

DRAFT

STANDARD FOR FISH SAUCE (*PATIS*) AND FISH FLAVORED SAUCE

1. SCOPE

This standard applies to fish sauce (*patis*) and fish flavored sauce produced by means of salt fermentation and may include other ingredients added to assist the fermentation process. The product is intended for direct consumption as a seasoning, or condiment or ingredient for food.

2. DEFINITION OF TERMS

For the purpose of this standard, the following terms shall mean:

Container means any form of packaging material, which completely or partially encloses the food (including wrappers). A container may enclose the food as a single item or several units or types of prepackaged food when such is presented for sale to the consumer.

Current Good Manufacturing Practices (cGMP) is a quality assurance system aimed at ensuring that products are consistently manufactured, packed, repacked or held to a quality appropriate for the intended use. It is thus concerned with both manufacturing and quality control procedures.

Fermentation is the breakdown of organic substances into simpler components mainly by the action of enzymes produced by microorganisms (Mackie and Whittle, 1971).

Fish means any of the cold-blooded (ectothermic) aquatic vertebrates. Amphibians and aquatic reptiles are not included (Codex, 2003).

Food is any processed substance which is intended for human consumption and includes drink for man, beverages, chewing gum and any substances, which have been used as an ingredient in the manufacture, preparation or treatment of food. (RA 9711 Food and Drug Administration (FDA) Act of 2009)

Food Additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities (Codex GSFA 2013).

Food Standard is a regulatory guideline that defines the identity of a given food product (i.e. its name and the ingredients used for its preparation) and specifies the minimum quality factors and, when necessary, the required fill of container. It

1 may also include specific labeling requirements other than or in addition to the
2 labeling requirements generally applicable to all prepackaged foods.

3
4 **Histamine** is a biogenic amine formed in fish muscle by decarboxylation of the
5 amino acid histidine by bacteria (Whittle and Howgate, 2002).

6 **Ingredient** is any substance including food additives, used as a component in the
7 manufacture or preparation of a food and present in the final product in its original
8 or modified form.

9 **Label** includes any tag, brand, mark, pictorial, or other descriptive matter, written
10 printed, marked, embossed or impressed on, or attached to a container of food.

11 **Labeling** means any written, printed or graphic matter (1) upon any article or any
12 of its container or wrappers or (2) accompanying the packaged food.

13 **Lot** is food produced during a period of time and under more or less the same
14 manufacturing conditions as indicated by a specific code.

15 **Packaging** is the process of packing that is part of the production cycle applied
16 to a bulk product to obtain the finished product. Any material, including painted
17 material, employed in the packaging of a product including any outer packaging
18 used for transportation of shipment. Packaging materials are referred to as
19 primary or secondary according to whether or not they are intended to be in direct
20 contact with the product.

21 **pH** is the measure of the intensity or degree of acidity of a food material.

22 **Prepackaged** means packaged or made up in advance in a container, ready for
23 sale to the consumer (BFAD Administrative Order No. 88-B s. 1984).

24 **Processed Food** shall refer to food that has been subjected to some degree of
25 processing (e.g. milling, drying, concentration and canning, etc.), which partially
26 or completely change the physico-chemical and/or sensory characteristics of the
27 raw material.

28 **Proteins** are nitrogenous organic compounds consisting of linked amino acids
29 that are distributed widely in plants and animals. The sequence of amino acids in
30 proteins is determined by the base sequence of their encoding genes. They serve
31 many roles, such as enzymes, structural elements and hormones, and are
32 essential nutrients (IFIS, 2005).

33 **Putrefaction** is a typically anaerobic, microbial decomposition of substances
34 (especially proteinaceous and fatty products such as meat and fish) with the
35 production of foul-smelling compounds (e.g. ammonia, hydrogen sulfide,
36 cadaverine and putrescine). (IFIS, 2005).

37
38 **Salt Content** is the amount of salt (as NaCl) in the product, usually expressed as
39 percentage.

1 **3. DESCRIPTION**

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3 **3.1 Product Definition**

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5 Fish sauce and fish flavored sauce are clear liquid products, with a salty taste
6 and fish flavor, obtained from fermentation of a mixture of fish and salt. Color
7 may vary from straw-yellow to amber.

8 Fish Sauce has a minimum of 4.0% protein while Fish Flavored Sauce has a
9 protein content of below 4.0% but not lower than 1.0%. Protein content shall
10 only come from the fish material. Fish flavored sauce may also be the product
11 of the final extraction and referred to as such due to its low protein content.

12
13 **3.2 Process Description**

14
15 The products are prepared by mixing fish with salt and fermenting the mixture
16 in covered containers or tanks and allowed to ferment until the liquid formed is
17 of the desired quality.

18
19 **4. ESSENTIAL COMPOSITION AND QUALITY FACTORS**

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21 **4.1 Ingredients**

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23 **4.1.1 Basic Ingredients**

- 24
25 a. **Raw materials (Fish)** - shall be sound, wholesome and fit for human
26 consumption. Species of fish used may include, but not limited to those
27 listed in Annex 1. This also includes fish parts generated from other fish
28 processing operations, which may include fish heads, flesh/meat, skins
29 tails and internal organs.
- 30 b. **Salt** - shall be of food grade quality and meets the requirements and
31 standards for iodized salt as per R.A. No. 8172: An Act Promoting Salt
32 Iodization Nationwide and for Related Purposes (Annex 2) including
33 Bureau Circular No. 2007-009 Updated Standards for Iodine Level of
34 Salts.
- 35 c. **Water (for preparing brine)** - shall be water fit for human consumption
36 and meets the potability requirements prescribed in the Philippine National
37 Standards for Drinking Water as per DOH Administrative Order No. 2007-
38 0012 (Annex 3), and/or its future amendments.

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40 **4.1.2 Other Ingredients**

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

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45 **4.2. Quality and Safety Criteria**

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47 **4.2.1 Physico-chemical Properties**

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49 The products shall conform to the physico-chemical requirements specified in
50 Table 1.

Table1. Physico-chemical requirements for fish sauce and fish flavored sauce

Parameter	Fish Sauce		Fish Flavored Sauce
	Special/ Premium	Regular	
Protein, % (min)*	6.0	4.0	1.0
Salt, as NaCl, % (min)	24	24	24
pH	5.0– 6.5	5.0 – 6.5	5.0 – 6.5
Histamine, ppm (max)	400	400	400

* % Protein = Total Nitrogen (g/liter) x PF/actual specific gravity of fish sauce x 10
PF – Protein Factor – 6.25

4.2.2 Sensory Properties

- a. The product shall be clear to translucent, and free from sediments except salt crystals
- b. The products shall have odor and taste characteristic of the product

5. DEFECTS

A sample unit shall be considered defective when it exhibits any of the defects as defined and described in the following subsections.

5.1 Types of Defects

5.1.1 Foreign Matters

The presence in the sample unit of any matter which has not been derived from the components or constituents of ingredients used in the product and listed in subsection 4.1.1; and, does not pose a threat to human health and can be 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.

5.1.2 Appearance

- a. Cloudiness
- b. Presence of any sediments or suspended solids, except salt crystals
- c. Visible mold growth

5.1.3 Odor and Flavor

- a. Objectionable odors or flavors indicative of decomposition or deterioration, like putrefaction
- b. Odors and flavors, not characteristic of the product

5.2 Classification of Defectives

1 A container whose contents exhibit any of the defects described in
 2 subsections 5.1.1 to 5.1.3 and in which the number of defects observed per
 3 unit lot exceeds the acceptance number prescribed in the appropriate
 4 sampling plan (Annex 3) shall be considered as “defective”.

6. LOT ACCEPTANCE

8 A lot shall be considered acceptable when it complies with the applicable Quality
 9 and Safety Criteria as prescribed in Sub-section 4.2 and the number of
 10 “defectives”, as defined in Sub-section 5.2, does not exceed the acceptance
 11 number prescribed in the appropriate sampling plan (Annex 3).

7. FOOD ADDITIVES

15 Food additives when used shall be in accordance with the regulations prescribed
 16 by Food and Drug Administration (FDA) B.C. No. 016, s. 2006: Updated List of
 17 Food Additives) and the Codex General Standard for Food Additives (GFSA)
 18 Codex Stan 192-1995; 2011 Revision), and/or its future amendments. The food
 19 additives listed but not limited to those in Table 2 may be used for the manufacture
 20 of fish sauce and fish flavored sauce.

23 Table 2. Food Additives for Fish Sauce (*Patis*) and Fish Flavored Sauce in accordance
 24 with the regulations of the FDA and the Codex General Standard for Food
 25 Additives (GFSA)
 26

Functional Class	Codex INS No.	Food Additive	Maximum Use Level
Colors	150c	Caramel Color Class III and IV	50,000 mg/kg
Sweeteners	950	Acesulfame K	1000mg/kg
	955	Sucralose	120 mg/kg
	951	Aspartame	350 mg/kg
Preservatives		Sodium benzoate	1000 mg/kg
		Potassium sorbate	1000 mg/kg
Preservatives/ Antioxidants	223	Sodium metabisulfite	300mg/kg* (residual)
Flavor Enhancer	621	Monosodium glutamate	GMP
	630	Inosinic acid	GMP
	631	Disodium Inosine 5' monophosphate	GMP
	627	Disodium 5' guanylate	GMP

* Based on the Food Category System No. 12.6 – Sauces and like products

8. CONTAMINANTS

32 The final covered by the Standard shall comply with the Maximum Levels of
 33 the Codex General Standard for Contaminants and Toxins in Food and Feed
 34 (CODEX STAN 193-1995) and/or its future amendments.

36 Raw material fish for fish sauce shall not contain marine biotoxins (e.g.
 37 Ciguatoxin, Tetrodotoxin, and PSP) in amounts which could present a risk to
 38 human health.

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2 Product made using aquaculture fish shall comply with the maximum limits for
3 veterinary drugs established by the Codex Alimentarius Commission.
4
5

6 **9 WEIGHTS AND MEASURES**

7 8 **9.1 Fill of Containers**

9 10 **9.1.1 Minimum Fill**

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12 a. The container should be well filled with the fish sauce, which should
13 occupy not less than 90% (minus any necessary head space according to
14 good manufacturing practices) of the water capacity of the container. The
15 water capacity of the container is the maximum volume of distilled water at
16 20°C that the sealed container can hold when completely filled.
17

18 b. Flexible containers should be filled as full as commercially practicable.
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20 **9.1.2 Classification of “Defectives”**

21
22 A container that fails to meet the requirement for the minimum fill (90%
23 container capacity) of subsection 8.1.1 shall be considered as defective.
24

25 **9.1.3 Lot acceptance**

26
27 A lot will be considered as meeting the requirements of sub-section 8.1.1
28 when the number of “defectives” as defined in subsection 9.1.2 does not
29 exceed the acceptance number (c) of the appropriate sampling plan (Annex
30 4).
31

32 **10. HYGIENE**

33
34 The products covered by the provisions of this standard shall be prepared and
35 handled in accordance with the appropriate sections of the Codex
36 Recommended International Code of Practice – General Principles of Food
37 Hygiene (CAC/RCP 1 – 1969, Rev. 4-2003) and/or the FDA A.O. No. 153 s.
38 2004 - Guidelines, Current Good Manufacturing Practices in Manufacturing,
39 Packing, Repacking or Holding Food and processed according to the
40 Recommended Code of Practice for the Processing of Fish Sauce (*Patis*) and
41 Fish Flavored Sauce(PNS/FDA _____).
42
43

44 **11. PACKAGING AND LABELING**

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46 **11.1** The product shall be packed in appropriate primary packaging material that
47 will maintain its integrity during storage and transport.
48

49 **11.2.** Labeling of *retail* packages/container - Each retail container shall be labeled
50 and marked with the information in accordance with current FDA Labeling
51 Regulations and shall contain the following information:
52

- a. The name of the product shall be “**Fish Sauce (*Patis*)**” or “**Fish Flavored Sauce (*Patis*Flavor)**”. The products may be called by other common names provided that such names are accepted in the country of distribution.
- b. The name and the address of the manufacturer, packer, distributor, importer, exporter or vendor of the food.
- c. The complete list of ingredients and food additives used in the preparation of the product in descending order of proportion. The common, local or usual name of the raw material used shall be specified.
- d. The net content by weight in the metric system. Other systems of measurement required by importing countries shall appear in parenthesis after the metric system unit.
- e. The words “Use by date” / “Consume Before” indicating end of period at which the product shall retain its optimum quality attributes at defined storage conditions.
- f. Lot identification marked in code identifying product lot.
- g. The words “Product of the Philippines” or the country of origin if imported.
- h. Additional requirements
A pictorial representation of the product(s) on the label should not mislead the consumer with respect to the product so illustrated

11.3 Labeling of Non-retail, Bulk Containers

The name of the product, lot identification code and the name and address of the manufacturer or packer shall appear in the container. However, the name and address of the manufacturer may be replaced by identification marks provided that such mark is clearly identified with accompanying documents.

11.4 Nutrition Labeling

Nutrition labeling shall conform to established regulations by the FDA.

12. METHOD OF SAMPLING AND ANALYSIS

12.1. Method of Sampling

Sampling shall be in accordance with the FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods (CAC/RM 42-1969), Codex Alimentarius Volume 13, 1994 (Annex 4).

12.2. Methods of Analysis

- 12.2.1. Determination of Total Nitrogen (TN) for Protein Determination (Annex 5).
According to the AOAC Official Methods of Analysis, 18th ed., 2005 Method No. 940.25.
- 12.2.2. Determination of Salt Content, as Sodium Chloride (NaCl) (Annex 6)
According to the AOAC Official Methods of Analysis, 18th ed., 2005. Method No 973.13. 976.18 or 976.19.
- 12.2.3. Determination of pH (Annex 7)
According to the AOAC Official Methods of Analysis, 18th ed., 2005. Method No. 981.12.

1 **12.2.4. Histamine Content (Annex 8)**

2 According to the AOAC Official Methods of Analysis, 18th ed., 2005. Method
3 No. 977.13 or other scientifically equivalent method.

4 **12.2.5. Determination of Net Weight**

5 According to the procedure described in Annex 9 - Determination of Net
6 Weight

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11 **13. REFERENCES**
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33 **Prepackaged Foods** (CAC/RM 42-1969) *In* Joint FAO/WHO Food Standards
34 Program: Codex Alimentarius Commission Volume 13: Methods of Analysis and
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2 **Stan 302-2011)**. Codex Alimentarius Commission. Food and Agriculture
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- 4 DOH. 2007. **Philippine National Standards for Drinking Water 2007 (AO 2007-**
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15 **Specifications**. Philippine National Standards (PNS), Bureau of Product Standards
16 (BPS)

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ANNEX 1**Species of Finfishes Utilized in the Production of Fish Sauce (*Patis*) and Fish Flavored Sauce**

English Name	Local Name	Scientific Name
1. Anchovy	<i>Dilis</i>	<i>Stolephorus</i> spp
2. Barracuda	<i>Tursillo</i>	<i>Spyraena</i> spp.
3. Big-eyed scad	<i>Matangbaka</i>	<i>Selarcrumenophthalmus</i>
4. Croaker	<i>Alakaak</i>	<i>Penahias</i> spp/
5. Fimbriated sardines	<i>Tunsoy</i>	<i>Sardinellafimbriata</i>
6. Frigate tuna	<i>Tulingan</i>	<i>Auxisthazard</i>
7. Fusilier	<i>Dalagangbukid</i>	<i>Caesio</i> spp.
8. Indian mackerel	<i>Alumahan</i>	<i>Rastrelligerkanagurta</i>
9. Indian oil sardines	<i>Tamban</i>	<i>Sardinellalongiceps</i>
10. Japanese scad	<i>Galunggong</i>	<i>Decapterusmaruadsi</i>
11. Lizard fish	<i>Kalaso</i>	<i>Saurida</i> spp.
12. Mackerel scad	<i>Galunggong</i>	<i>Decapterusmacarellus</i>
13. Parrotfish	<i>Loro, Molmol</i>	<i>Leptuscarus</i> sp.
14. Round sardinella	<i>Lapad</i>	<i>Sardinellaaurita</i>
15. Short bodied mackerel	<i>Hasa-hasa</i>	<i>Rastrelligerbrachysoma</i>
16. Short finned scad or roundscad	<i>Galunggong</i>	<i>Decapterusmacrosoma</i>
17. Slipmouth	<i>Sap-sap</i>	<i>Leignatuhs</i> spp.
18. Spotted sardinella	<i>Tamban, tunsoy</i>	<i>Amblygastersirm</i>
19. Smooth belly sardinella	<i>Tamban</i>	<i>Amblygasterleiogaster</i>
20. Striped mackerel	<i>Alumahan</i>	<i>Rastrelligerkanagurta</i>
21. Threadfin bream	<i>Bisugo</i>	<i>Nemipterus</i> spp.
22. White sardinella	<i>Tunsoy</i>	<i>Sardinellaalbella</i>
23. Whiting, common	<i>Asuhos</i>	<i>Sillagosihama</i>

Reference:

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ANNEX 2

Standard for Iodized Salt

1. SCOPE

This standard applies to iodized salt used as condiment or an ingredient in the preparation of food in households, food service and food manufacturing establishments.

2. DESCRIPTION

Iodized salt is food grade salt that contains the prescribed level of iodine. It shall be produced refined or unrefined (crude) salt obtained from underground rock salt deposits or by evaporation of seawater or natural brine. The finished product shall be in the form of solid crystal or powder, white in color, without visible spots of clay, sand, gravel or other foreign matter.

3. IODIZATION PROCESS

3.1 Salt may be iodized with potassium iodate (KIO_3) or potassium iodide (KI) by means of any of the following methods:

- a) dry mixing of salt in powdered form
- b) dip feeding or spray mixing if salt is in crystal form
- c) submersion of ice crystals in iodated brine

4. ESSENTIAL COMPOSITION AND QUALITY FACTORS

To ensure the stability of iodine, salt to be iodized must conform with the following quality requirements:

Moisture, minimum	4 % for refined salt 7 % for unrefined salt
NaCl minimum	97 % dry basis
Calcium and magnesium, maximum	2 %
Water insolubles, maximum	0.2 %
Heavy metal contaminants	
Arsenic as As	0.5 mg/kg
Cadmium as Cd	0.5 mg/kg
Lead as Pb	2.0 mg/kg
Mercury as Hg	0.1 mg/kg

1 **4.1 Naturally Present Secondary Products and Contaminants in Raw Salt**

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3 Notwithstanding the purity requirements in section 4.1. the raw salt may
4 naturally contain secondary products, which are present in varying amounts
5 depending on the origin and method of production of salt, and which are
6 composed mainly of calcium, potassium, magnesium and sodium sulphates,
7 carbonates, bromides and of calcium, potassium chlorides as well as natural
8 contaminant may also be present in amounts varying with the origin and
9 method of production of the salt.

10
11 **5. LABELLING**

12
13 5.1 Iodized salt for commercial distribution shall carry appropriate labeling in
14 accordance with BFAD rules and regulations on labeling of prepackaged
15 foods. Specifically, the following information shall be declared in every
16 container of iodized salt whether in bulk or retail package.

17
18 5.1.1 For locally produced iodized salt

- 19
20 a) The name of the product, "IODIZED SALT", printed in bold capital
21 letters
22 b) Name and address of manufacturer
23 c) Net weight
24 d) Iodine compound used
25 e) Chemical additives, e.g. anti-caking agents, emulsifiers
26 f) Open date marking, e.g. "Best Before" or "Consume Before" Date
27 g) Lot identification code (replacers must use manufacturer's lot i.d code)
28 h) Storage Instruction: STORE IN COOL DRY PLACE
29

30 5.1.2 For imported Iodized salt

- 31
32 a) same as 5.1.1 (a), (c) to (h)
33 b) Name and address of Importer/Local Distributor
34 c) Country of Origin
35

36 5.2 Labeling of Non-retail Containers

37
38 In the case of non-retail containers of at least 25 kg of iodized salt, the
39 labelling information required in sections 5.1.1. (b), (d) or in 5.1.2 (b) may not
40 be declared if such bulk packages are intended for delivery to distributors of
41 food manufacturers/institutional users, provided every shipment or delivery is
42 accompanied by a document containing all information in 5.1.1. or 5.1.2.
43

44 5.3 Iodine levels based on WHO recommendation

45
46 In order to meet national needs, the prescribed levels of iodized salt be
47 indicated below:
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49
50
51
52

	Type of Container	Packages
Sampling point	Bulk (>2 kg)	Retail (<2 kg)
Production site	70-150 g/kg	60-100 mg/kg
Port of entry*	70-150 mg/kg	60-100 mg/kg
Retail site	> 50 mg/kg	> 40 mg/kg

* For imported iodized salt, also at importer's/distributor warehouse

6. FOOD ADDITIVES

6.1 All additives used, including KIO and KI, and shall be of food grade quality and shall conform to the specifications prescribed by JECFA of the Food Chemicals Codex.

6.1.1	Anti-caking Agents	Maximum Level in the Final Product
6.1.1.1	Coating agents; Carbonate.) Calcium/magnesium, Magnesium oxide;) Phosphate, Tricalcium; Silicon dioxide,) amorphous; Silicates, calcium ,) magnesium, sodium alumino or sodium or) sodium calcium alumino)	20 g/kg singly or in Combination
6.1.1.2.	Coating hydrophobic agents, aluminum,) calcium, magnesium, potassium or sodium) salts of myristic, palmitic or stearic acid))	
6.1.1.3	Crystal modifiers: ferrocyanide, calcium,) potassium combination or sodium))	10 mg/kg singly or in combination, expressed as {Fe(CN)}
6.1.2.	Emulsifiers) Polysorbate 80)	10 mg/kg
6.1.3	Processing Aid) Dimethylpolysiloxane)	10 mg of residue/kg

7. PACKAGING

All iodized salt shall be packed in woven propylene bags, clean and unused jute bags, or other non-porous material with a lining of high density polyethylene to ensure the retention of appropriate iodine level at the time of consumption.

8. STORAGE, TRANSPORT AND DISPLAY AT RETAIL

In order to minimize avoidable losses of iodine, iodized salt shall not be exposed to any of the following conditions during storage, transport and display at retail outlets:

- a) direct sunlight or near source of strong light
- b) high temperature and humidity
- c) contamination with moisture, e.g. rain, flood, etc.
- d) contamination with dust or filth from the environment

October 10, 2007

Bureau Circular
No. 2007-009

Subject: Updated Standards for Iodine level of Salts

I. RATIONALE

Rule VI, Section 1 a) of the Revised Implementing Rules and Regulations (RIRR) of Republic Act (RS) No. 8172 also known as “An Act Promoting Salt Iodization Nationwide and for other Purposes” identifies Department of Health (DOH), as the lead agency in the implementing the said Act, and that through the Bureau of Food and Drugs (BFAD), the DOH shall set and enforce standards for food-grade iodized salt and monitor the compliance thereof by the food-grade manufacturers/importers, distributors and traders as specified in Section 2 Rule VIII.

The Food Nutrition and Research Institute (FNRI) on 26 May 2007 referred to the BFAD its recommendation on the possible levels of iodine across distribution stages. In particular, the FNRI proposed the following standard for iodine content:

	Type of containers/packaging	
	Bulk (<2 kilograms)	Retail (<2 kilograms)
Iodine Content	40-70 mg/kg	15-40 mg/kg

Also, attached with said letter are syntheses of studies conducted in other countries that provided empirical basis for regulatory decision.

It is emphasized that lowering the standard will harmonize the iodine level with other countries, will reduce cost and will encourage compliance. Also emphasized in the attachments is the international iodine standard which is 15-20 mg/kg.

II. DIRECTIVE

In view of the foregoing considerations, and ease of administration of regulatory standards, the BFAD hereby adopts the following standard for iodine content in pursuant of its mandate provided for in RA 8172.

Iodine Content : 20-70 mg/kg across distribution channels, whether bulk or retail, imported or local

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III. REPEALING CLAUSE

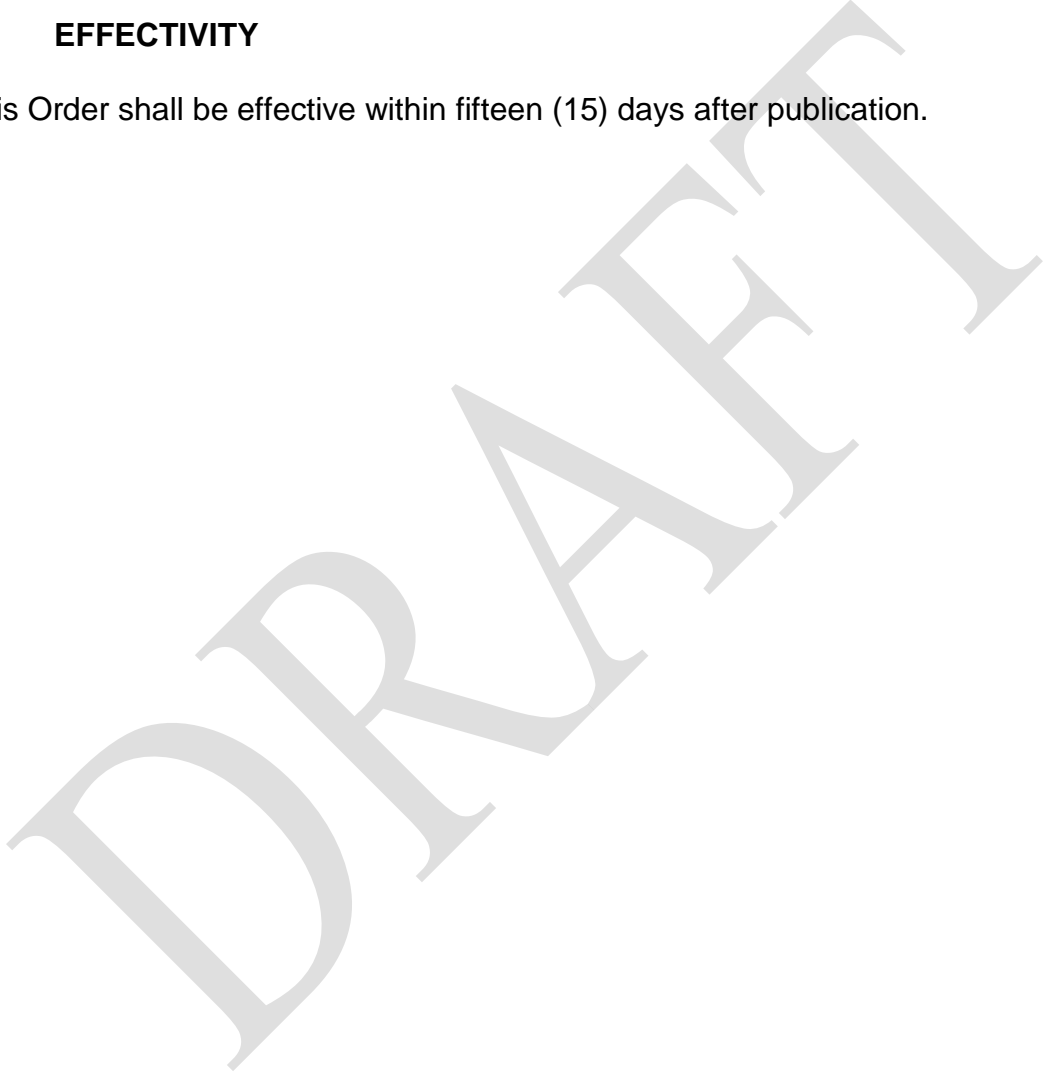
Provisions of previous issuances which are contrary to those reflected hereon are modified, and/or repealed accordingly.

IV. SEPARABILITY

If any provisions of this Order is declared as unconstitutional, or not valid, the rest of the provisions thereon shall still subsist given their effect in entirety.

V. EFFECTIVITY

This Order shall be effective within fifteen (15) days after publication.



ANNEX 3

Standard Parameters and Values for Drinking Water
Philippine National Standards for Drinking Water 2007 (DOH AO 2007-0012)

Table 1. Standard values for bacteriological quality

Parameter	Value/Unit	Point of Compliance
Total Coliform	< 1.1 MPN/100 ml	Service Reservoir Water treatment works Consumers' taps Refilling stations Water haulers Water vending machines
Fecal Coliform	< 1.1 MPN/100 ml	Service Reservoir Water treatment works Consumers' taps Refilling stations Water haulers Water vending machines Point sources - Level 1
Heterotropic Plate Count	< 500 CFU/ml	Service Reservoir Water treatment works Consumers' taps nearest meter Refilling stations Water vending machines

Table 2. Standard values for Physical and Chemical Quality for Acceptability Aspects for Drinking Water

Constituents	Maximum Level (mg/L) or Characteristic	Constituents	Maximum Level (mg/L) or Characteristic
Taste	No objectionable taste	Hydrogen Sulfide	0.05
Odor	No objectionable odor	Iron	1.0
Color	Apparent = 10 color units True = 5 color units	Manganese	0.4
Turbidity	3 NTU	pH	6.5 – 8.5
Aluminum	0.2	Sodium	200
Chloride	250	Sulfate	250
Copper	1.0	Total Dissolved Solids	500
Hardness	300 as CaCO ₃	Zinc	5.0

Table 3. Standard Values for Organic and Inorganic Chemical Constituents of Health Significance in Drinking Water

Inorganic Chemical	Constituents	Maximum Level (mg/L)	Constituents	Maximum Level (mg/L)
	Antimony	0.02	Fluoride	1.0
	Arsenic	0.05	Lead	1.01
	barium	0.7	Mercury (total)	0.001
	Boron	0.5	Nickel	0.02
	Cadmium	0.003	Nitrate	50
	Chromium (Total)	0.05	Nitrite	3.0
	Cyanide (Total)	0.07	Selenium	0.01
Organic Chemical	Constituents	Maximum Level (mg/L)	Constituents	Maximum Level (mg/L)
	Benzene	0.01	Ethylbenzene	0.30
	Carbon tetrachloride	0.004	Nitritotriacetic acid (NTA)	0.20
	1,2-Dichlorobenzene	0.1	Polyaromatic hydrocarbons (PAHs)	0.20
	1,4-Dichlorobenzene	0.5	Polynuclear aromatic	0.0007
	1,2-Dichloroethane	0.003	Tetrachloroethene	0.02
	1,1-Dichloroethene	0.05	Styrene	0.04
	1,2-Dichloroethene	0.07	Tetrachloroethene	0.70
	Dichloromethane	1.0	Trichloroethene	0.07
	Di(2-ethylhexyl) phthalate	1.01	Vinyl chloride	0.0003
	Edetic Acid (ADTA)	0.001	Xylene	0.5
Organic Pesticide	Constituents		Maximum Level (ug/L)	Status in the Philippines
	Aldrin and Dieldrin (combined)		30.0	Banned
	Atrazine		0.03	Registered
	Carbofuran		2.0	Registered
	Chlordane		7.0	Banned
	DDT **		0.2	Banned
	1,2-Dibromo-3-chloropropane (DBCP)		1.0	Banned
	2,4-Dichlorophenoxyacetic acid (2,4-D)		1.0	Registered
	Endrin		30.	Banned
	1,2-Dibromomethane (Ethylene dibromide)		0.6	Banned
	Heptachlor and Heptachlor epoxide (combined)		0.03	Banned
	Lindane		2.0	Restricted
	MCPA (4-(2-methyl-4-chloro) phenoxy acetic acid		2.0	Registered
	Pendimethalin		20.0	Registered
	Pentachlorophenol (PCP)		9.0	Banned

ANNEX 4

**Codex Alimentarius Sampling Plans for Prepackaged Foods (AQL 6.5)
(CAC/RM 42-1969)**

**Sampling Plan No. 1 – Normal Operations
Inspection Level 1, AQL 6.5)**

1. Net weight: ≤ 1 kg

Lot Size (N)	Sample size	Acceptance Number (C)
4,800 or less	6	1
4,801 – 24,000	13	2
24,001 – 48,000	21	3
48,001 – 84,000	29	4
94,001 – 144,000	48	6
144,001 – 240,000	84	9
More than 240,000	126	13

2. Net weight: >1 kg ≥ 4.5 kg

Lot Size (N)	Sample size	Acceptance Number (C)
2,400 or less	6	1
2,401 – 15,000	13	2
15,001 – 24,000	21	3
24,001 – 42,000	29	4
42,001 – 72,000	48	6
72,001 – 120,000	84	9
More than 120,000	126	12

3. Net weight > 4.5 kg

Lot Size (N)	Sample size	Acceptance Number (C)
600 or less	1	1
601 – 2,000	13	2
2,001 – 7,200	21	3
7,201 – 15,000	29	4
15,001 – 24,000	48	6
24,001 – 42,000	84	9
More than 42,000	126	13

Sampling Plan 2 - In Case of Disputes
Inspection Level 2, AQL 6.5)

1. Net weight: ≥ 1 kg

Lot Size (N)	Sample size	Acceptance Number (C)
4,800 or less	13	2
4,801 – 24,000	21	3
24,001 – 48,000	29	4
48,001 – 84,000	48	6
94,001 – 144,000	84	9
144,001 – 240,000	126	13
More than 240,000	200	19

2. Net weight: >1 kg ≥ 4.5 kg

Lot Size (N)	Sample size	Acceptance Number (C)
2,400 or less	13	2
2,401 – 15,000	21	3
15,001 – 24,000	29	4
24,001 – 42,000	48	6
42,001 – 72,000	84	9
72,001 – 120,000	126	13
More than 120,000	200	19

3. Net weight > 4.5 kg

Lot Size (N)	Sample size	Acceptance Number (C)
600 or less	13	2
601 – 2,000	21	3
2,001 – 7,200	29	4
7,201 – 15,000	48	6
15,001 – 24,000	84	9
24,001 – 42,000	126	13
More than 42,000	200	19

Source: Codex Alimentarius Sampling Plans for Prepackaged Foods - CAC/RM 42-1969, Codex Alimentarius
Volume13.

ANNEX 5

**Determination of Total Nitrogen (TN) for Protein Determination
(Improved Kjeldahl Method)**

1. Reagents:

- a. Sulfuric acid. – 93-98% H₂SO₄, N-free
- b. Mercuric oxide or metallic mercury. – HgO or Hg, reagent grade, N-free
- c. Potassium sulfate (or anhydrous sodium sulfate). Reagent grade, N-free
- d. Salicylic acid, Reagent grade, N-free
- e. Sulfide or thiosulfate solution. – Dissolve 40g com. K₂S in 1L H₂O. (Soln of 40 g Na₂S or 80 g Na₂S₂O₃·5H₂O in 1L may be used.)
- f. Sodium hydroxide.- Pellets or solution, nitrate-free. For soln, dissolve ca 450g solid NaOH in H₂O, cool, and dilute to 1L. (Specific gravity should be ≥1.36)
- g. Zinc granules. – Reagent grade.
- h. Zinc dust. – Impalpable powder.
- i. Methyl red indicator. – Dissolve 1g methyl red in 200 ml alcohol.
- j. Hydrochloric or sulfuric acid standard solution. – 0.5or0.1M when amount of N is small, or (sulfuric acid – 0.25 or 0.05M when amount of N is small)
- k. Sodium hydroxide standard solution. –0.1M

Standardize each standard solution with primary standard and check one against the other. Test reagents before use by blank determination with 2 g sugar, which ensures partial reduction of any nitrates present.

[*Caution:* Use only freshly opened H₂SO₄ or add dry P₂O₅ to avoid hydrolysis of nitrites and cyanates. Ratio of salt to acid (w:v) should be ca 1:1 at the end of digestion for proper temperature control. Digestion maybe incomplete at lower ratio; nitrogen may be lost at higher ratio. Each g fat consumes 10 mL H₂SO₄ and each g carbohydrates 4 mL during digestion]

2. Apparatus:

- a. For digestion. Use Kjeldahlflask or hard moderately thick, well-annealed glass with total capacity ca 500-800ml. Conduct digestion over heating device to bring 250 mL H₂O at 25C to rolling boil in ca 5 min or other time as specified in method. To test heaters, preheat 10 min if gas or 30min if electric. Add 3-4 chips to prevent superheating.
- b. For distillation. Use 50-800 Kjeldahl or other suitable flask, fitted with rubber stopper through which passes lower end of efficient scrubber bulb or trap to prevent mechanical carryover of NaOH during distillation. Connect upper end of the bulb tube to condenser tube by rubber tubing. Trap outlet of condenser

1 in such a way as to ensure complete absorption of NH_3 distilling over into acid
 2 in receiver.

3
 4 **3. Determination**
 5

- 6 a. Place weighed test portion of fish sauce (0.7-2.2 g) in a digestion flask. Add
 7 0.7g HgO or 0.65g metallic mercury, 15 g powdered K_2SO_4 or anhydrous
 8 Na_2SO_4 , and 25ml H_2SO_4 . If test portion > 2.2g is used, increase H_2SO_4 by 10
 9 ml for each g test portion. Place flask in inclined position and heat gently until
 10 frothing ceases (if necessary, add a small amount of paraffin to reduce
 11 frothing); Boil briskly until solution clears and then add then \geq min longer (2h
 12 for materials containing organic material)
- 13 b. Cool, add 200 ml H_2O , cool below 25° add 25 ml of the sulfide or thiosulfate
 14 solution, and mix to precipitate Hg. Add few Zn granules to prevent bumping,
 15 tilt flask and add layer of NaOH without agitation. (For each 10ml H_2SO_4 used,
 16 or its equivalent in diluted H_2SO_4 , add 15 g solid NaOH or enough solution to
 17 make contents strongly alkaline.) (Thiosulfate or sulfide solution may be
 18 mixed with the NaOH solution before addition to flask.) Immediately connect
 19 flask to condenser and, with the tip of condenser immersed in standard acid
 20 and 5-7 drops indicator in receiver, rotate flask to mix contents thoroughly;
 21 then heat until all (NH_3) has distilled (≥ 150 ml distillate). Remove receiver,
 22 wash tip of condenser, and titrate excess standard acid in distillate with
 23 standard (NaOH) solution. Correct for blank determination on reagents.

24
 25 **4. Calculation of Percent Nitrogen (N) of Fish Sauce**
 26

- 27 a. When standard HCl is used:

28
 29
$$\text{Percent N} = (\text{mL of standard acid} \times \text{molarity of acid}) - (\text{ml of standard NaOH} \\ \times \text{molarity of NaOH}) \times 1.4007 / \text{g fish sauce sample}$$

- 30
 31
 32 b. When standard H_2SO_4 is used:

33
 34
$$\text{Percent N} = (\text{mL of standard acid} \times 2 \times \text{molarity of acid}) - (\text{ml of standard} \\ \text{NaOH} \times \text{molarity of NaOH}) \times 1.4007 / \text{g fish sauce sample}$$

- 35
 36
 37 c. Calculation of Nitrogen Content from Percent to g/liter is computed as follows:

38
 39
$$\text{Nitrogen (g/liter)} = \% \text{ N} \times 10 / \text{specific gravity of fish}$$

40
 41
$$\text{Specific gravity of fish sauce} = 1.20$$

42
 43
 44
 45 **5. Calculation of Total Nitrogen to Percent Protein**
 46

47
$$\% \text{ Protein} = \text{Nitrogen (g/liter)} \times \text{PF} / \text{specific gravity} \times 10$$

1 PF = Protein Factor needed to convert nitrogen concentration to protein. For fish
 2 sauce and other fishery products, a PF of 6.25 is used. This is equivalent to
 3 0.16 g nitrogen per gram of protein.
 4

5 The conversion of total nitrogen (g/liter) to percent protein is provided in Table 1.
 6

7 **Table 1. Calculated percent protein from total nitrogen (TN) in g/liter**
 8

Total Nitrogen (gram/liter)	Protein (%)	Total Nitrogen (gram/liter)	Protein (%)	Total Nitrogen (gram/liter)	Protein (%)
0.5	0.26	10.5	5.47	21.0	10.94
1.0	0.52	11.0	5.73	21.5	11.20
1.5	0.78	11.5	5.99	22.0	11.46
2.0	1.04	12.0	6.25	22.5	11.72
2.5	1.30	12.5	6.51	23.0	11.98
3.0	1.56	13.0	6.77	24.0	12.50
3.5	1.82	13.5	7.03	24.5	12.76
4.0	2.08	14.0	7.29	25.0	13.02
4.5	2.34	14.5	7.55	25.5	13.28
5.0	2.60	15.0	7.81	26.0	13.55
5.5	2.86	15.5	8.07	26.5	13.81
6.0	3.13	16.0	8.33	27.0	14.07
6.5	3.39	16.5	8.59	28.0	14.56
7.0	3.65	17.0	8.85	28.5	14.85
7.5	3.91	17.5	9.11	29.0	15.11
8.0	4.17	18.0	9.38	30.0	15.63
8.5	4.43	18.5	9.64	30.5	15.89
9.0	4.69	19.0	9.90	31.0	16.15
9.5	4.95	20.0	10.42	31.5	16.41
10.0	5.21	20.5	10.68	32.0	16.67

33 **References:**

- 35 1. AOAC. 2005. Official Methods of Analyses of AOAC: Method 940.25, 955.04. AOAC International 18th Edition
 36 2. JAOAC 38, 56(1955)
 37
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ANNEX 6**Determination of Salt (Chlorine as Sodium Chloride)
(Volumetric Method)****1. Reagents**

- a. Silver nitrate standard solution -0.1 N. Prepare by dissolving slightly more than theoretical weight of AgNO_3 (Equivalent weight, 169.87) in halogen-free H_2O and dilute to volume. Thoroughly clean glassware, avoid contact with dust, and keep prepared solution in amber glass-stoppered bottles away from the light. Standardize against 0.1N NaCl containing 5.844g of dry NaCl/L.
- b. Ammonium thiocyanate standard solution – 0.1N. Prepare ca 0.1N solution from reagent that shows no chlorine, using 7.612 g NH_4SCN or KSCN /L.
- c. Determine working titer by accurately measuring 40-50mL standard AgNO_3 solution adding 2 mL ferric alum solution and 5mL HNO_3 (1 + 1), and titrating with thiocyanate solution until solution appears pale rose after vigorous shaking.
- d. Ferric indicator – Saturated solution of $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$
- e. Nitric acid solution – 6N. Transfer 380 mL concentrated HNO_3 in 1L volumetric flask. Volume to 1L with distilled H_2O . Transfer to amber bottle.

2. Determination

- a. Weigh 10 gram triplicate samples into 250 mL Erlenmeyer flask.
- b. Add 20 ml of the standardized 0.1 N AgNO_3 or more than enough to precipitate all Cl as AgCl .
- c. Add 20 ml of HNO_3 . Boil gently on a hot plate or sand bath until all solids except AgCl solids dissolve (usually 15 min).
- d. Cool, add 50 mL H_2O and 50 mL indicator and titrate with 0.1N NH_4SCN solution until solution becomes permanent light brown.
- e. Subtract mL 0.1N NH_4SCN used from mL 0.1N AgNO_3 added and calculate difference as NaCl. With 10 g sample each mL 0.1N AgNO_3 = 0.058% NaCl.

References :

1. AOAC. 2005. Official Methods of Analyses of the AOAC: Official Method 937.09. AOAC International 18th Edition, 2005
2. JAOAC 20, 410 (1937); 23, 589(1940)
3. CAS-7647-14-5 (Sodium Chloride)

ANNEX 7

Determination of pH

1. Principle

pH is measurement of H ion activity and indicates acidity. It may be measured by determining electric potential between glass and reference electrodes, using commercial apparatus standardized against primary standard pH buffers.

2. Apparatus and Reagents

- (a) pH meter. Commercial instrument with scale graduated in ≤ 0.1 pH unit and repeatability of ≤ 0.05 unit. Some instruments permit expansion of any 2 pH unit range to cover entire scale and have accuracy of ca 0.01 pH unit and repeatability of 0.005 pH unit. Other instruments have digital read-outs with similar capabilities. Operate meter in accordance with manufacturer's instructions.
- (b) Standard buffer solutions – pH 4 buffer and pH 7 buffer.
- (c) Electrodes – Glass membrane indicator electrode and calomel reference electrode (single or combination). Keep calomel electrodes filled with saturated KCl solution.
- (d) Balance – With capacity of ≤ 2 kg and sensitivity of 0.1 g.

3. Standardization and Operation of pH meter

- (a) Switch instrument on and let electronic components warm up and stabilize before proceeding.
- (b) Standardize specific instrument according to manufacturer's instructions, using NIST SRM2 buffers. Equilibrate electrodes, buffers, and samples at same temperature (ca 2 °C) before pH measurements. Set temperature compensator control of instrument at observed temperature. When determining pH of either unknown sample or buffer, gently stir solution before testing.

4. pH Determination

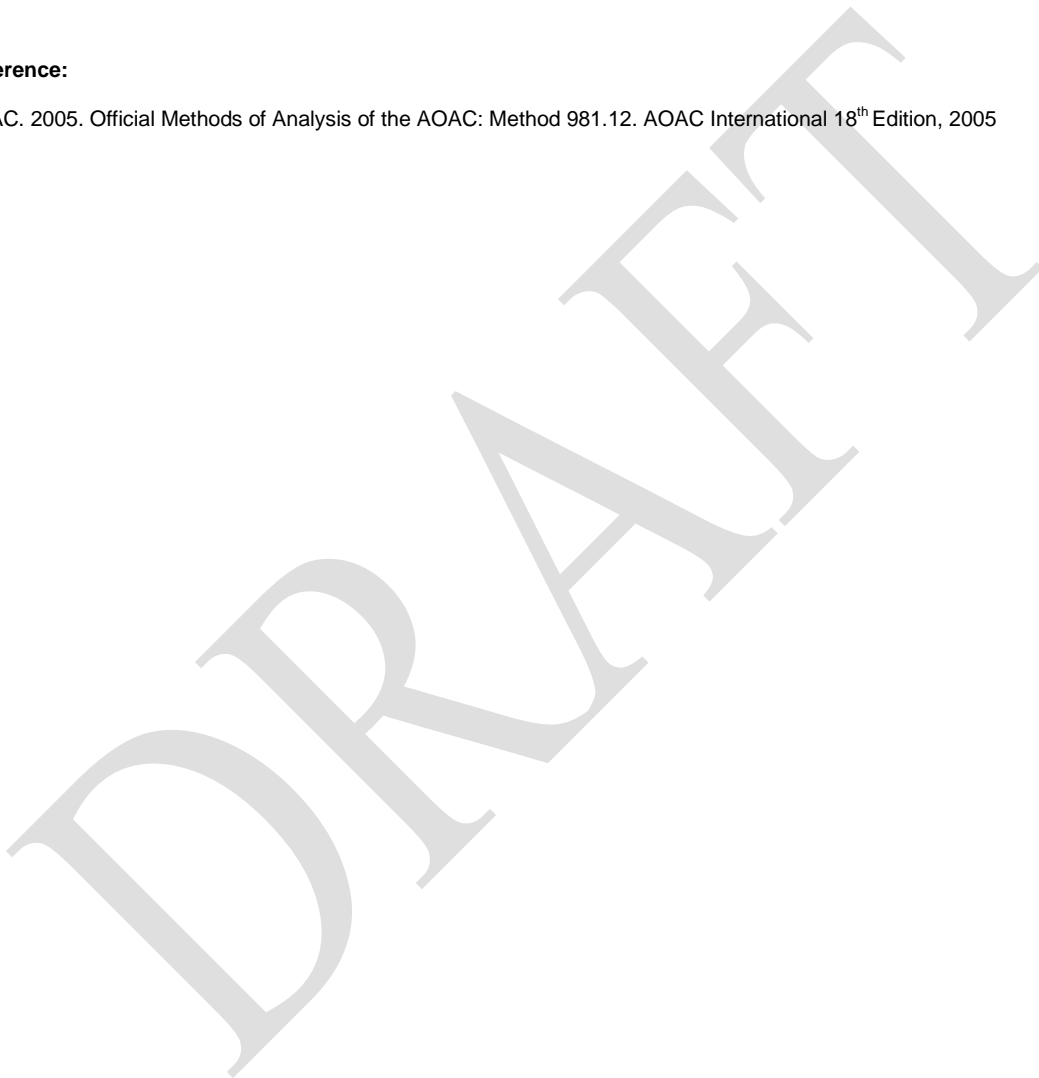
- (a) Obtain test portions of fish sauce for pH determination.
- (b) Dilute fish sauce to 1:10 with distilled water by weight. The dilution of fish sauce is necessary because of the high ionic strength in the undiluted sauce (Codex Standard for Fish Sauce (Codex Stan 302-2011)).

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- (c) Mix diluted sample and allow to equilibrate to ca 25°C. If pH meter is equipped with temperature compensator, then it may be used in lieu of equilibrating samples to specified temperature, provided it is $\pm 15 - 25^\circ\text{C}$.
- (d) pH measurement. Rinse and blot electrodes. Immerse electrodes in sample and read pH, letting meter stabilize 1 min. Rinse and blot electrodes and repeat on fresh portion of sample. Determine 2 pH values on each sample. Readings in close agreement indicate that sample is homogenous. Report values to 2 decimal places.

Reference:

AOAC. 2005. Official Methods of Analysis of the AOAC: Method 981.12. AOAC International 18th Edition, 2005



ANNEX 8

Determination of Histamine
(Fluorometric Method)

1. Principle

Product 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.

2. Apparatus

(a) *Chromatographic tube* – 200 x 7 id mm propylene tube fitted with small plastic stopcocks and ca 45 cm Teflon tubing. Control flow rate at >3mL/min by adjusting height of column relative to tubing outlet. Alternatively, use 2-way valve in place of tubing.

(b) *Photofluoremeter*– With medium pressure Hg lamp with excitation at 350 nm and measuring emission at 444 nm.

(c) *Repipets* – 1 or 5 mL

3. Reagents:

(a) *Ion exchange resin* - - Bio-Rad AG 1-X8, 50-100 mesh (Bio-Rad Laboratories, 1000 Alfred Nobel Dr. Hercules, CA 94547, USA: www.biorad.com) or Dowex 1-X8, 50-100 mesh. Convert to –OH form by adding ca 15mL 2M NaOH/g resin to beaker. Swirl mixture and let stand <30 min. 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, **B(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* – 1.19M as H₃PO₄. Dilute 121.8 mL 85% H₃PO₄ to L. For other concentration H₃PO₄, the volume required for 1L 1.19M H₃PO₄ = 17493/(density H₃PO₄ x % 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%. 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 (1) *Stock solution.* – 1 mg/mL as free base. Accurately weigh ca 169.1 mg
2 Histamine·2HCl (98%) into 100 mL volumetric flask, and dissolve and
3 dilute to volume with 0.1M HCl. Prepare fresh weekly.
4
- 5 (2) *Intermediate solution* - 10µg/mL. Pipet 1mL stock solution into 100 mL
6 volumetric flask, and dilute to volume with 0.1M HCl. Prepare fresh
7 weekly.
8
- 9 (3) *Working solutions* - 0.5, 1.0 and 1.5µg/mL. Pipet 1,2 and 3 mL
10 intermediate solution into separate 100 mL volumetric flasks, and dilute
11 each to volume with 0.1M HCl. Prepare fresh daily.
12

13 4. Preparation of Standard Curve

- 15 (1) Pipet duplicate 5 mL aliquots of each working standard solution into
16 separate 50mL glass or polypropylene Erlenmeyers. Pipet in 10 mL
17 0.1M HCl to each flask and mix. Pipet in 3 mL 0.1M HCl and mix. Pipet
18 in 3mL 1M NaOH and mix. Within 5 min, Pipet in 1mL OPT solution and
19 mix immediately.
20
- 21 (2) After exactly 4 min. pipet in 3 mL 3.57 N H₃PO₄ and mix immediately. It
22 is important to mix thoroughly after each addition and at least once
23 during OPT reaction. (Run 6-10 OPT reactions simultaneously by adding
24 reagents to Erlenmeyers in set order).
25
- 26 (3) Prepare blank by substituting 5 mL 0.1M HCl for histamine solution.
27 Within 1.5 h record fluorescence intensity (*I*) of working standard
28 solutions with H₂O in reference cell, using excitation wavelength 350 nm
29 and emission wavelength of 444 nm. Plot (*I*) (corrected for blank) using
30 µg histamine/5mL aliquot.
31

32 5. Determination

- 34 (1) .Extract prepared 10g test portion with 75% (v/v) methanol as in 957.07C
35 , paragraph 1. (Transfer 10 g prepared test portion in semimicro
36 container of high speed blender , add ca. 50mL methanol, blend in high-
37 speed blender for ca 2 min.
38
- 39 (2) Transfer to 100mL glass-stoppered volumetric flask, rinsing lid and
40 blender jar with methanol and adding rinsing to flask. Heat in H₂O bath
41 to 60°C and let stand for 15 min at this temperature. Cool to 25°C, dilute
42 to volume with methanol, and filter through folded paper. Alcohol filtrate
43 may be stored in refrigerator several weeks.) (Light powdery precipitate
44 separating on storage may be ignored).
45
- 46 (3) Pass 4-5 mL H₂O through column, **B(a)**,and discard eluate. Pipet 1mL
47 extract onto column and add 4-5 mL H₂O. Immediately initiate column
48 flow into 50 mL volumetric flask containing 5.00mL 1.00M HCl. When
49 liquid level is ca 2 mm above resin, add 5 mL H₂O and let elute. Follow
50 with H₂O in larger portions until ca 35 mL has eluted. Stop column flow,
51 dilute to volume with H₂O, stopper, and mix. Refrigerate eluate.

- 1
2 (4) Pipet 5 mL eluate into 50 mL Erlenmeyer, add pipet in 10 mL 0.1M HCl.
3 Proceed as in preparation of calibration curve beginning "Pipet in 3 mL
4 1M NaOH....."

5
6 If test sample contains >15mg histamine/100g fish sauce, pipet 1 mL test
7 solution-OPT mixture into 10 mL beaker containing exactly 2 mL blank-OPT
8 mixture, and mix thoroughly. Read fluorescence of new solution. Dilute and
9 mix aliquots with blank-OPT mixture as needed to obtain measurable
10 reading. This approximation indicates proper dilution of eluate required prior
11 to second OPT reaction needed for reliable quantitation of test solution.
12 Alternatively, use sensitivity range control of fluorometer (if instrument has
13 one) to estimate dilution. Use these approximations to prepare appropriate
14 dilutions of aliquot of eluate with 0.1M HCl, and proceed as in the
15 Preparation of Standard Curve, beginning "Pipet in 3mL 1M NaOH....."

16 6. Calculation

17
18 Plot of I (measured by meter of deflection or recorder response and corrected
19 for blank) against μg histamine/5mL test solution should be straight line
20 passing through origin with slope= $m = [(I_a/1.5) + I_b + 2I_c]/3$.

21
22
23
24
$$\text{mg Histamine/100 g Fish sauce} = (10)(F)(1/m)(I_s)$$

25
26
$$\mu\text{g Histamine/g Fish sauce} = 10 \times (\text{mg histamine/100g fish sauce})$$

27
28 where I_s, I_a, I_b and I_c = fluorescence from samples, 1.5, 1.0, and 0.5 μg
29 histamine standards respectively; and F = dilution factor = (mL eluate = mL
30 0.1M HCl)/mL eluate. $F = 1$ for undiluted eluate.

31
32 If calibration plot is not linear, use standard curve directly from quantitation.
33 Each subdivision on abscissa should be $\leq 0.1\mu\text{g}$ histamine/5 mL test
34 solution.

35 Read all values from curve to nearest 0.05 μg histamine/5 mL test solution

36
37
$$\text{mg Histamine/100 g Fish sauce} = (10)(F)(W)$$

38
39 where W = μg histamine/5 mL test solution as determined from standard
40 curve.

41 References:

- 42
43
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45 1. AOAC. 2005. Official Methods of Analysis of the AOAC: Method 977.13. AOAC International 18th Edition
46 2. JAOAC 60, 1125, 1131 (1977)
47 3. CAS-51-45-6 (histamine)
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ANNEX 9

Determination of Net Weight

1. Apparatus:

Weighing balance (sensitivity: 0.10 gram)

2. Procedure:

- (a) Weigh the sample unit on its original sample packed container. This is the gross weight.
- (b) Open and pour out the contents of each individual package. Wash the empty package and blot dry.
- (c) Weigh out the washed empty package. This is the weight of the packaging material.
- (d) Subtract the weight of the empty package from the gross weight. The resulting figure is the net weight of the individual package (net weight = gross weight – weight of packaging).
- (e) Average the results from all package of a sample representing a lot. Report result as the average net weight of the product.