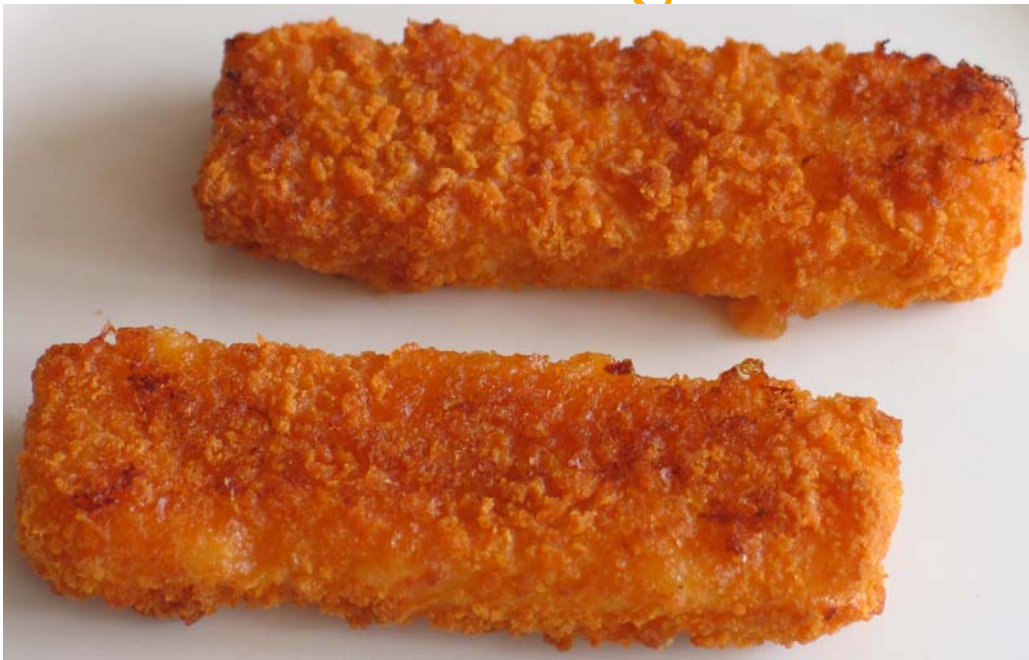




EAST AFRICAN STANDARD

Quick frozen fish sticks (fish fingers), fish portions and fish fillets
— Breaded or in batter — 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 166:1989(Rev. 2:2004), *Standard for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets - Breaded or in Batter*

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.

Contents

1	Scope	1
2	Normative references	1
3	Description	2
4	Essential composition and quality factors	3
5	Food additives	3
6	Hygiene and handling	5
7	Labelling	6
8	Sampling, examination and analyses	7
9	Definition of defects	8
10	Lot acceptance	8
	Annex A (normative) Sensory and physical examination	12
	Annex B (normative) Estimation of proportion of fish fillets and minced fish flesh	13
	Annex C (normative) Determination of histamine	14
	Annex D (normative) Determination of moisture in meat	16
	Annex E (normative) Fish flesh content (FFC) in frozen coated fish products	17

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Quick frozen fish sticks (fish fingers), fish portions and fish fillets — Breaded or in batter — Specification

1 Scope

This standard applies to quick frozen fish sticks (fish fingers) and fish portions cut from quick frozen fish flesh blocks, or formed from fish flesh, and to natural fish fillets, breaded or batter coatings, singly or in combination, raw or partially cooked and offered for direct human consumption without further industrial 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

3.1.1 A fish stick (fish finger) is the product including the coating weighing not less than 20 g and not more than 50 g shaped so that the length is not less than three times the greatest width. Each stick shall be not less than 10 mm thick.

3.1.2 A fish portion including the coating, other than products under 3.1.1, may be of any shape, weight or size.

3.1.3 Fish sticks or portions may be prepared from a single species of fish or from a mixture of species with similar sensory properties.

3.1.4 Fillets are slices of fish of irregular size and shape which are removed from the carcass by cuts made parallel to the back bone and pieces of such fillets, with or without the skin.

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 or further industrial processing of intermediate quick frozen material under controlled conditions which maintains the quality of the product, followed by the re-application of the quick freezing process, is permitted.

3.3 Presentation

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

3.3.1 meets all the requirements of the standard, and

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

4 Essential composition and quality factors

4.1 Raw material

4.1.1 Fish

Quick frozen breaded or battered fish sticks (fish fingers) breaded or battered fish portions and breaded or battered fillets shall be prepared from fish fillets or minced fish flesh, or mixtures thereof, of edible species which are of a quality such as to be sold fresh for human consumption.

4.1.2 Coating

The coating and all ingredients used therein shall be of food grade quality and conform to all applicable Codex standards.

4.1.3 Frying fat (oil)

A fat (oil) used in the cooking operation shall be suitable for human consumption and for the desired final product characteristic (see also Section 5).

4.2 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 Section 8.

4.3 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.

5 Food additives

Only the use of the following additives is permitted.

CD/K/535:2010

Additive

Maximum level in the final product

For Fish Fillets and Minced Fish Flesh Only

Moisture/Water Retention Agents

- 339(i) Monosodium orthophosphate
- 340(i) Monopotassium orthophosphate
- 450(iii) Tetrasodium diphosphate
- 450(v) Tetrapotassium diphosphate
- 451(i) Pentasodium triphosphate
- 451(ii) Pentapotassium triphosphate
- 452(i) Sodium polyphosphate
- 452(iv) Calcium, polyphosphates

10 g/kg expressed as P_2O_5 singly or in combination (includes natural phosphate)

401 Sodium alginate

GMP

Antioxidants

- 300 Ascorbic acid
- 301 Sodium ascorbate
- 303 Potassium ascorbate
- 304 Ascorbyl palmitate

GMP

1 g/kg

In Addition, for Minced Fish Flesh Only

Acidity Regulator

- 330 Citric acid
- 331 Sodium citrate
- 332 Potassium citrate

GMP

Thickeners

- 412 Guar gum
- 410 Carob bean (Locust bean) gum
- 440 Pectins
- 466 Sodium carboxymethyl cellulose
- 415 Xanthan gum
- 407 Carrageenan and its Na, K, NH_4 salts (including Furcelleran)
- 407a Processed Euchema seaweed (PES)
- 461 Methyl cellulose

GMP

Food Additives for Breaded or Batter Coatings

Leavening Agents

- 341(i) Monocalcium orthophosphate
- 341(ii) Dicalcium orthophosphate
- 541 Sodium aluminium phosphate, basic and acidic
- 500 Sodium carbonates
- 501 Potassium carbonates
- 503 Ammonium carbonates

1 g/kg expressed as P_2O_5 singly or in combination

GMP

Flavour Enhancers

- 621 Monosodium glutamate
- 622 Monopotassium glutamate

GMP

Additive	Maximum level in the final product
<u>Colours</u>	
160b Annatto extracts	20 mg/kg expressed as bixin
150a Caramel I (plain)	GMP
160a(i) β -carotene (Synthetic)	100 mg/kg singly or in combination
160e β -apo-carotenal	
<u>Thickeners</u>	
412 Guar gum	GMP
410 Carob bean (Locust bean) gum	
440 Pectins	
466 Sodium carboxymethyl cellulose	
415 Xanthan gum	
407 Carrageenan and its Na, K, NH ₄ salts (including Furcelleran)	
407a Processed <i>Eucheama</i> Seaweed (PES)	
461 Methyl cellulose	
401 Sodium alginate	
463 Hydroxypropyl cellulose	
464 Hydroxypropyl methylcellulose	
465 Methylcellulose	
<u>Emulsifiers</u>	
471 Monoglycerides of fatty acids	GMP
322 Lecithins	
<u>Modified Starches</u>	
1401 Acid treated starches	GMP
1402 Alkaline treated starches	
1404 Oxidized starches	
1410 Monostarch phosphate	
1412 Distarch phosphate esterified with sodium trimetaphosphate; esterified with phosphorus oxychloride	
1414 Acetylated distarch phosphate	
1413 Phosphated distarch phosphate	
1420 Starch acetate esterified with acetic anhydride	
1421 Starch acetate esterified with vinyl acetate	
1422 Acetylated distarch adipate	
1440 Hydroxypropyl starch	
1442 Hydroxypropyl starch phosphate	

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;
- (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 in any sample unit. This applies only to species of *Clupeidae*, *Scombridae*, *Scombresocidae*, *Pomatomidae* and *Coryphaenidae* families;

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 quick frozen fish fillets, minced fish fillet and mixtures

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 Sections 2, 3, 7 and 8 of EAS 38 the following specific provisions apply:

7.1 Name of the food

7.1.1 The name of the food to be declared on the label shall be "breaded" and/or "battered", "fish sticks" (fish fingers), "fish portions", or "fillets" as appropriate or other specific names used in accordance with the law and custom of the country in which the food is sold and in a manner so as not to confuse or mislead the consumer.

7.1.2 The label shall include reference to the species or mixture of species.

7.1.3 In addition there shall appear on the label either the term "quick frozen" or the term "frozen" whichever is customarily used in the country in which the food is sold, to describe a product subjected to the freezing processes as defined in subsection 3.2.

7.1.4 The label shall show whether the products are prepared from minced fish flesh, fish fillets or a mixture of both in accordance with the law and custom of the country in which the food is sold and in a manner so as not to confuse or mislead the consumer.

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

7.2 Storage instructions

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

7.3 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 of the manufacturers or packer, 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 product shall be in accordance with the FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods (AQL-6.5) (CODEX STAN 233-1969). For prepackaged goods the sample unit is the entire container. For products packed in bulk the sample unit is at least 1 kg of fish sticks (fish finger), fish portions or fillets.
- (ii) Sampling of lots for examination of net weight shall be carried out in accordance with an appropriate sampling plan meeting the criteria established by the Codex Alimentarius Commission.

8.2 Determination of net weight

The net weight (exclusive of packaging material) is determined on each whole primary container of each sample representing a lot and shall be determined in the frozen state.

8.3 Sensory and physical examination

Samples taken for sensory and physical examination shall be assessed by persons trained in such examination and in accordance with procedures elaborated in Sections 8.4 through 8.7, Annex A and the *Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories (CAC/GL 31 - 1999)*.

8.4 Estimation of fish core

According to the method described in Annex E.

8.5 Determination of gelatinous conditions

According to the method described in Annex D.

8.6 Estimation of proportion of fish fillets and minced fish flesh

According to the method described in Annex B.

8.7 Cooking methods

The frozen sample shall be cooked prior to sensory assessment according to the cooking instructions on the package. When such instructions are not given, or equipment to cook the sample according to

the instructions is not obtainable, the frozen sample shall be cooked according to the applicable method(s) given below:

For fish blocks or other unbreaded test samples, cut 3 test portions, each 4 × 3 × 0.5 in. (ca 10 × 7.5 × 1.2 cm) from test sample.

Cooking procedure is based on heating product to internal temperature 160°F (70°C). Cooking times vary according to size of product and equipment used. To determine cooking time, cook extra test portion same way using temperature measuring device with probe of known length to determine internal temperature. Cooking equipment, including cooking oil for deep fat frying, shall be free from substances that interfere with sensory evaluation of cooked product.

Methods of heating product include, but are not limited to, baking, bake-in-foil, broiling, boil-in-bag, shallow pan frying, deep fat frying, oven frying, grilling, poaching, steaming, and microwave heating.

8.8 Determination of histamine

According to the method in Annex C.

9 Definition of defects

The sample unit shall be considered defective when it exhibits any of the properties defined below:

9.1 Foreign matter

The presence in the sample unit of any matter which has not been derived from fish (excluding packing 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.

9.2 Bones (cooked state) in packs designated boneless

More than one bone per kg greater or equal to 10 mm in length, or greater or equal to 1 mm in diameter; a bone less than or equal to 5 mm in length, is not considered a defect if its diameter is not more than 2 mm. The foot of a bone (where it has been attached to the vertebra) shall be disregarded if its width is less than or equal to 2 mm, or if it can easily be stripped off with a fingernail.

9.3 Odour and flavor (cooked state)

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

9.4 Flesh abnormalities

Objectionable textural characteristics such as gelatinous conditions of the fish core together with greater than 86% moisture found in any individual fillet or sample unit with pasty texture resulting from parasites affecting more than 5% of the sample unit by weight.

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) (CODEX STAN 233-1969);
- (ii) the average percent fish flesh of all sample units is not less than 50% of the frozen weight;
- (iii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any container; and
- (iv) the Food Additives, Hygiene and Labelling requirements of Sections 5, 6 and 7 are met.



Fish sticks



Draft for comments only

ward



Example of packaged fish sticks

Draft for comments only — Not for



fish sticks

Draft for comments only — Not to be used

Annex A
(normative)

Sensory and physical examination

The sample used for sensory evaluation should not be the same as that used for other examinations.

1. Complete net weight determination, according to defined procedures in Section 8.2.
2. Complete fish core determination on one set of the sample units according to defined procedures in Section 8.4.
3. Complete the estimation of the proportion of fillets and minced flesh, if required.
4. Cook the other set of sample units and examine for odour, flavour, texture, foreign matter, and bones.
5. In cases where a final decision on gelatinous conditions 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 8.7 or by using the procedure in Section 8.5 to determine if greater than 86% moisture is present in any product unit. If a cooking evaluation is inconclusive, then procedure in 8.5 would be used to make the exact determination of moisture content.

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Annex B
(normative)**Estimation of proportion of fish fillets and minced fish flesh**

(West European Fish Technologists Association — WEFTA Method)

a) Equipment

Balance, sensitive to 0.1 g

Circular sieve - 200 mm diameter, 2.5 or 2.8 mesh opening (ISO) soft rubber edge (or blunt) spatula, forks, suitable sized plates, water tight plastic bags.

b) Preparation of Samples

Fish Portions/Sticks: Take as many portions as needed to provide a fish core sample of about 200g (2kg). If breaded and/or battered first strip coating according to the method describer in section 7.4.

c) Determination of Weights "A" of the Frozen Fish Samples

Weight the single fish portions/decoated fish cores while they are still frozen. Smaller portions are combined to a sample sub-units of about 200 g (e.g. 10 fish sticks of about 20 g each). Record the weight "A" of the sub-units. Place the pre-weighed sample sub-units into water tight bags.

d) Thawing

Thaw the samples by immersing the bags into a gently agitated water bath of about 20°C, but not more than 35°C.

e) Draining

After thawing has been completed (duration about 20-30 min.) take each sample unit, one at a time, and drain the exuded fluid (thaw drip) for 2 minutes on a pre-weighed circular sieve inclined at an angle of 17-20 degrees. Remove adhering drip from the bottom of the sieve by use of a paper towel when draining is completed.

f) Determination of weight "B" of the Drained Fish Sample and Weight "C" of the Thaw Drip

Determine the weight of the drained fish sample "B" - sieve plus fish minus sieve weight. The difference of "A" - "B" is the weight of exuded fluid - thaw drip.

g) Separation

Place the drained fish core on a plate and separate the minced flesh from the fillet using a fork to hold the fillet flesh and a soft, rubber edge spatula to scrape off the minced flesh.

Annex C (normative)

Determination of histamine

C.1 Principle

Sample is extracted with 75% (v/v) methanol. Extract is passed through ion exchange column. *o*-Phthaldialdehyde solution is added to eluate to form fluorescent histamine derivatives. Fluorescent intensity of derivatives is measured using fluorometer and histamine is quantified using external standards.

C.2 Apparatus

Rinse all plastic and glass containers with HCl (1 + 3) and H₂O before use.

- (a) **Chromatographic tube** — 200 ×7 id mm polypropylene tube fitted with small plastic stopcocks and ca 45 cm Teflon tubing. Control flow rate at >3 ml/min by adjusting height of column relative to tubing outlet. Alternatively, use 2-way valve in place of tubing.
- (b) **Photofluorometer** — Equipped with medium pressure Hg lamp with excitation at 350 nm and measuring emission at 444 nm.
- (c) **Repipets** — 1 and 5 ml.

C.3 Reagents

- (a) **Ion-exchange resin** — Bio-Rad AG 1-X8, 50–100 mesh or Dowex 1-X8, 50–100 mesh. Convert to -OH form by adding ca 15 ml 2M NaOH/g resin to beaker. Swirl mixture and let stand <30min. Decant liquid and repeat with additional base. Thoroughly wash resin with H₂O, slurry into fluted paper and wash again with H₂O. Prepare resin fresh weekly and store under H₂O. Place glass wool plug in base of tube, C.2(a), and slurry in enough resin to form 8 cm bed. Maintain H₂O level above top of resin bed at all times. Do not regenerate resin in packed column; rather, use batch regeneration in beaker when necessary. Wash column with ca 10 ml H₂O before applying each extract.
- (b) **Phosphoric acid** — 3.57N. Dilute 121.8 ml 85% H₃PO₄ to 1 L. For other concentration H₃PO₄, volume required for 1 L 1.19M acid = 17493/(density H₃PO₄ × percent H₃PO₄). Standardize 5.00 ml by titration with 1.00M NaOH to phenolphthalein end point, and adjust concentration if necessary.
- (c) ***o*-Phthaldialdehyde (OPT) solution** — 0.1% (w/v). Dissolve 100 mg OPT in 100 ml distilled-in-glass methanol. Store in amber bottle in refrigerator. Prepare fresh weekly.
- (d) **Histamine standard solutions** — Store in refrigerator.
 - (1) **Stock solution** — 1 mg/ml as free base. Accurately weigh ca 169.1 mg histamine 2HCl (98%) into 100 ml volumetric flask, and dissolve and dilute to volume with 0.1M HCl. Prepare fresh weekly.
 - (2) **Intermediate solution** — 10 µg/ml. Pipet 1 ml stock solution into 100 ml volumetric flask, and dilute to volume with 0.1M HCl. Prepare fresh weekly.
 - (3) **Working solutions** — 0.5, 1.0, and 1.5 µg/5 ml. Pipet 1, 2, and 3 ml intermediate solution into separate 100 ml volumetric flasks, and dilute each to volume with 0.1M HCl. Prepare fresh daily.

- (e) **Methanol** — 75% (v/v). Place 75 ml MeOH (distilled in glass) into 100 ml volumetric flask or stoppered graduated cylinder. Dilute to volume with H₂O. Swirl flask while adding H₂O.

C.4 Preparation of standard curve

Pipet duplicate 5 ml aliquots of each working standard solution into separate 50 ml glass or polypropylene Erlenmeyers. Pipet in 10 mL 0.1M HCl to each flask and mix. Pipet in 3 ml 1M NaOH and mix. Within 5 min, pipet in 1 ml OPT solution and mix immediately. After exactly 4 min, pipet in 3 ml 3.57NH₃PO₄ and mix immediately. It is important to mix thoroughly after each addition and at least once during OPT reaction. (Run 6– 10 OPT reactions simultaneously by adding reagents to Erlenmeyers in set order.) Prepare blank by substituting 5 ml 0.1M HCl for histamine solution. Within 1.5 h, record fluorescence intensity (*I*) of working standard solutions with H₂O in reference cell, using excitation wavelength of 350 nm and emission wavelength of 444 nm. Plot *I* (corrected for blank) against µg histamine/5 ml aliquot.

C.5 Determination

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

Pipet 5 ml eluate into 50 ml Erlenmeyer, and pipet in 10 ml 0.1M HCl. Proceed as in C.4, beginning "Pipet in 3 ml 1M NaOH . . .".

If test sample contains >15 mg histamine/100 g fish, pipet 1 ml sample–OPT mixture into 10 ml beaker containing exactly 2 ml blank–OPT mixture, and mix thoroughly. Read fluorescence of new solution. Dilute and mix aliquots with blank–OPT mixture as needed to obtain measurable reading. This approximation indicates proper dilution of eluate required prior to second OPT reaction needed for reliable quantitation of test sample. Alternatively, use sensitivity range control of fluorometer (if instrument has one) to estimate dilution. Use these approximations to prepare appropriate dilution of aliquot of eluate with 0.1NHCl, and proceed as in C.4, beginning "Pipet in 3 ml 1M NaOH . . .".

C.6 Calculations

Plot of *I* (measured by meter deflection or recorder response and corrected for blank) against µg histamine/5 ml test solution should be straight line passing through origin with slope = $m = [(I_a / 1.5) + I_b + 2I_c] / 3$.

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

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

where *I_s*, *I_a*, *I_b*, and *I_c* = fluorescence from test sample, 1.5, 1.0, and 0.5 µg histamine standards, respectively; and *F* = dilution factor = (ml eluate + ml 0.1M HCl)/ml eluate. *F* = 1 for undiluted eluate.

If calibration plot is not linear, use standard curve directly for quantitation. Each subdivision on abscissa should be ≤0.1 µg histamine/5 ml test solution. Read all values from curve to nearest 0.05 µg histamine/5 ml test solution.

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

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

where *W* = µg histamine/5 ml test solution as determined from standard curve.

Annex D
(normative)**Determination of moisture in meat****D.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 $\leq 70^\circ\text{C}$ and pressure ≤ 50 mm Hg. Use covered Al dish ≥ 50 mm diameter and 40 mm deep.

D.2 Air drying

D.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.

D.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.

D.3 Calculations

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

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

Annex E (normative)

Fish flesh content (FFC) in frozen coated fish products

(Applicable to the determination of the FFC in frozen coated fish products.)

NOTE The coated fish products industry uses the term "portions" in the name of some of its products. The term "test portion" may be misunderstood as only applicable to these products. Therefore, in this method the term "test unit" is understood to apply to whatever form is being examined.

Caution! Use protective gloves when immersing and holding test sample in water bath set at $>43^{\circ}\text{C}$.

E.1 Principle

Method uses combination of (1) heat and H_2O to breakdown adhesive properties of coating (batter and/or breading) and (2) hands to assist in determining when coating's ability to adhere to flesh's frozen surfaces is diminished and can be easily removed.

E.2 Apparatus

- (a) Water baths — Primary ($17\text{--}49^{\circ}\text{C}$) and secondary ($17\text{--}30^{\circ}\text{C}$).
- (b) Thermometers — Two; immersion type, accurately measuring to $\pm 1^{\circ}\text{C}$.
- (c) Thermometer holders — Two; with clips.
- (d) Balance — Accurately weighing to 0.1 g.
- (e) Stop watch — Reading seconds.
- (f) Paper towels.
- (g) Spatula — 4 in. (ca 10 cm) blade with rounded tip.
- (h) Nut pick.

E.3 Preparation of Test Sample

Maintain integrity of frozen test samples by storing in freezer until ready to remove batter and/or breading. Take into account all applied coating when weighing coated test samples.

E.4 Determination

Set primary H_2O bath temperature between $17\text{--}49^{\circ}\text{C}$. Set secondary H_2O bath temperature between $17\text{--}30^{\circ}\text{C}$.

Weigh and record weight of each test unit while it is hard frozen. Using hands, immerse and hold test unit in primary H_2O bath until coating becomes soft and can be removed easily from still-frozen flesh.

Remove test unit from H_2O bath and blot lightly with enough paper towel to absorb excess H_2O . Complete blotting in ≤ 7 s. Scrape and remove coating from flesh with spatula. If coating is difficult to remove using hands, redip and hold partially debattered or debreaded test unit in secondary H_2O bath until coating becomes soft and can be removed easily from still-frozen flesh.

Remove test unit from H_2O bath and blot lightly with enough paper towel to absorb excess H_2O . Complete blotting in ≤ 7 s. Scrape and remove coating from flesh with spatula. When necessary, repeat redipping procedure and use nut pick to remove coating from any voids (holes, spaces, or

depressions) until all coating has been removed from still-frozen flesh. Reweigh and record weight of debattered and/or debreaded test unit.

NOTE Several preliminary trials may be necessary to determine optimum H₂O bath temperatures, dip times, and number of dips required for debattering and/or debreading test units. The correct dip time is the minimum time of immersion in H₂O baths required before coating on test unit can be scraped off easily, provided that coated test unit is still solidly frozen.

As a guide, no more than 1 initial dip (17–49°C) and 2 redips (17–30°C) for a maximum of 2.5, 0.5, and 0.5 min, respectively, should be necessary.

E.5 Calculations

Calculate content of fish flesh, %, in test sample as follows:

$$\text{Flesh, \%} = (W_d / W_b) \times 100$$

where W_d = weight of debattered and/or debreaded test unit; W_b = weight of coated test unit.

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