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EAST AFRICAN STANDARD

Fish protein concentrate — 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

The nutritive value of proteins from fish in many instances is the same as that of milk proteins. Fish proteins are fairly well-balanced in most of the essential amino acids, and are also generally rich in lysine, which is known to be deficient in most cereals, millets, root crops, etc, which form the bulk of the more than two-thirds of the world population. It is now well established that supplementation of diets based on cereals and such other products with fish even at low levels greatly improves the growth-promoting value of the former.

Increasing attention has been paid during recent years to the conversion of fish material into an edible quality of fish flour or fish protein concentrate conforming to prescribed nutritional and hygienic standards. Fish protein concentrate is a colourless, odourless, tasteless, bland and dry powder prepared from fish by a hygienic solvent extraction process for human consumption. It contains a high percentage of protein and less than 0.75 % of fat.

The formulation of this Standard is expected to help in exercising proper quality control in fish protein concentrate prepared particularly from that variety of fish which may not otherwise be consumed.

A recommended method for the preparation of fish protein concentrate is given in Annex K.

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

IS 9808:1981(R2005), *Specification for Fish Protein Concentrate*

CKS 286:1971, *Fish protein concentrate for human consumption*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp

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

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSV1.0/Standards>

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

Assistance derived from these sources is hereby acknowledged.

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Fish protein concentrate — Specification

1 Scope

This standard prescribes the requirements and the methods of sampling and test for fish protein concentrate.

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

CAC/RCP 52, *Code of practice for fish and fishery products*

EAS 38, *Labelling of prepackaged foods — Specification*

EAS 79, *Cereals, pulses and milled products — Sampling of static batches*

ISO 936, *Meat and meat products — Determination of total ash*

ISO 1736, *Dried milk and dried milk products — Determination of fat content — Gravimetric method (Reference method)*

ISO 3310-2, *Test sieves — Technical requirements and testing — Part 2: Test sieves of perforated metal plate*

ISO 5537, *Dried milk — Determination of moisture content (Reference method)*

ISO 20483, *Cereals and pulses — Determination of the nitrogen content and calculation of the crude protein content — Kjeldahl method*

3 Definitions

For the purpose of this standard the following definitions shall apply:

acceptable

acceptable to the purchaser

fish protein concentrate

the dried powdered product produced (in a clean plant) from fresh fish, fish meal, or fish presscake after extraction with ethanol or isopropanol

4 Raw material

4.1 The fish used shall itself be in a condition fit for human consumption.

4.2 The fish used may preferably be inexpensive varieties, for example, silver belly (*Leiognathus* sp), dhoma, jew fish (*Pseudorciaena* sp) or malabar sole (*Cyanolossus* sp) and shall be free from selenium, neurotoxins and other organo-metallic impurities (see Table 1).

4.3 The fish should be dressed and eviscerated to remove less desirable portions such as the intestines and then washed with water.

5 Requirements

5.1 Description

Fish protein concentrate shall be in the form of a fine free-flowing colourless powder that is easy to blend and shall not have more than a faint odour and taste when added to boiling water in a closed container. It shall be prepared by a hygienic solvent extraction process. It shall be free from dirt, or other extraneous matter, added colouring and flavouring agents. It shall not contain formalin.

5.2 Solvents

Any of the following solvents shall be used in its preparation:

- a) Ethanol — 96 percent (v/v) (food grade),
- h) Iso-propanol — food grade, and
- c) Hexane (food grade)

The solvent residues in the finished product shall not exceed the limits compatible with good manufacturing practices.

5.3 Particle Size

The material shall be such that it passes completely through 150-micron sieve (see ISO 3310-2), or as agreed to between the buyer and the supplier.

5.4 Physical and chemical requirements

The concentrate, when tested in accordance with the relevant methods, shall comply with the requirements given in Table 1 and Table 2.

Table 1 — Requirements for fish protein concentrate

SI/No.	Characteristic	Requirement	Method of test CD-K-567-2010
(i)	Moisture, % by mass, Max	8.0	ISO 5537
(ii)	Crude protein content ($N \times 6.25$), on dry basis, % by mass, Min	80.0	ISO 20483
(iii)	Total available lysine, g/100 g of protein, Min	6	C
(iv)	Fat content, on dry basis, % by mass, Max	0.75	ISO 1736
(v)	Ash, on dry basis, % by mass, Max	18	ISO 936
(vi)	Acid insoluble ash, on dry basis, % by mass, Max	0.5	F
(vii)	Available lysine, g/100 g of protein, Min	6.5	Annex B
(viii)	Fluoride (as F), mg/kg, Max	250	G
(ix)	Sand content, % by mass, Max	0.5	
(x)	Fineness (residue remaining on a sieve of nominal aperture size 210 μm)	Nil	

Table 2 — Microbiological and heavy metal limits for fish protein concentrate

Characteristic		Requirement	Method of test
(1)	(2)	(3)	(4)
i)	Total bacterial count/g, in the finished product, Max	10 000	ISO 4833
ii)	<i>Escherichia coli</i> count/g, Max	Absent	ISO 7251
iii)	Faecal <i>Streptococci</i> count/g, Max	Absent	Annex H
iv)	Coagulase positive <i>Staphylococci</i> /g, Max	Absent	ISO 6888
v)	<i>Salmonella</i> , per 25 g	Absent	ISO 6579
vi)	<i>Shigella</i> , per 25 g	Absent	ISO 21567
vii)	<i>Vibrio cholerae</i> , per 25 g	Absent	ISO/TS 21872
viii)	<i>Listeria monocytogenes</i> , per 25 g	Absent	ISO 11290
ix)	Heavy metals:		
	a) Mercury, mg/kg, Max	0.5	EAS 41
	b) Copper, mg/kg, Max	20.0	EAS 41
	c) Zinc, mg/kg, Max	50.0	EAS 41
	f) Arsenic, mg/kg, Max	0.1	EAS 41
	e) Lead, mg/kg, Max	0.3	EAS 41
	g) Cadmium	0.3	EAS 41
	h) Methylmercury	0.5	EAS 41

5.5 Protein Efficiency Ratio (PER) — The PER shall not be less than 2.5.

5.6 Any odour or flavour (or both) imparted by the concentrate to cooked porridge, prepared from maize meal containing 5 % (m/m) of fish flour, shall be acceptable and almost neutral.

6 Packing and marking

6.1 Packing

The fish protein concentrate (FPC) shall be packed in clean, sound containers made of tinsplate, PCRC sheets, cardboard, paper or other material agreed to between the purchaser and vendor in such a way as to protect it from spillage, contamination, migration of moisture or air from atmosphere and seepage of fat into the material through the packing material. When packed in flexible material, the packaging material should be capable of withstanding rough handling during transportation. The FPC shall not come in direct contact with packaging materials other than grease-proof or sulphate paper, cellulose paper or any other non-toxic packing material which may be covered into moisture-proof laminate or coated paper. When packed in metallic containers, the container shall be airtight and completely filled so as to have minimum air, or the space shall be filled with inert gas, or the contents held in vacuum.

6.2 Marking

6.2.1 The following shall be marked clearly and legibly on the container.

- a) Name of the material,

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- b) Name and address of the manufacturer.
- c) Batch/Code number,
- d) Minimum net mass and gross mass,
- e) Date of manufacture, and
- f) The words 'Best before (month and year to be indicated)'; and
- g) Any other requirement as given OIML R87, *Quantity of product in prepackages*.

6.2.2 Each container may also be marked with a certification Mark.

7 Sampling

Representative samples of material for test and criteria for conformity shall be drawn according to the method prescribed in EAS 79.

8 Tests

Tests shall be carried out as prescribed in 5.5, 5.6 and Tables 1 and 2 using pure chemicals and distilled water (see EAS 123).

NOTE 'Pure chemical' shall mean chemicals that do not contain impurities which affect the test result.



Annex A
(normative)

Determination of moisture

A.1 Procedure

Weigh accurately about 5 g of the material in an aluminium dish having a diameter of at least 50 mm and a depth not exceeding 40 mm, previously dried in an oven and weighed. Shake the dish until the contents are evenly distributed. With the cover removed, place the dish to dry in an air-oven maintained at 100 ± 2 °C. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing *until* the difference in mass between two successive weighings is less than one milligram. Record the lowest mass.

A.2 Calculation

$$\text{Moisture, percent by mass} = \frac{100(M_1 - M_2)}{M_1 - M}$$

where

M_1 = mass, in g, of the dish with the material before drying;

M_2 = mass, in g, of the dish with the material after drying; and

M = mass, in g, of the empty dish.

Annex B
(normative)**Determination of available lysine****B.1 Principle**

The method depends upon the reaction of fluorodinitrobenzene (FDNB) with the end-NH₂ groups of the lysine units in intact food proteins, and colourimetric estimation of the DNP-lysine obtained by subsequent acid hydrolysis

B.2 Apparatus

B.2.1 Photo-electric Absorptionmeter — Set at 435 nm or fitted with a blue filter having a maximum transmission close to 435 nm

B.2.2 Round-Bottom Flask Fitted with Reflux Condenser — 100 ml capacity.

B.2.3 Water Bath — Maintained at 100 °C

B.2.4 Volumetric Flasks — 100-ml and 200-ml capacity.

B.2.5 Burette — 25-ml capacity, graduated at 0.05 ml intervals.

B.2.6 Conical Flask

B.2.7 Pasteur Pipette

B.3 Reagents

B.3.1 Sodium Bicarbonate Solution — 8 percent (m/v).

B.3.2 Fluorodinitrobenzene (FDNB) Solution — Prepare fresh daily and for every sample use approximately 0.3 ml of fluorodinitrobenzene (pipetted after warming the bottle) dissolved in approximately 12 ml of ethanol.

B.3.3 Standard Hydrochloric Acid — 1 and 8.1 N.

B.3.4 Sodium Hydroxide Solution — 2 N.

B.3.5 Phenolphthalein Solution — 1 percent in 70 percent ethanol.

B.3.6 Buffer Solution — 19 parts of 8 percent sodium bicarbonate and one part of 8 percent sodium carbonate, adjusted suitably to 8.5 pH with the addition of a little acid or alkali.

B.3.7 Methyl Chloroformate

B.3.8 Di-ethyl ether — free from peroxide.

B.3.9 Standard DNP-Lysine Hydrochloride Monohydrate — Dissolve accurately weighed, 24 mg of DNP-lysine hydrochloride monohydrate in 500 ml of 1 N hydrochloric acid solution. Two millilitres of this solution will contain 39.85 µg of available lysine.

B.4 Procedure

B.4.1 The procedure shall be followed in duplicate and away from direct or strongly reflected sunlight.

NOTE Routine pipetting of ether and fluorodinitrobenzene by mouth is not recommended. An automatic sucker, such as the

pro-pipette is recommended and the same sucker may be used for several pipettes.

B.4.1 Weigh accurately about 0.75 g of the material into a 100 ml round bottom flask and shake gently with 8 ml of sodium bicarbonate solution for 10 minutes. Add fluorodinitrobenzene solution. Shake gently but continuously for 2 hours. Evaporate off the ethanol on a boiling water-bath. Add 24 ml of 8.1 N hydrochloric acid and reflux gently for 16 hours. (Some yellow crystals may appear on the condenser. These are not DNP-lysine and need not be recovered). Cool suitably for easy filtration. Filter the contents with water washings and make up the volume of the filtrate to 200 ml.

B.4.2 Transfer accurately 30 ml of the aliquot of the filtrate to a 50-ml flask and make up to volume.

B.4.3 Transfer 2 ml of the diluted filtrate to a glass stoppered tube. Extract with 5 ml portions of diethyl ether each time until the final ether fraction is colourless, sucking off the ether layer with the pasteur pipette. Remove dissolved ether by standing the tube in hot water.

B.4.4 Make up the volume to 10 ml with 1 N hydrochloric acid and read the extinction in a 1 cm cell with the photo-electric absorption meter at 435 nm.

B.4.5 For the blank, take a second aliquot of 2 ml diluted filtrate (see B.4.2) in a tube and extract with ether as for the first, also take a third aliquot of 2 ml in a small conical flask for a dummy titration. Dilute and add phenolphthalein indicator and titrate with 2 N sodium hydroxide taken in a burette. Note the volume of sodium hydroxide needed and discard the flask. Add the same volume to the second tube and then 2 ml of buffer solution of pH 8.5 (continue without pause from this stage onwards as DNP-compounds are less stable at this pH).

B.4.6 Add 0.05 ml of methyl chloroformate to the second tube. Shake and wait for 5 to 10 minutes. Then add 0.75 ml of concentrated hydrochloric acid continuously to avoid excessive effervescence. Extract again with two 5 ml portions of diethyl ether. Evaporate off any residual ether and make up to 10 ml with water. Read the extinction of the tube.

B.4.7 Carry out the experiment through B.4.3 to B.4.6 with the standard DNP-lysine solution.

B.5 Calculation

Available lysine, g/100 g of crude protein:

$$= \frac{X_1 - X_2}{S_1 - S_2} \times \frac{39.85}{1000000} \times \frac{50}{2} \times \frac{200}{30} \times \frac{100}{W(100 - M)} \times \frac{100}{P}$$

where

X_1 = absorptionmeter reading with unknown;

X_2 = blank absorptionmeter reading with unknown;

S_1 = Absorption meter reading with standard;

S_2 = blank absorptionmeter reading with standard;

W = mass, in g, of the material;

M = moisture, percent by mass, in the material; and

P = crude protein (on dry basis) percent by mass, in the material.

Annex C
(normative)**Determination of acid insoluble ash****C.1 Reagent****C.1.1 Dilute Hydrochloric Acid** — 5N, prepared from concentrated hydrochloric acid.**C.2 Procedure****C.2.1** Weigh accurately about 2 g of the dried material in a tared porcelain, silica or platinum dish. Ignite with a meker burner for about 1 hour. Complete the Ignition by keeping in a muffle furnace at 500 °C to 570 °C until grey ash results.

Cool and filter through whatman filter paper No. 42 or its equivalent. Wash the residue with hot water until the washings are free from chlorides as tested with silver nitrate solution and return the filter paper and residue to the dish. Keep it in an electric air oven maintained at 135 ± 2 °C for about 3 hrs. Ignite the dish again for about 30 minutes, cool and weigh. Repeat this process till the difference between two successive weighings is less than 1 mg. Note the lowest weight.

C.3 Calculation**C.3.1** Acid insoluble ash, per cent by weight

$$= \frac{100(M_2 - M)}{M_1 - M}$$

where,

- M_2 = the lowest weight, in g, of the dish with the acid insoluble ash;
 M = weight, in g, of the empty dish; and
 M_1 = weight, in g, of the dish with the dried product taken for the test.

Annex D (normative)

Determination of fluorine

Principle

The sample is ashed with $\text{Ca}(\text{OH})_2$ as a fluorine fixative, and fluorine is isolated by distillation from HClO_4 and estimated in the distillate by thorium nitrate back-titration.

D.1 Apparatus

D.1.1 Steam distillation apparatus (see Figure 1)

D.1.2 Nessler Tubes — 100 ml capacity.

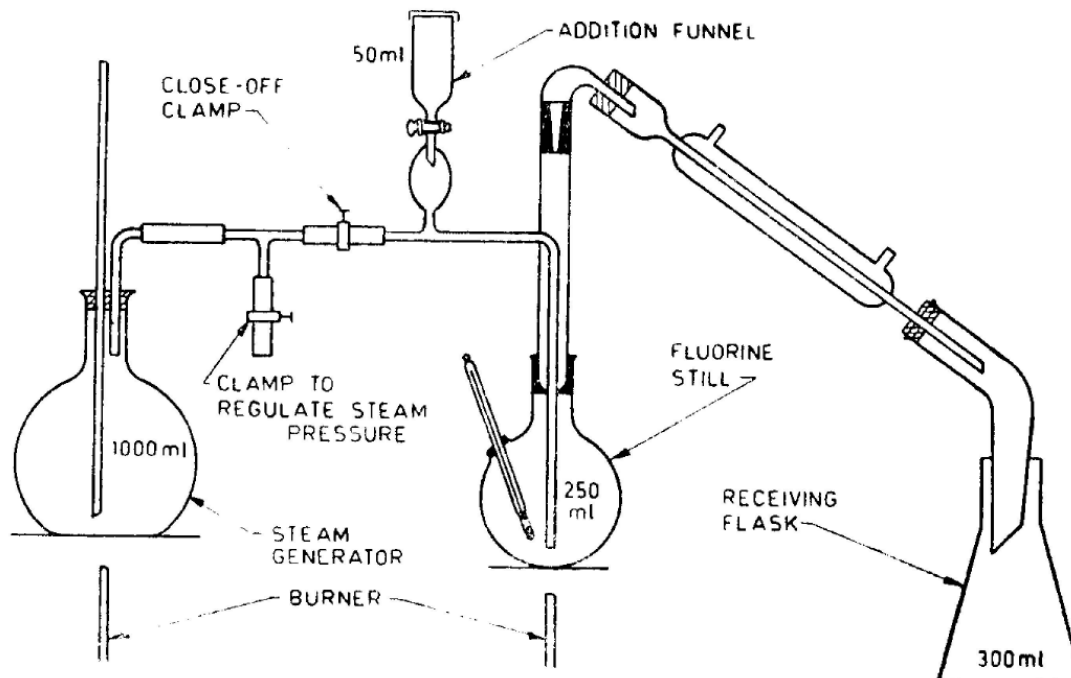


Figure D.1 — Steam distillation apparatus for the isolation of fluorine by the modified method

D.1.3 Platinum crucibles

D.1.4 Heating Lamp

D.1.5 Muffle Furnace

D.1.6 Vacuum Desiccator containing Silica Gel

D.2 Reagents

D.2.1 Calcium Hydroxide — 1 mole $\text{Ca}(\text{OH})_2$ /litre.

D.2.2 Thorium Nitrate Solution — 2.7×10^{-4} M.

D.2.3 Silver Perchlorate Solution — 50 g/100 ml.

D.2.4 Perchloric Acid — 60 per cent.

D.2.5 Sodium Hydroxide — 0.05 N.

D.2.6 p-Nitrophenol — 0.5 per cent alcoholic solution.

D.2.7 Buffer — Mix 400 ml, 0.2 N HCl and 4.4 g $\text{NH}_2\text{OH}\cdot\text{HCl}$ and dilute to 2 litres with water.

D.2.8 Indicator — 0.01 percent sodium alizarin sulphonate.

D.3 Preparation of sample

D.3.1 Weigh 0.5 – 1.0 g sample directly into platinum crucibles. Add glycerine to hydrophobic samples to make them wettable. Fix fluorine by adding 20 ml $\text{Ca}(\text{OH})_2$ suspension. Place the mixture under a heating lamp and bring to just dryness, and ash for about 16 hours in a muffle furnace at 550°C . Store ashed sample in a vacuum desiccator over silica gel.

D.4 Isolation of fluorine

D.4.1 Add excess silver perchlorate (usually 2 g) to still pot to suppress formation of HCl. Also add 10 -15 small (1 mm) borosilicate glass beads and about 0.05 g finely-ground silica sand. Use a total of about 40 ml perchloric acid for distillation. Cover ashed sample in platinum crucible with watch glass and dissolve sample in minimum of acid. Introduce sample and remainder of acid into still pot immediately, through addition funnel. Thoroughly rinse all apparatus used to transfer sample and add rinse to still with enough water to make total volume about 150 ml. Distil at $125 \pm 5^\circ\text{C}$ at 3-4 ml/min until about 300 ml distillate is collected. Keep distillate just alkaline to p-nitrophenol by drop-wise additions of 0.05 N sodium hydroxide. Generally not more than 2 ml of sodium hydroxide solution is necessary.

D.5 Titration of fluoride

D.5.1 Prepare calibration curve of 10-50 μg fluoride standards every time titrations are performed. Determine fluorine by direct titration with the thorium nitrate solution in matched, 100-ml Nessler tubes, using fluorescent light for illumination. Add 10 ml HCl-hydroxylamine buffer and 1 ml indicator solution to each titration tube. After all tubes are thus prepared, add just enough thorium nitrate solution to one tube to produce first pink colour (as viewed through depth of tube) and fill to the 100-ml mark with water. Volume of thorium nitrate solution added is the titration blank. Use this tube as colour standard to compare all subsequent titration end-points. Determine end-point only when tubes are filled to the 100-ml mark.

D.5.2 Neutralize excess NaOH in distillate to p-nitrophenol end-point (colourless) with buffer solution and make to 300 ml with water. Titrate suitable aliquots (25 to 75 ml) with thorium nitrate against colour standard tubes. Correct for titration blank. Determine amount of fluorine in aliquot from calibration curve. Correct fluoride content for distillation blank which should be determined periodically to ensure accurate results.

D.6 Calculation

$$\text{Fluoride, mg/kg} = \frac{M}{W}$$

where

M = μg of fluorine in sample, and

W = mass of sample in g.

Annex E (normative)

Method for the preparation of fish protein concentrate

E.1 Raw material

Fish protein concentrate can be prepared from a single species or a mixture of trash fish comprising jew fish (*Pseudorciaena* sp), silver belly (*Leiognathus* sp), malabar sole (*Cyanolossus* sp), dhoma, etc.

E.2 Preparation of pressed cake

The raw material is washed to remove extraneous matter and minced without dressing. The minced mass is suspended in water in the ratio of 1:1 by mass and glacial acetic acid is added to the extent of 0.5 percent (*m/v*) on the basis of the mass of minced muscle. The material is cooked in an open-jacketted kettle at 70-80°C with constant stirring for about 30 minutes. The water is removed by decantation and the mass is pressed in a hydraulic press. The pressed cake goes for solvent extraction.

E.3 Solvent extraction

E.3.1 The wet cake is charged into the extraction cell having heating, stirring and reflux arrangements. The first extraction is carried out using ethyl alcohol in the ratio of two parts by volume of alcohol to one part by mass of cake and kept at boiling temperature for half an hour under constant agitation. The three subsequent extractions, after draining out solvent alcohol are carried out in a similar way using an azeotropic mixture of hexane and ethyl alcohol (bp 58.7 °C) at its boiling point, adding the azeotrope to the cake in the ratio of 1:5 (v/v). Other solvents (food grade) such as isopropanol can also be used to remove fat and odour-bearing compounds from the fish cake.

Extraction should continue till the final oil content of the cake is reduced to 0.5 percent or less on dry mass basis. After the final extraction, residual solvent from the mass is removed by centrifugation and subsequent steam stripping and vacuum drying to remove solvent as completely as possible. The dried matter is reduced to the desired particle size by means of a pulveriser. The spent solvent can be recovered by evaporation and condensation and re-used in the process.

Annex F
(normative)

Determination of lead content

The lead content shall be determined in accordance with:

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

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Annex G
(normative)

Determination of cadmium content

The lead content shall be determined in accordance with:

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

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

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Annex H
(normative)

Determination of mercury content

The lead content shall be determined in accordance with:

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

H.1 Scope and field of application

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

H.2 Principle

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

H.3 Reagents

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

H.3.1 Sulfuric acid ($\rho_{20} = 1.84$ g/ml).

H.3.2 Nitric acid ($\rho_{20} = 1.38$ g/ml).

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

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

H.3.5 Urea, 400 g/l solution.

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

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

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

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

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

1 ml of this standard solution contains 0.1 μ g of mercury.

H.4 Apparatus

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

Usual laboratory equipment, and

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

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

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

- a) Element (A) is a modified Soxhlet extractor of capacity 200 ml, fitted with a stop-cock and a side tube linking this element directly to the round-bottomed flask (D).
- b) Element (B) is a dropping funnel of capacity 75 ml, fitted to the second neck of the round-bottomed flask (D).
- c) Element (C) is a condenser 35 cm long, fitted to the top of the Soxhlet extractor (A).
- d) Element (D) is a round-bottomed flask, of capacity 500 ml, fitted below the Soxhlet extractor (A), and in the second neck (30 mm from the first neck) of which is fitted the dropping funnel (B).

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

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

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

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

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

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

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

H.4.4 Pipettes and burettes, of appropriate capacities.

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

H.4.6 Analytical balance.

H.5 Procedure

H.5.1 Preparation of test sample

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

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

H.5.2 Test portion

H.5.2.1 Liquid products

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

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

H.5.2.2 Doughy, solid or dehydrated products

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

H.5.3 Decomposition

H.5.3.1 Decomposition of the test portion

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

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

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

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

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

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

H.5.3.2 Blank test

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

H.5.4 Determination

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

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

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

H.5.5 Preparation of the calibration graph

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

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

H.5.6 Number of determinations

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

H.6 Expression of results

H.6.1 Method of calculation and formulae

H.6.1.1 Test portions taken by volume

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

where

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

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

H.6.1.2 Test portions taken by mass

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

where

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

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

H.6.1.3 Result

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

H.6.2 Repeatability

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

H.6.3 Other method of expression of results

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

H.7 Test report

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

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

Dimensions in millimetres

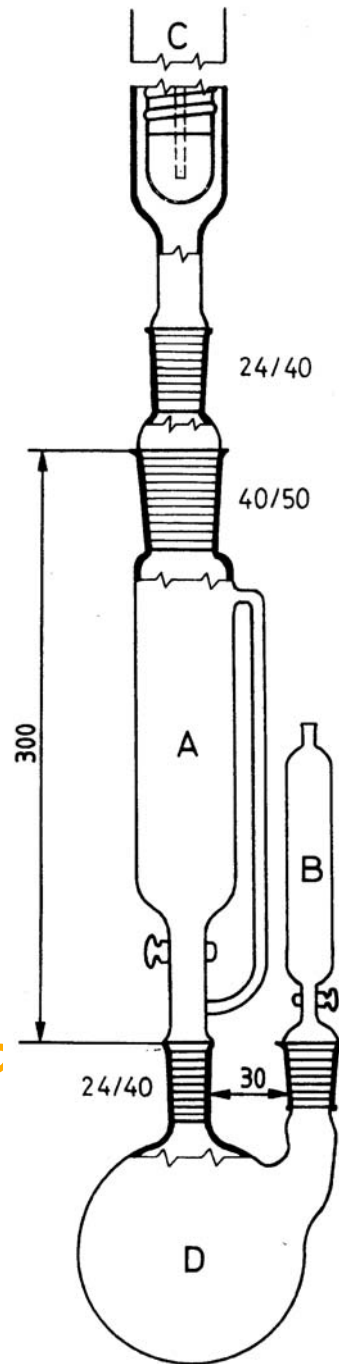


Figure H.1 — Decomposition apparatus

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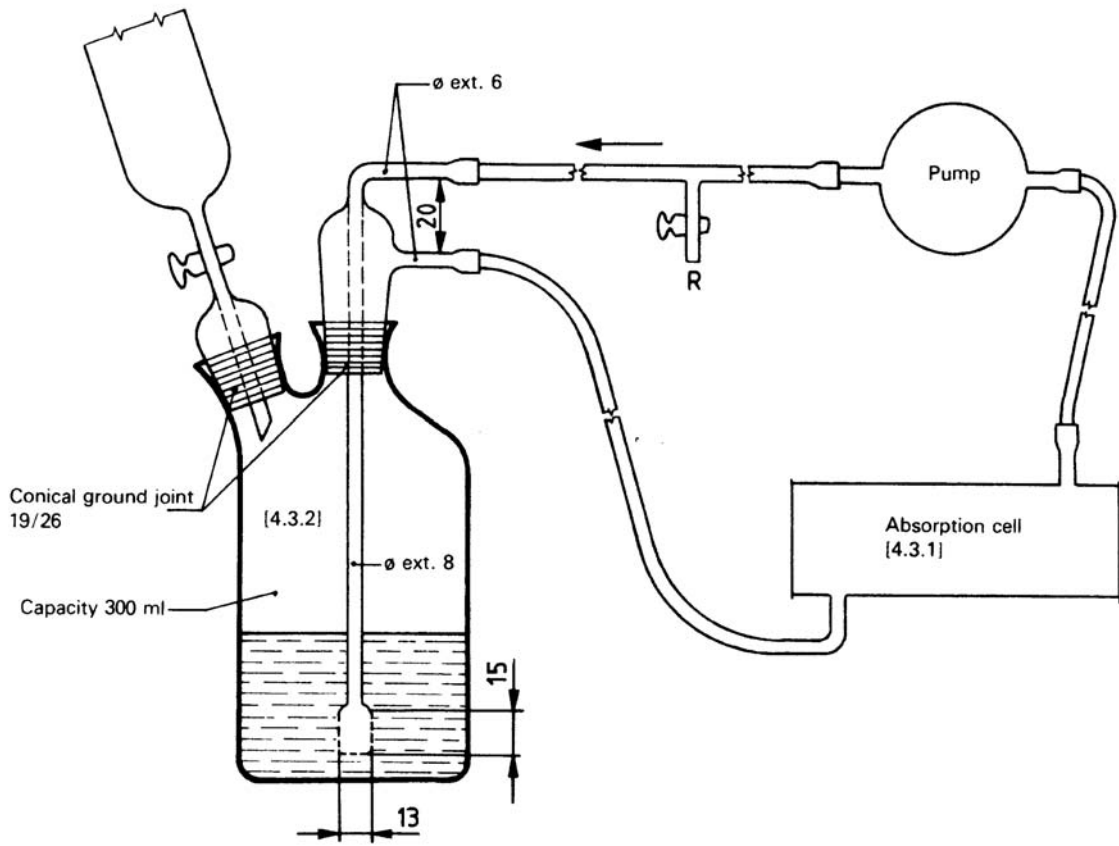


Figure H.2 — Atomic absorption apparatus

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