.
3. Examine product for discolouration, foreign and objectionable matter.
4. Assess odour, flavour and texture in accordance with CAC/GL 31

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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 μ g 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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