

FINAL DRAFT
**STANDARDS FOR ETHNIC FLOUR-BASED CONFECTIONERIES (*POLVORON,*
PIAYA, AND BARQUILLOS)**

1. SCOPE

This standard shall apply to ethnic flour-based confectioneries, specifically *polvoron*, *piaya*, and *barquillos*, in suitable packaging materials or containers.

2. DEFINITION OF TERMS

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

Confectionery - a group of food items primarily made of sugar and other sweeteners, and includes candies, caramels, toffees, and chocolate bars. It is a generic term for sweetened food products. Sugar confectionery refers to products such as sweets, candy and chocolates. These products are shelf-stable and usually have water activity below 0.85. (Dictionary of Food Science and Technology, International Food Information Service, Blackwell Publishing, UK, 2005.)

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

Flavor and flavoring substances - substances which are added to impart flavor which are either natural, nature identical or artificial flavoring substances (A.O. No.

88-B s. 1984; Rules and Regulations governing the Labeling of Prepackaged Food Products distributed in the Philippines).

(a) natural flavor

flavoring substances derived through appropriate physical processes from spices, herbs, fruit or fruit juices, vegetable or vegetable juices, edible yeast, bark, bud, root, leaf or plant materials, meat, fish, poultry, eggs, dairy products or fermentation products thereof.

(b) nature-identical flavoring substances

substances chemically derived from aromatic materials or obtained synthetically, which are chemically identical to substances present in natural products intended for human consumption.

(c) artificial flavoring substances

substances that impart flavor but which have not been identified in natural products or natural sources of flavorings.

Flour - a powdery substance that is the product of grinding or milling of cereal grains (such as but not limited to wheat, rice, barley, and maize), root crops and legumes.

Food - 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 additives - any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures to be safe under the conditions of the intended use (R.A. 3720. Food, Drug and Cosmetic Act).

Food and Drug Administration or FDA - formerly known as Bureau of Food and Drug (BFAD) of the Department of Health (DOH); which was renamed in accordance to RA 9711 (Food and Drug Administration (FDA) Act of 2009).

Food standard - 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 the container. It may also include specific labeling requirements other than or in addition to the labeling requirements generally applicable to all prepackaged foods.

Ingredient - any substance including food additive, used as a component in the manufacture or preparation of a food and present in the final product in its original or modified form.

Label - includes any tag, brand, mark, pictorial, or other descriptive script, written, printed, marked, embossed or impressed on, or attached to the container.

Labeling - any written, printed or graphic matter (1) upon any article or any of its container or wrappers and/or (2) accompanying the packaged food.

Lot - food produced during a period of time and under more or less the same manufacturing condition indicated by a specific code.

Milk - the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing (CODEX STAN 206-1999).

Milk product - a product obtained by any processing of milk, which may contain food additives, and other ingredients functionally necessary for the processing (CODEX STAN 206-1999).

Muscovado sugar

a non-centrifugal sugar which is primarily composed of sucrose and molasses. It includes three forms, namely: amorphous; lump or molded locally known as “*panocha*” or “*sangkaka*”; and thick, concentrated syrup locally known as “*inuyat*”. (BAFPS Draft Standards for Muscovado Sugar)

Packaging - the process of packing that is part of the production cycle applied to a bulk product to obtain the finished product. It also refers to any material, including painted material, employed in the packaging of a product including any outer packaging used for transportation of shipment. Packaging materials are referred to as primary or secondary according to whether or not they are intended to be in direct contact with the product.

Rancidity - formation of off-flavors in food due to lipid oxidation (oxidative rancidity) and/or release of free fatty acids by lipolysis (hydrolytic rancidity).

Water activity - the ratio of vapor pressure of water in the food substrate to the vapor pressure of pure water at the same temperature (Jay *et. al.*, 2005). It is also a measure of water available for chemical reactions and microbial growth (Fennema, 1996).

3. DESCRIPTION OF PRODUCTS

3.1 Product Definition

Ethnic flour-based confectioneries are confectionery products with flour and sugar as basic ingredients. The ethnic flour-based confectioneries specifically covered by this standard are the following:

3.1.1 Polvoron –The product is usually made from a mixture of toasted flour, sugar, butter and milk or milk products. Other ingredients such as *pinipig*, chocolate and nuts could be added. The product mixture may be formed into oval or round shapes or by using appropriate *polvoron* molders.

3.1.2 Piaya – *Piaya* is a traditional flat, disc- shaped unleavened pastry with muscovado sugar filling. Other ingredients such as but not limited to purple yam and pandan could also be added.

3.1.3 Barquillos – *Barquillos* may also be called as wafer rolls. The product is a wafer with thin and delicate texture, traditionally made using a special wafer iron (*barquillera*) or baked in an oven, and then immediately rolled to form cylinders. *Barquiron* is a variant of *barquillos* with *polvoron* filling.

3.2 Process Description

The product shall have undergone a process sufficient to ensure quality and shelf life stability at ambient conditions and shall be packed in any suitable packaging material and/or container.

4 ESSENTIAL COMPOSITION AND QUALITY FACTORS

4.1 Raw Materials

4.1.1 Basic Ingredients

(a) Flour – May come from cereal grains, root crops, or legumes, and must conform to RA 8976 (Philippine Food Fortification Act of 2000) (for wheat flour), and other applicable food standards.

(b) Sugar and other sweeteners – May include table sugar, muscovado sugar, corn syrup, and other similar food items, and must conform to RA 8976 (Philippine Food Fortification Act of 2000) (for refined sugar), and other applicable food standards. In some cases, sweetened condensed milk and similar milk products also act as the source of sugar and/or sweetener.

(c) Milk and milk products – Applicable as basic ingredient only for *polvoron*. Must conform to requirements prescribed by PNS/BAFPS 36:2008 (Philippine National

Standard for Fresh Milk), FDA A.O. No. 132 s. 1970 (Regulation Prescribing the Standard of Identity and Quality of Milk and Milk Products, B-4.12-01), and other applicable food standards.

(d) Butter, margarine, lard, oil, and other similar food items – Applicable as basic ingredient for *polvoron* and *piaya*. Must comply with RA 8976 (Philippine Food Fortification Act of 2000) (for cooking oil), FDA A.O. No. 230 s. 1974 (Regulation: B-4 Definition and Standards for Food; B-4.15 Fats and Oils, B-4.15-01 Shortening), FDA A.O. No. 243 s. 1975 (Regulation: B-4 Definition and Standards of Food; B-4.18 Margarine), and other applicable food standards.

4.1.2 Optional Ingredients

(a) Eggs –Must be fresh eggs, and must comply with the requirements prescribed by PNS/BAFPS 35: 2005 (Philippine National Standards for Table Egg), and other applicable food standards. Other forms of processed eggs can be used.

(b) Fruit, vegetables, nuts, and root crops – May include fresh items or preserves and must conform to all applicable food standards

(c) Flavor and Flavoring Substances - All flavor/flavoring substances as defined in subsection 2.4 shall be food grade, and must comply with FDA's B.C. No. 016 s.2006 (Updated List of Food Additives).

(d) Potable Water – Must be water fit for human consumption and potability determined by health authorities as cited in Philippine National Standards for drinking water (Department of Health A. O. No. 2007-0012. Philippine National Standards for Drinking Water 2007).

(e) Sesame Seeds – Must conform to applicable food standards.

4.2 Quality Criteria

4.2.1 General Requirements

(a) **Water Activity** – The water activity (a_w) for *polvoron*, *piaya* and *barquillos* should not be greater than 0.60 at 25°C.

(b) Microbiological limits

The microbiological limits of ethnic flour-based confectioneries shall conform to the standards set for Processed Foods, under the food description Breakfast Cereals and Snack Foods (Bureau Circular No. 1-A, 2004; Guidelines for the Assessment of Microbiological Quality of Processed Foods). The microbiological limits of ethnic flour-based confectioneries shall be as follows*:

Table 1. **Microbiological limits of ethnic flour-based confectioneries**

Test Microorganism	n	C	m	M
Yeast and Molds, cfu/g	5	2	10	10^3
Coliforms, MPN/g	5	2	10	10^2

n – number of samples tested

c – maximum number of samples > m but not more than M

m – guide level

M – Maximum acceptance level

4.2.2 Types of Defects

(a) Odor/flavor/color

A sample unit affected by objectionable odors, flavors and colors which are indicative of rancidity and yeast and mold growth.

(b) Presence of yeasts and molds

The presence of yeasts and molds may signify spoilage and may be due to improper handling of raw materials and finished products during processing, storage, and distribution.

(c) Foreign matter

The presence in the sample unit of any matter, which has not been derived from ingredients or processing aids used, does not pose a threat to human health and is readily recognized without magnification or is present at a level determined by magnification method or any equivalent methods that indicates non-compliance with good manufacturing practices and sanitation practices.

4.2.3 Classification of “Defectives”

A container that has any of the type of defects set in 4.2.2 shall be considered as “defective”.

4.2.4 Lot Acceptance

A lot shall be considered as meeting the applicable quality requirements when the number of “defectives”, as defined in sub-section 4.2.3, does not exceed the acceptance number of the appropriate sampling plan.

5 FOOD ADDITIVES

5.1 Food additives when used shall be in accordance with the regulations established by the Food and Drugs Administration (FDA) (Bureau Circular No. 016 s.2006. Updated List of Food Additives) and/or the Codex Alimentarius Commission.

The following food additives listed in, but not limited to, Table 2, may be used for the manufacture of ethnic flour-based confectioneries.

5.2 All others that have not been included in the above list shall be allowed as carry-over provided they are approved by FDA regulation (B.C. No. 016 s. 2006; Updated List of Food Additives) and shall be in accordance to the Section 4 of the Preamble of the General Standard for Food Additives (GFSA) (Codex Stan 192-1995, Rev. 5 (2004)). These additives include those that are used for the raw materials and other ingredients.

Table 2. **Food Additives for Ethnic flour-based confectioneries (*polvoron*, *piaya*, and *barquillos*)** (B.C. No.016 s. 2006. Updated List of Food Additives)

Function	Food Additive	Max. level of usage
Anti-caking agent	Polydimethylsiloxane	10 mg/kg ^d
Antioxidant	Ascorbyl Esters	200 mg/kg (As ascorbyl stearate) ^d
		100 mg/kg (Fat or oil basis) ^b
		200 mg/kg (Fat or oil basis) ^b
	BHT	50 mg/kg ^c
		200 mg/kg (Fat or oil basis) ^b
		50 mg/kg ^c
		25 mg/kg ^d

	Gallate, propyl	200 mg/kg (Fat or oil basis) ^b	
	Tertiary Butylhydroquinone	200 mg/kg (Fat or oil basis) ^b	
		200 mg/kg ^c	
	Tocopherols	500 mg/kg (Fat or oil basis) ^b	
Color	Allura Red AC	348 mg/kg ^b	
	Amaranth	100 mg/kg ^b	
	Annatto Extracts	25 mg/kg (As total bixin or norbixin) ^b	
	Brilliant Blue FCF	300 mg/kg ^b	
	Caramel Colour, Class III	GMP ^b	
		GMP ^c	
		GMP ^d	
	Caramel Colour, Class IV	GMP ^b	
		GMP ^c	
	Carotenoids	GMP ^c	
		200 mg/kg ^d	
Fast Green FCF	100 mg/kg ^b		
	100 mg/kg ^d		
Sunset Yellow FCF	400 mg/kg ^b		
Preservative	Benzoates	1500 mg/kg (As benzoic acid) ^b	
	Hydroxybenzoates, p-	2000 mg/kg (As p-hydroxybenzoic acid) ^b	
		300 mg/kg (As p-hydroxybenzoic acid) ^d	
Stabilizer	Polysorbates	10000 mg/kg ^b	
		5000 mg/kg (Flour basis) ^c	
		5000 mg/kg ^d	
	Sorbates	2000 mg/kg (As sorbic acid) ^b	
	Sorbitan Esters of Fatty Acids	20000 mg/kg ^a	
		5000 mg/kg (Flour basis) ^c	
		10000 mg/kg ^d	
Sweetener	Alitame	300 mg/kg ^a	
	Acesulfame Potassium	3500 mg/kg ^b	
	Aspartame	10000 mg/kg ^b	
		5000 mg/kg ^d	
	Saccharin	3000 mg/kg ^b	
		2000 mg/kg ^d	
	Sucralose	1500 mg/kg ^b	

^a Based on the Food Category System: 5.0 Confectionery;

^b Based on the Food Category System: 5.2 Sugar-based confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3, and 05.4;

^c Based on the Food Category System: 7.1.2. Crackers, excluding sweet crackers

^d Based on the Food Category system: 7.2 Fine bakery wares

6 CONTAMINANTS

The products covered by this standard shall comply with the maximum limits for contaminants and the maximum residue limits for pesticides and veterinary drugs established by the Codex Alimentarius Commission.

7 HYGIENE

7.1 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1 – 1969, Rev. 4-2003), Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004), Recommended International Code Of Hygienic Practice for Egg Products CAC/RCP 15-1976 (amended 1978, 1985), and/or the FDA A.O. No. 153 s. 2004 - Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food

7.2 When tested by appropriate methods of sampling and examination, the product:

- shall be free from filth that may pose a hazard to health;
- shall be free from parasites which may represent a hazard to health;
- shall not contain any substance originating from microorganisms in amounts which may represent a hazard to health;
- shall be free from microorganisms capable of development under normal conditions of storage; and
- shall be free from container integrity defects which may compromise the hermetic seal.

8 PACKAGING AND LABELING

8.1 The packaging used for ethnic flour-based confectioneries should be made of suitable food-grade materials, and should be clean, and hygienic. The packaging materials used should not adversely affect product quality and safety.

The product must also be packed and sealed in suitable containers that will provide additional protection against contamination. The packaging material used should be able to withstand mechanical, chemical and thermal stresses encountered during normal product handling and distribution.

8.2 The net weight of any sample unit shall be in accordance with FDA's B.C. No. 6-A s. 1998 (Permissible Net Content Variation in Prepackaged Food).

8.3 Labeling

Each container shall be labeled and marked with the following information in accordance with FDA's Labeling Regulation (A.O. 88-B s. 1984; Rules and Regulations governing the Labeling of Prepackaged Food Products distributed in the Philippines):

(a) The name of the product shall be known as *Polvoron* (Molded Toasted Flour with Butter), *Piaya* (Native Pastry with Melted Sugar Fillings), or *Barquillos* (Wafer Rolls). Additional descriptors pertaining to the ingredients used or the product form may also be included (e.g. *Barquiron*, Purple yam *piaya*; Chocolate *polvoron*, Pandan-flavored *barquillos*). Other local or regional names referring to products similar to those defined under subsection 3.1 may also be included, provided that these names are acceptable in the area of distribution, and in accordance with the customs or practices of the area or country in which the product is distributed.

(b) The complete list of ingredients and food additives used in the preparation of the product in descending order of proportion.

(c) The net quantity of content by weight in the metric system. Other systems of measurement required by importing countries shall appear in parenthesis after the metric system unit.

(d) The name and address of the manufacturer, packer and/or distributor of the food.

(e) Open date marking

The words "Best/Consume Before" or "Expiry Date" or "Use by" indicating end of period at which the product shall retain its optimum quality attributes at defined storage conditions.

(f) Lot or code number identifying product lot.

(g) The words “Product of the Philippines”, or the country of origin if imported.

(h) Additional requirements

A pictorial representation of raw material or end-product on the label should not mislead the consumer with respect to the raw material or end-product so illustrated.

(i) Optional information

Any special conditions for the storage of the food may be indicated in the label.

Where practicable, storage instructions shall be in close proximity to the open date marking.

8.4 Nutrition Labeling

Nutrition labeling shall conform to the established regulations of FDA and/or authority.

9 METHODS OF ANALYSIS AND SAMPLING

9.1 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.

9.2 Determination of Water Activity

According to the AOAC Official Methods of Analysis, 16th ed., 1995. Method No. 978.18.

9.3 Enumeration of Yeast and Mold Count

According to the USFDA Bacteriological Analytical Manual (2001)

9.4 Enumeration of Coliform and *E.coli*

According to the USFDA Bacteriological Analytical Manual (2001)

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ANNEX 1

FAO/WHO Alimentarius Sampling Plan for Prepackaged Foods (AQL=6.5) CAC/RM 42-1969

1. Sampling Plan 1 (Inspection Level I, AQL = 6.5)

1.1 Net Weight is equal to or Less Than 1 kg (2.2 lb)

Lot size (N)	Sample size (n)	Acceptance No. (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
84,001 – 144,000	48	6
144,001 – 240,000	84	9
More than 240,000	126	13

1.2 Net Weight is Greater than 1 kg (2.2 lb) but not more than 4.5 kg (10 lb)

Lot size (N)	Sample size (n)	Acceptance No. (c)
2,400 or less	6	1
2,841 – 15,000	13	2
15,001 – 24,000	21	3
24,001 – 42,000	29	4
44,001 – 72,000	48	6
72,001 – 120,000	84	9
More than 120,000	126	13

1.3 Net Weight Greater Than 4.5 kg (10 lb)

Lot size (N)	Sample size (n)	Acceptance No. (c)
600 or less	6	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

2.Sampling Plan 2 (Inspection Level II, AQL = 6.5)

2.1 Net Weight is Equal To or Less Than 1 kg (2.2 lb)

Lot size (N)	Sample size (n)	Acceptance No. (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
84,001 – 144,000	84	9
144,001 – 240,000	126	13
More than 240,000	200	19

2.2 Net Weight is Greater Than 1 kg (2.2 lb) but not more than 4.5 kg (10 lb)

Lot size (N)	Sample size (n)	Acceptance No. (c)
2,400 or less	13	2
2,841 –15,000	21	3
15,001 – 24,000	29	4
24,001 – 42,000	48	6
44,001 – 72,000	84	9
72,001 – 120,000	126	13
More than 120,000	200	19

2.3 Net Weight Greater Than 4.5 kg (10 lb)

Lot size (N)	Sample size (n)	Acceptance No. (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

ANNEX 2

Determination of Water Activity (AOAC 978.18)

A. Principle

Water activity, a_w , is ratio of vapor pressure of H₂O in product to vapor pressure of pure H₂O at same temperature. It is numerically equal to 1/100 of relative humidity (RH) generated by product in closed system. RH can be calculated from direct measurement of partial vapor pressure or dew point or measured indirectly by sensors whose physical or electric characteristics are altered by RH to which they are exposed. Instruments are checked or calibrated on basis of RH generated by standard salt slushes.

B. Instruments and Systems

(Select 1 of following instruments or systems to perform test. Each has different application limitations because of interferences from other volatile components of products being measured. check with instrument manufacturer for more specific limitations.)

- (a) Change in electrical conductivity of immobilized salt solution. – Instrument available from Beckman Industrial, Rosemount Analytical Div., 89 Commerce Rd, Cedar Grove, NJ 07009; Nova Sina AG, Andreastrasse 7-11, CH 8050, Zurich, Switzerland; Rotronic Instrument Corp., 160 E. Main St, Huntington, NY 11743. Immobilized salt sensors are affected by polyols such as glycerol and glycol and by volatile amines
- (b) Change in electrical capacitance of polymer thin films. – Instrument available from General Eastern Instruments, 50 Hunt St, Watertown, MA 02172. Polymer thin film sensors are affected by CH₃COOH.
- (c) Dew point by chilled mirror technique. – Instrument available from EG&G, Environmental Equipment Division, 217 Middlesex Turnpike, Burlington, MA 01803 or General Eastern Instruments. Dew point measurements can be affected by condensables with lower critical temperature than H₂O.
- (d) Longitudinal change in dimensions of water-sorbing fiber. – Instrument available from G Lufft Metallbarometerfabrik, D-7, Postfach 692, Neue Weinsteige 22, Stuttgart, Germany.
- (e) Partial water vapor pressure by manometric system. – Partial H₂O vapor pressure measurements can be made useless by living products that respire, such as grains or nuts; by active fermentation; or by products that expand excessively when subjected to high vacuum.
- (f) Relative weight of moisture sorbed by anhydrous hydrophilic solid, e.g., microcrystalline cellulose.-see J. Agr. Food chem. 22, 326(1974).

C. Apparatus and Reagents

(As needed for instrument or system selected.)

- (a) Dew point instrument. – Equipped to measure temperature to $\pm 0.1^\circ$. See 978.18B(c).
- (b) Forced-draft cabinet. – Constant temperature, set to maintain $25 \pm 1^\circ$; capacity $\geq 0.06 \text{ m}^3$ (2 cu ft); with access port to accommodate instrument sensor leads. Use in conjunction with (c).
- (c) Insulated box with cover. – Large enough to hold test container, (e), and small enough to fit in forced-draft cabinet, (b); with access port to accommodate instrument sensor leads. Protect test container from short-term temperature fluctuations.
- (d) Manometric system. – Sensitive to pressure differential of $\pm 0.01 \text{ mm Hg}$ (1.33 Pa). See 978.18B(e).
- (e) Test containers. – 120 or 240 mL (4 or 8 oz) wide-mouth or Mason glass jars with Al- or Teflon-lined screw caps and gaskets. Check integrity of cap seals and sensor leads by any means available, e.g., ability of system to hold vacuum, using Tesla coil.

- (f) Water bath. – Capable of maintaining temperature constant within 0.1° at $25\pm 1^\circ$; capacity sufficient to hold measuring chamber of selected apparatus.
- (g) Hydrophilic solid. – Microcrystalline cellulose, Type PH-101 (FMC Corp., Pharmaceutical and Bioscience Division, 1735 Market St, Philadelphia, PA 19103, or equivalent).
- (h) Reference salts. – ACS reagent grade, fine crystal. see Table 978.18.

Table 978.18 Water Activity of Reference Salt Slushes at 25°

Salt	a_w	Salt	a_w
MgCl ₂	0.328	KBr	0.809
K ₂ CO ₃	0.432	(NH ₄) ₂ SO ₄	0.810
Mg(NO ₃) ₂	0.529	KCl	0.843
NaBr	0.576	Sr(NO ₃) ₂	0.851
CoCl ₂	0.649	BaCl ₂	0.902
SrCl ₂	0.709	KNO ₃	0.936
NaNO ₃	0.743	K ₂ SO ₄	0.973
NaCl	0.753		

D. Preparation of Reference Salt Slushes

Place selected reference salt in test container to depth of ca 4 cm for more soluble salts (lower a_w), to depth of ca 1.5 cm for less soluble salts (higher a_w), and to intermediate depth for intermediate salts. Add H₂O in ca 2 mL increments, stirring well with spatula after each addition, until salt can absorb no more H₂O as evidenced by free liquid. Keep free liquid to minimum needed to establish saturation of salt with H₂O. Slushes are ready for use upon completion of mixing, and are usable indefinitely (except for some high a_w salts susceptible to bacterial attack), if contained in manner to prevent substantial evaporation losses. Some slushes, e.g., NaBr, may solidify gradually by crystal coalescence, with no effect on a_w .

E. Calibration

Select ≥ 5 salts to cover a_w range of interest or range of sensor being used. Measure humidity generated by each salt slush in terms of instrument readout, as in 978.18F. Plot readout against a_w values given in Table 978.18 for selected salts, using cross-section paper scaled for reading to 0.001 a_w unit. Draw best average smooth line through plotted points. Use this calibration line to translate sensor instrument readout of samples to a_w or to check vapor pressure or dew point instruments for proper functioning.

F. Determination

Place calibration slush or sample in forced-draft cabinet, (b), or H₂O bath, (f), until temperature is stabilized at $25\pm 1^\circ$. Transfer salt slush or sample to test container, (e), seal container with sensing device attached, and place in temperature control device. Use volume of sample or slush $>1/20$ total volume sample container plus any associated void volume of sensing system, but not so much as to interfere with operation of system. Record instrument response at 15, 30, 60, and 120 min after test container is placed in temperature control device, or record response on strip chart. Two consecutive readings, at indicated intervals, which vary by <0.01 a_w unit are evidence of adequately close approach to equilibrium. Continue readings at 60-min intervals, if necessary. Convert last reading to a_w by calculation from physical measurements or by reference to calibration line. Make all measurements within range of calibration points; do not extrapolate calibration line. Make all measurements in same direction of change, and, if required by properties of sensor, expose sensor to controlled RH below ambient before starting each measurement.

ANNEX 3

Enumeration of Yeast and Mold Count

Enumeration of Yeasts and Molds in Food--Dilution Plating Technique (USFDA, 2001)

A. Equipment and materials

1. Basic equipment (and appropriate techniques) for preparation of sample homogenate
2. Equipment for plating samples
3. Incubator, 25°C
4. Arnold steam chest
5. pH meter
6. Water bath, 45 ± 1° C

B. Media and reagents

1. Dichloran rose bengal chloramphenicol (DRBC) agar
2. Dichloran 18% glycerol (DG18) agar
3. Plate count agar (PCA), standard methods; add 100 mg chloramphenicol/liter when this medium is used for yeast and mold enumeration. This medium is not efficient when "spreader" molds are present.
4. Malt agar (MA)
5. Malt extract agar (Yeasts and Molds) (MEAYM)
6. Potato dextrose agar (PDA), dehydrated; commercially available

C. Procedures

Sample preparation

Analyze 25-50 g from each subsample; generally, larger sample sizes increase reproducibility and lower variance compared with small samples. Test individual subsamples or composite according to respective Compliance Program for the food under analysis. Add appropriate amount of 0.1% peptone water to the weighed sample to achieve 10⁻¹ dilution, then homogenize in a stomacher for 2 min. Alternatively, blending for 30-60 sec can be used but is less effective. Make appropriate 1:10 (1+9) dilutions in 0.1% peptone water. Dilutions of 10⁻⁶ should suffice.

Plating and incubation of sample

Spread-plate method. Aseptically pipet 0.1 ml of each dilution on pre-poured, solidified DRBC agar plates and spread inoculum with a sterile, bent glass rod. DG18 is preferred when the water activity of the analyzed sample is less than 0.95. Plate each dilution in triplicate.

Pour-plate method. Use sterile cotton-plugged pipet to place 1.0 ml portions of sample dilution into pre-labeled 15 x 100 mm Petri plates (plastic or glass), and immediately add 20-25 ml tempered DG18 agar. Mix contents by gently swirling plates clockwise, then counterclockwise, taking care to avoid spillage on dish lid. After adding sample dilution, add agar within 1-2 min; otherwise, dilution may begin to adhere to dish bottom (especially if sample is high in starch content and dishes are plastic) and may not mix uniformly. Plate each dilution in triplicate.

From preparation of first sample dilution to pouring or surface-plating of final plate, no more than 20 min (preferably 10 min) should elapse. **Note:** Spread plating of diluted sample is

considered better than the pour plate method. When the pour plate technique is used, fungal colonies on the surface grow faster and often obscure those underneath the surface, resulting in less accurate enumeration. Surface plating gives a more uniform growth and makes colony isolation easier. DRBC agar should be used for spread plates only.

Incubate plates in the dark at 25°C. Do not stack plates higher than 3 and do not invert. **Note:** Let plates remain undisturbed until counting.

Counting of plates

Count plates after 5 days of incubation. If there is no growth at 5 days, re-incubate for another 48 h. Do not count colonies before the end of the incubation period because handling of plates could result in secondary growth from dislodged spores, making final counts invalid. Count plates containing 10-150 colonies. If mainly yeasts are present, plates with 150 colonies are usually countable. However, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may have to be lowered at the discretion of the analyst. Report results in colony forming units (CFU)/g or CFU/ml based on average count of triplicate set. Round off counts to two significant figures. If third digit is 6 or above, round off to digit above (e.g., 456 = 460); if 4 or below, round off to digit below (e.g., 454 = 450). If third digit is 5, round off to digit below if first 2 digits are an even number (e.g., 445 = 440); round off to digit above if first 2 digits are an odd number (e.g., 455 = 460). When plates from all dilutions have no colonies, report mold and yeast counts (MYC) as less than 1 times the lowest dilution used.

Isolate individual colonies on PDA or MA, if further analysis and species identification is necessary.

ANNEX 4

Enumeration of *E.coli* and Coliform Count (Enumeration of *Escherichia coli* and the Coliform Bacteria (USFDA, 2001))

Conventional Method for coliforms, fecal coliforms and *E. coli*

A. Equipment and materials

1. Covered water bath, with circulating system to maintain temperature of $45.5 \pm 0.2^{\circ}\text{C}$. Water level should be above the medium in immersed tubes.
2. Immersion-type thermometer, $1\text{-}55^{\circ}\text{C}$, about 55 cm long, with 0.1°C subdivisions, certified by National Institute of Standards and Technology (NIST), or equivalent
3. Incubator, $35 \pm 1.0^{\circ}\text{C}$
4. Balance with capacity of ≥ 2 kg and sensitivity of 0.1 g
5. Blender and blender jar
6. Sterile graduated pipets, 1.0 and 10.0 mL
7. Sterile utensils for sample handling
8. Dilution bottles made of borosilicate glass, with polyethylene screw caps equipped with Teflon liners. Commercially prepared dilution bottles containing sterile Butterfield's phosphate buffer can also be used.
9. Quebec colony counter, or equivalent, with magnifying lens
10. Longwave UV light [~ 365 nm], not to exceed 6 W.
11. pH meter

B. Media and Reagents

Brilliant green lactose bile (BGLB) broth, 2% (M25)

Lauryl tryptose (LST) broth (M76)

EC broth (M49)

Levine's eosin-methylene blue (L-EMB) agar (M80)

Butterfield's phosphate-buffered water (R11) or equivalent diluent (except for shellfish)

Lauryl tryptose MUG (LST-MUG) broth (M77)

Peptone Diluent, 0.1% (R56)

MPN - Presumptive test for coliforms, fecal coliforms and *E. coli*

Weigh 50 g food into sterile high-speed blender jar. (see Chapter 1 and current FDA compliance programs for instructions on sample size and compositing) Frozen samples can be softened by storing it for ≤ 18 h at $2\text{-}5^{\circ}\text{C}$, but do not thaw. Add 450 mL of Butterfield's phosphate-buffered water and blend for 2 min. If < 50 g of sample are available, weigh portion that is equivalent to half of the sample and add sufficient volume of sterile diluent to make a 1:10 dilution. The total volume in the blender jar should completely cover the blades.

Prepare decimal dilutions with sterile Butterfield's phosphate diluent. Number of dilutions to be prepared depends on anticipated coliform density. Shake all suspensions 25 times in 30 cm arc or vortex mix for 7 s. Do not use pipets to deliver $< 10\%$ of their total volume. Transfer 1 mL portions to 3 LST tubes for each dilution for at least 3 consecutive dilutions. Hold pipet at angle so that its lower edge rests against the tube. Let pipet drain 2-3 s. Not more than 15 min should elapse from time the sample is blended until all dilutions are inoculated in appropriate media.

NOTE: Use 5-tube MPN for analysis of shellfish and shellfish harvest waters.

Incubate LST tubes at 35°C. Examine tubes and record reactions at 24 ± 2 h for gas, i.e., displacement of medium in fermentation vial or effervescence when tubes are gently agitated. Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 2 h. Perform confirmed test on all presumptive positive (gas) tubes.

MPN - Confirmed test for coliforms

From each gassing LST tube, transfer a loopful of suspension to a tube of BGLB broth, avoiding pellicle if present. Incubate BGLB tubes at 35°C and examine for gas production at 48 ± 2 h. Calculate most probable number (MPN) of coliforms based on proportion of **confirmed** gassing LST tubes for 3 consecutive dilutions.

MPN - Confirmed test for fecal coliforms and *E. coli*

From each gassing LST tube from the Presumptive test, transfer a loopful of each suspension to a tube of EC broth (a sterile wooden applicator stick may also be used for these transfers). Incubate EC tubes 24 ± 2 h at 45.5 °C and examine for gas production. If negative, reincubate and examine again at 48 ± 2 h. Use results of this test to calculate fecal coliform MPN. To continue with *E. coli* analysis, proceed to Section F under Enumeration of Escheria coli and the Coliform Bacteria of the USFDA Bacteriological Analytical Manual (2001). The EC broth MPN method may be used for seawater and shellfish since it conforms to recommended procedures (1). (Caution: see Note below).

NOTE: Fecal coliform analyses are done at 45.5± 0.2°C for all foods, except for water testing and in shellfish and shellfish harvest water analysis, which uses an incubation temperature of 44.5± 0.2°C.