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SPECIFICATION FOR QUICK FROZEN WHOLE FISH, FISH FILLETS, STEAKS AND MINCED FISH

SRI LANKA STANDARDS INSTITUTION

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SLS 1141 : 1996

Gr. 10

SRI LANKA STANDARDS INSTITUTION
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Sri Lanka.

Sri Lanka Standard
SPECIFICATION FOR QUICK FROZEN WHOLE FISH, FISH FILLETS, STEAKS AND MINCED FISH

FOREWORD

This standard was finalized by the Sectoral Committee on Agriculture and Food Technology - 2 and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 1996-10-17.

The seasonal nature of the fishery industry and high demand for fish in cities away from the coastal area, necessitate preservation of fish by freezing. Freezing fish allows them to be kept in good condition for comparatively long periods. Fish, that can be kept in ice for only a matter of days will remain wholesome for many months once frozen. However, care is still needed to produce a high quality product.

Presently fish is frozen as whole, or presented in any other style in consumer packs of various masses.

In order to establish and maintain high quality standards and to assist the industry to exercise proper quality control procedures, the formulation of this specification was considered necessary.

Guidelines for the determination of a compliance of a lot with the requirements of this standard based on statistical sampling and inspection are given in Appendix A.

During the formulation of this specification due consideration has been given to the relevant provisions made under the Sri Lanka Food Act No. 26 of 1980. Specific requirement given in this specification, wherever applicable, are in accordance with the relevant regulations. However, general provisions made under the Sri Lanka Food Act have not been included in this specification and therefore, the attention of the user of this specification is drawn to the general provisions made in the regulations framed under the food Act.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or an analysis, shall be rounded off in accordance with CS 102. The number of significant figures to be retained in the rounded off value shall be the same as that of the specified value in this standard.

In the preparation of this standard, the assistance derived from the following publications is gratefully acknowledged:

i) CODEX STAN 165 : 1989 Standard for quick frozen blocks of fish fillets, minced fish flesh and mixtures of fillets and minced fish flesh.

ii) IS 10763 : 1983 Indian Standard Specification for frozen minced fish meat.

1 SCOPE

This specification prescribes the requirements and methods of test for quick frozen whole fish, fish fillets, steaks and minced fish which are intended for further processing.

2 REFERENCES

SLS 79 Edible common salt
CS 102 Presentation of numerical values
SLS 311 Determination of lead
SLS 312 Determination of arsenic
SLS 345 Determination of mercury
SLS 428 Random sampling methods
SLS 467 Labelling of prepackaged foods
SLS 516 Microbiological test methods
Part 1 General guidance for enumeration of micro-organisms colony count technique at 30 °C
Part 3 Detection and enumeration of coliforms faecal coliforms and *Escherichia coli*
Part 5 General guidance for detection of *Salmonella*
Part 6 General guidance for enumeration of *Staphylococcus aureus*
SLS 614 Potable water
SLS 974 Code of hygienic practice for fresh fish
SLS 975 Code of hygienic practice for frozen fish

3 DEFINITIONS

For the purpose of this specification, the following definitions shall apply :

3.1 quick frozen blocks : Rectangular or other uniformly shaped masses of cohering fish fillets, pieces of fillets, minced fish or a mixture subjected to a freezing process comprising of:

i) a single species ; or
ii) a mixture of species with similar sensory characteristics.

3.2 fillets : Slices of fish of irregular size and shape which are removed from the carcass by cuts made parallel to the back bone.

3.3 minced fish flesh : Particles of skeletal muscle which have been separated from and are essentially free from bones and skin.

3.4 whole fish : Fish with its natural shape.

3.5 steak : A section of fish, removed by cutting approximately at right angle to the backbone.

3.6 chunk/cubes : Cross cuts taken from gutted, headless and dressed fish.

3.7 defects : for definitions see Appendix B.

4 STYLE OF PRESENTATION

The product shall be presented in the following styles :

4.1 Whole fish ;

a) With the head as "head-on"
b) Without the head as "headless"

4.2 Fillets ;

a) skin on, unscaled, pin bone present or removed
b) skin on, scaled, pin bones present or removed
c) skinless, pin bones present or removed

4.3 Minced fish flesh ; and

4.4 Steaks, chunks, cubes and slices. (see Note)

NOTE

Steaks, chunks, cubes and slices of the product shall be permitted provide that it:

a) is sufficiently distinctive from other forms of presentation laid down in this standard ;

b) meets all other requirements of this standard ; and

c) is adequately described on the label to avoid confusing or misleading the consumer.

5 OPTIONAL INGREDIENTS

One or more of the following ingredients may be used.

5.1 Salt

Conforming to SLS 79. (see Note 1)

5.2 Moisture/water retention agents

i)	Monophosphate, monosodium or monopotassium (Monosodium or Monopotassium orthophosphate);	) Not exceeding) 10g per kg,) expressed) as P_2O_5,) singly or in) combination) (See Note 2)
ii)	Diphosphate, tetrasodium or tetrapotassium (Sodium or Potassium pyrophosphate);	
iii)	Triphosphate, pentasodium or pentapotassium or calcium (Sodium, Potassium or Calcium tripolyphosphate);	
iv)	Polyphosphate, sodium (Sodium hexametaphosphate);	
v)	Sodium alginate, maximum level of 5 g per kg in the final product.	

5.3 **Antioxidants**

i)	Ascorbic acid or its sodium or potassium salts;	) Not exceding 1 g per kg,) expressed as ascorbic acid,
ii)	Ascorbyl palmitate ;	) singly or in combination.

5.4 **Thickeners**

i)	Guar gum	)
ii)	Carob bean (locust bean) gum	)
iii)	Pectins	)
iv)	Carboxymethyl cellulose, sodium salt	)not exceeding 5 g per kg
v)	Xanthan gum	)singly or in combination
vi)	Carageenan	)
vii)	Methyl cellulose	)

5.5 Any other permitted food additives may be added.

NOTES

1. *Salt shall be present not exceeding 1.0 per cent by mass.*
2. *Includes the phosphates naturally present in the fish fillet and minced fish flesh.*

6 REQUIREMENTS

6.1 Raw material

The quality of fresh fish used for freezing or for processing shall be of such a quality that it is suitable for human consumption.

6.2 Processing

6.2.1 The product shall be prepared and processed in accordance with SLS 974 and SLS 975. The recognized practice of further processing of intermediate quick forzen material under controlled conditions followed by the reapplication of the quick freezing process is permitted.

6.2.2 Water used for processing of frozen fish shall conform to SLS 614.

6.2.3 The product shall either be quick frozen individually or in mass.

6.2.4 The product after any suitable preparation shall be subjected to a freezing process and shall comply with the conditions prescribed in this standard.

6.2.5 The freezing process shall be carried out in such a way that the range of temperature of maximum crystallization (between 0 °C to -5 °C) within 90 minutes.

6.2.6 The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18 °C at the thermal center after thermal stabilization.

6.2.7 The product shall be maintained under such conditions as to maintain the quality during transportation, storage and distribution .

6.3 Final product

6.3.1 Appearance

The final product shall be reasonably free from dehydration.

The final product after thawing as in Appendix C, shall have the following appearance and characteristics:

a) The product shall be of characteristic colour, and the units in a package shall be acceptably uniform in colour. The flesh shall be free from blood clots, bruises and discolouration. In case of skin-on products it shall be free from discoloured skin and readily discernible damage to the skin.

b) The product described as boneless shall be free from cartilage and bones that are capable of piercing or hurting the palate after being cooked.

c) The skinless product shall not have residual skin or surface damage that detracts materially from its appearance.

d) The product that is claimed or implied to be free from scales shall be acceptably free from scales.

e) In the case of whole fish head-on, the product shall be free from body deformations.

f) In the case of gutted fish, the product shall be free from viscera.

g) The product shall be easily separated when labelled as individually quick forzen.

h) The product shall be free from black membrane where its presence will deleteriously affect the appearance.

j) The fish and fish product shall be, to the extent possible in good manufacturing practice, free from parasites.

k) The product shall be free from foreign matter.

m) The product shall be free from poisonous or harmful substances.

6.3.2 Odour and flavour

The product after thawing as in Appendix C, shall have a good, characteristic odour. After cooking in accordance with Appendix D, have a good, characteristic flavour and odour. It shall be free from any objectionable flavour and odour.

6.3.3 Texture

After thawing as in Appendix C and cooking as in Appendix D, the flesh of fish and fish products shall be firm. The flesh shall not be mushy or gelatinious.

6.3.4 Microbiological limits

The product shall conform to the microbiological limits given in Table 1 when tested according to the method given in Column 7 of the table.

TABLE 1 - Microbiological limits

Sl No.	Test-organism	n	c	Limit per g		Method of test Ref. to SLS 516
				m	M	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i	Aerobic plate count	5	3	10^5	10^6	Part 1
ii	*Staphylococcus aureus*	5	3	100	500	Part 6
iii	*E. coli*	5	3	11	500	Part 3
iv	*Salmonella*	5	0	0	-	Part 5

where,

n is the number of sample units to be tested;
c is the maximum allowable number of sample units yielding values between m and M;
m is the limit below which a count is acceptable for any sample unit; and
M is the limit above which a count is unaccepatable for any sample unit.

6.3.5 **Limits for heavy metals**

The product shall conform to the tolerance limits for heavy metals given in Table 2 when tested according to the methods prescribed in Column 4 of the table.

TABLE 2 - Limits for heavy metals

Sl. No. (1)	Heavy metals (2)	Limit (3)	Method of test (4)
i	Arsenic, mg/kg, max.	1.0	SLS 312
ii	Mercury, mg/kg, max.	0.5	SLS 345
iii	Lead, mg/kg, max.	1.0	SLS 311

6.3.6 The product when prepared using Tuna species should not contain more than 100 mg/kg histamine when determined in accordance with Appendix E.

7 PACKAGING

7.1 The material used in packaging shall be new and shall not impart any injurious substances or any off flavour to the product.

7.2 The packaging shall ensure that the product is properly protected from mechanical damage, leakage and dehydration.

7.3 Material which imparts flavour or in any way causes discolouration of the product or which is itself discoloured by contact with the product shall not be used as the inner carton.

7.4 Inner cartons shall be placed in master cartons which shall be wirebound or strapped with wire or any other suitable material.

7.5 Only one style of product (See 4) and preferably one size (See Note) shall be packed in any master carton.

NOTE

Inner cartons containing fish of different sizes may be packed in a master carton but this should be avoided as much as possible.

8 MARKING

8.1 The following information and other information which may be required by the importing country shall be marked clearly, legibly and indelibly on every (mastor) carton, or on a lable securely attached therto:

a) The Name of the product;

i) The name of the product as " XY " where "X" represents the style of presentation and "Y" represents the common name of the species packed.

ii) The term " quick frozen" or "frozen" ; which ever is customarily used in the country in which the product is distributed, to describe a product subjected to the freezing process.

b) The proportion of mince in percentage of net fish content by the percentage ranges : less than 25, 25 to 35 etc. Packets with more than 90 per cent mince are regarded as mince packages;

c) Net contents either in the system international units or avoirdupois units or both systems of measurement (See Note);

NOTE

Where the product has been glazed, the declaration of net content of the product shall be exclusive of the glaze

d) Name and address of the manufacturer and/or distributor (including the country of origin);

e) Batch or code number;

f) Registered trade mark, if any;

g) Date of packaging.

h) Date of expiary; and

j) Instructions for storage.

8.2 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 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 identificable with the accompanying documents.

8.3 Marking and labelling shall also be in accordance with SLS 467.

NOTE

Attention is drawn to certification marking facilities offered by the Sri Lanka Standards Institution. See the inside back cover of the standard.

9 METHODS OF TEST

9.1 Thawing shall be done in accordance with the method prescribed in Appendix C.

9.2 Cooking shall be done in accordance with the method prescribed in Appendix D.

9.3 The product shall be visually examined for frozen state and appearance defined in Appendix B.

9.4 Tests shall be carried out as prescribed in SLS 311, SLS 312, SLS 345, Parts 1, 3 and 6 of SLS 516 and Appendix E of this specification.

APPENDIX A
COMPLIANCE OF A LOT

The sampling scheme given in this appendix should be applied where compliance of a lot to the requirements of the standard is to be assesed based on statistical sampling and inspection.

Where compliance with this standard is to be assured based on manufacturer's control systems coupled with type testing and check tests or any other procedure, appropriate schemes of sampling and inspection should be adopted.

A.1 LOT

In any consignment all packages of quick frozen blocks of fish of same style and belonging to one batch of manufacture or supply shall constitute a lot.

A.2 GENERAL REQUIREMENTS OF SAMPLING

When taking samples the following precautions shall be taken.

A.2.1 Samples for microbiological analysis shall be drawn first.

A.2.2 The samples shall be protected against adventitious contamination.

A.2.3 The sampling instruments shall be clean, sterile and dry when used. When taking samples for microbiological examination the sampling instruments shall be sterilized.

A.2.4 The samples shall be kept in glass or suitable containers. They shall be clean and dry when used. The samples for microbiological examination shall be kept in sterilized containers.

A.2.5 The samples shall be stored in such a manner that there will be no deterioration of quality of the material.

A.2.6 The sample containers shall be sealed air-tight after filling and marked with necessary details of sampling.

A.3 SCALE OF SAMPLING

A.3.1 Samples shall be tested from each lot for ascertaining conformity to the requirements of this specification.

A.3.2 The number of packages to be selected from a lot shall be in accordance with Table 2.

TABLE 2 - Scale of sampling

Number of packages in the lot (1)	Number of packages to be selected (2)
Up to 280	8
281 to 500	9
501 to 1200	10
1201 to 3200	12
3201 and above	13

A.3.3 If the lot consists of packages packed in (mastor) cartons then select 10 per cent of the cartons from the lot. Draw approximately equal number of packages from the cartons thus selected to form the sample size as given in Column 2 of the table.

A.3.4 The packages and cartons shall be selected randomly. In order to ensure randomness of selection tables of random numbers as given in SLS 428 shall be used.

A.4 NUMBER OF TESTS

A.4.1 Each package/carton selected as in A.3.2 and A.3.3 shall be inspected for marking and packaging requirements.

A.4.2 Take five packages selected as in A.3.2 and shall be tested for microbiological requirements given as in 6.3.4.

A.4.3 Remaining packages shall be individually inspected/tested for the requirements given as in 6.3.1, 6.3.2 and 6.3.3.

A.4.4 A composite sample shall be prepared by taking sufficient quantities of material from each package inspected as in A.4.3 and shall be tsted for the requirements given as in 6.3.5.

A.5 CRITERIA FOR CONFORMITY

The lot shall be declared as conforming to the requirements of this specification, if the following conditions are satisfied.

A.5.1 Each package/carton inspected as in A.4.1 satisfies the relevant requirements.

A.5.2 The five packages tested as in A.4.2 satisfy the relevant microbiological requirements.

A.5.3 Each package tested as in A.4.3 satisfies for relevant requirements.

APPENDIX B
DEFINITION OF PHYSICAL DEFECTS

For the purpose of this specification, the following definitions shall apply:

B.1 *DEHYDRATION (freezer burn)*

An excessive loss of moisture from the surface of the sample unit which shows clearly on the surface, penetrates below the surface, and cannot be easily removed by scraping.

B.2 *SCALES*

Attached to skin or readily noticeable loose scales.

B.3 *COLOUR DEFECTS*

B.3.1 **Blood clots** (Spots)

Any mass or lump of clotted blood.

B.3.2. **Bruises**

Diffused blood causing distinct reddish, brownish or other off colouration.

B.3.3 Discolouration

Appears as significantly intense discolouration due to melanin deposits, bile stains, liver stains or other causes.

Objectionable discolouration, spots or particles dervied from skin, black membrane, blood clots, blood spots, spinal cord or viscera.

B.3.4 Discoloured skin

Readily discernible deviation from the normal characteristic colour of the species.

B.4 *BONES*

A bone is regarded as a defect if its length is $\geq$ 10 mm or its diameter is $\geq$ 1 mm or if it is capable of piercing or hurting the palae after being cooked; a bone $\leq$ 5 mm length is not to be considered as a defect if its diameter is not $\geq$ 2 mm.

The foot of a bone (where it has been attached to the vertebra) shall be disregarded if its width is $\leq$ 2 mm or if it can be easily stripped off by a finger nail.

B.5 *VISCERA*

Any portion of viscera.

B.6 *PARASITES*

Parasites or parasitic infestation detected by the candling procedure (placing a sample on a 5 mm thick acryl sheet with 45 per cent translucency and candled with a light source giving 1500 lux, 30 cm above the sheet), by visual examination. Each parasitic infestation may be recognized by its colour, its effect on softening the fish flesh or by other physical indications.

B.7 *FOREIGN MATTER*

1). Any material not derived from fish or not permitted by the standard other than packaging.

ii) Packaging material.

B.8 BODY DEFORMATION

Deformation of the back (hump-back) or of the head if present (hooked snout) as a result of the extension of catilaginous material in these areas as the fish approaches spawning condition.

B.9 CUTS, WOUNDS AND OTHER SKIN BREAKS

Readily discernible damage to the skin.

B.10 DEFECTS OF ODOUR

Stale, sour, rancid or other objectionable odours indicative of decomposition or contamination.

B.11 TEXTURE

Any texture which is significantly different to the characteristics of the species and or distinctly objectionable, eg. the flesh is difinitely spongy, rubbery, mushy, soft, gelatinous, tough or gritty.

B.12 SKIN AND BLACK MEMBRANE SKIN

Does not include sub-cutaneous layer (silver lining). In flat fish white skin is not regarded as a defect. (see Note) Black membrane or belly lining does not include white membrane.

NOTE

In *skinless flat fish, small pieces or white skin are not regarded as defects, provided that the skin does not exceed more than 10 per cent of the surface of the fillets in the sample unit.*

APPENDIX C
THAWING METHOD

The sample is thawed by enclosing it in a film type bag and immersing in an agitated water bath held at ambient temperature.

If fish are individually quick frozen, complete thawing of the product is determined by gently squeezing the bag occasionally so as not to damage the texture of the fish product, and until no hard core or ice crystals are felt.

If the product is block frozen, turn the block over several times during thawing. The point at which thawing is complete can be determined by gently probing the block apart and until no hard core or ice crystals are felt.

APPENDIX D
COOKING METHOD

The following procedures are based on heating product to internal temperature higher than 70 °C. Cooking times vary according to size of product and equipment used. If determining cooking time, cook extra sample using temperature measuring device to determine internal temperature. An extra sample of 200.0 g is used.

D.1 BAKING PROCEDURE

Wrap product in aluminium foil and distribute evenly on flat cookie sheet or shallow flat pan. Heat in ventilated oven, preheated to 204 °C, until temperature of product reaches 70 °C or higher than 70 °C.

D.2. STEAMING PROCEDURE

Wrap product in aluminium foil and place on wire rack suspended over boiling water in covered container. Heat until internal temperature of product reaches 70 °C or higher than 70 °C.

D.3. BOILING IN BAG

Place the product into a boilable film-type pouch and seal. Immerse the pouch and its contents into boiling water and cook until the internal temperature of the product reaches 70 °C or higher than 70 °C.

APPENDIX E
DETERMINATION OF HISTAMINE CONTENT

Rinse all plastic and glass containers with 25 per cent (V/V) hydrochloric acid and water before use.

E.1 APPARATUS

E.1.1 Chromatographic tube, 200 mm x 7 mm, ploypropylene tube, flow rate controlled at more than 3 ml/minute.

E.1.2 Photofluorometer, with excitation at 350 nm and measuring emission at 444 nm.

E.2 REAGENTS

E.2.1 Ion exchange resin, Bio-Rad AG 1-X8, 50 - 100 mesh or Dowex 1-X8, 50 - 100 mesh.

Add 15 ml of 2 mol/l NaOH per 1 g resin, to a beaker to convert to OH form. Swirl the mixture and allow to stand for less than 30 minutes. Decant the liquid and repeat with additional base. Wash the resin thoroughly with water, slurry into fluted papaer (S x S No. 588, or equivalant) and wash again with water. Prepare resin fresh weekly and store under water.

Place glass wool plug in base of tube (D.1.1) and slurry in enough resin to form 8 cm bed. Maintain water level above to of resin bed at all times. Wash column with 10 ml of water before applying each extract.

NOTE

Do not regenerate resin in packed column; rather, use batch regeneration in beaker when necessary.

E.2.2 Phosphoric acid, standardized, c (H_3PO_4) = 1.78 mol/l

Dilute 121.8 ml of 85 per cent (V/V) Phosphoric acid to one litre. Standardize 5.00 ml by titrating with 1.00 mol/l sodium hydroxide using phenoltphalin as the indicator.

E.2.3 Ortho - Phthalic dicarboxaldehyde (OPT) solution, 0.1 per cent

Dissolve 100 mg OPT in 100 ml methyl hydroxide. Store in amber bottle in a refrigerator. Prepare fresh weekly.

E.2.4 Histamine standard solutions

E.2.4.1 Stock solution, 1 mg/ml

Weigh accurately about 169.1 mg histamine.2HCl into a 100-ml volumetric flask. Dissolve and dilute to volume with 0.1 mol/l hydrochloric acid. Prepare fresh weekly and store in refrigerator.

E.2.4.2 Intermediate solution, 10 µg/ml

Pipette 1 ml of stock solution (E.2.4.1) into 100-ml volumetric flask. Dilute to volume with 0.1 mol/l hydrochloric acid. Prepare freshly weekly and store in refrigerator.

E.2.4.3 Working solutions, 0.5 µg/5 ml, 1.0 µg/5 ml and 1.5 µg/5 ml Pipette 1 ml, 2 ml and 3 ml of intermidiate solution into 100 ml volumetric flasks separately. Dilute to volume with 0.1 mol/l hydrochloric acid. Prepare fresh daily and store in refrigerator.

E.3 PROCEDURE

E.3.1 Preparation of standard curve

Pipette in duplicate 5 ml aliquots of working standard solution (E.2.4.3) into 50-ml glass or polypropylene Erlenmeyer flasks. Pipette in 10 ml of 0.1 mol/l hydrochloric acid to each flask and mix. Pipette in 3 ml of 1 mol/l sodium hydroxide and mix. Within 5 minutes, pipette in 1 ml of OPT solution (E.2.3) and mix immediately. After exactly 4 minutes, pipette in 3 ml of phosphoric acid (E.2.2) and mix immediately.

NOTE

It is important to mix thoroughly after each addition and at least once during OPT reaction. Run 6 to 10 OPT reactions simultaneously by adding reagents to Erlenmeyers in set order.

Prepare blank by substituting 5 ml of 0.1 mol/l hydrochloric acid for histamine solution. Within 90 minutes, record fluorescence intensity (I) of working standard solution with water lin reference cell, using extinction warelength of 350 nm and emission wavelength of 444 nm.

Plot I (corrected for blank) against μg histamine/5 ml aliquot.

E.3.2 Determination

E.3.2.1 Transfer 10 g of the prepared sample to semimicro container of a high speed blender, add about 50 ml methyl alcohol. Blend for 2 minutes. Transfer to a 100 - ml glass-stoppered volumetric flask, rinsing lid and blender jar with methyl alcohol and adding rinsings to the flask.

Heat in a water bath at 60 °C for 15 minutes. Cool to 25 °C. Dilute to volume with methyl alcohol. Filter through a folded paper.

E.3.2.2 Pass 4 ml to 5 ml of water through the column (E.1.1) and discard eluate. Pipette 1 ml of extract (E.3.2.1) onto column and add 4 ml to 5 ml of water. Immediately initiate column flow into a 50-ml volumetric flask containing 5.00 ml of 1.00 mol/l hydrochloric acid. When liquid level is approximately 2 mm above the resin, add about 5 ml of water and elute. Follow with water in larger portions until about 35 ml has eluted. Stop column flow. Dilute to volume with water. Stopper and mix. Refrigerate the elute.

Pipette 5 ml of the eluate into 50 ml erlenmeyer flasks. Pipette in 10 ml of 0.1 mol/l hydrochloric acid. Pipette in 3 ml of 1 mol/l sodium hydroxide and mix. Proceed as in E.3.1.

E.3.2.3 If the sample contains more than 15 mg histamine per 100 g of fish, pipette 1 ml of sample - OPT mixture into 10-ml beaker containing exactly 2 ml of blank - OPT mixture. Mix thoroughly. Read fluorescence of new solution. Dilute and mix aliquots with blank -OPT mixture as needed to obtain a measurable reading.

NOTE

This approximation indicated proper dilution of elute required prior to second OPT reaction needed for reliable quantitation of sample. Alternatively, use sensitivity range control of fluorometer, if available, to estimate dilution. Use these approximations to prepare appropriate dilutions of aliquot of elute with 0.1 mol/l hydrochloric acid, proceeding as in D.3.1 commencing with the addition of 3 ml of 1 mol/l sodium hydroxide.

E.4 CALCULATION

Plot of (I) against µg histamine/5 ml of solution should be a straight line passing through the origin with a slope
$m = [(I_a/1.5) + I_b + 2I_c]/3$

E.4.1 Histamine content
mg/100 g of fish $= (10)(F)\ (1/m)(I_s)$

where

I_s is the fluorescence of the sample
I_a is the fluorescence of the 1.5 µg histamine standard solution;
I_b is the fluorescence of the 1.0 µg histamine standard solution;
I_c is the fluorescence of the 0.5 µg histamine standard solution;
and
F is the dilution factor

$$F = \frac{\text{eluate (ml)} + \text{hydrochloric acid (ml)}}{\text{eluate (ml)}}$$

F = 1 for undiluted eluate

E.4.2 If calibration plot is not linear, use the standard curve directly for quantitation. Each subdivision of abscissa should be 0.1 µg histamine /5 ml solution.

Histamine content
mg/100 g of fish = (10)(F)(W)

where,

F is the dilution factor, and
W is the µg of histamine /5 ml solution as determined from the curve.

SLS CERTIFICATION MARK

The Sri Lanka Standards Institution is the owner of the registered certification mark shown below. Beneath the mark, the number of the Sri Lanka Standard relevant to the product is indicated. This mark may be used only by those who have obtained permits under the SLS certification marks scheme. The presence of this mark on or in relation to a product conveys the assurance that they have been produced to comply with the requirements of the relevant Sri Lanka Standard under a well designed system of quality control inspection and testing operated by the manufacturer and supervised by the SLSI *which includes surveillance inspection of the factory, testing of both factory and market samples.*

Further particulars of the terms and conditions of the permit may be obtained from the Sri *Lanka Standards Institution, 17, Victoria Place, Elvitigala Mawatha, Colombo 08.*

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SRI LANKA STANDARDS INSTITUTION

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