

# PHILIPPINE NATIONAL STANDARD

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ICS 67.100.10

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## Ethnic milk-based confectioneries (Pastillas and Yema) – Specification



**BUREAU OF PRODUCT STANDARDS**

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## Foreword

The standards for the Philippine ethnic foods are being developed in response to the need for high standards of the products, guidance for assurance of its quality and safety, harmonization export requirements, therefore competitive in the world market.

The Standard for the Ethnic milk-based confectioneries (Pastillas and Yema) and its Recommended code of practice is one of the food standards developed under the project "Development of Standards for Selected Ethnic Food Products".

The standard was reviewed, finalized and endorsed for adoption by the Food and Drug Administration as the Philippine National Standard and Recommended Code of Practice.

Public consultation workshop was held in the region where the product is being manufactured abundantly. Stakeholders from different agencies and offices contributed their expertise for the finalization of the draft.

**Ethnic milk-based confectioneries (Pastillas and Yema) – Specification**

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**1 Scope**

This standard shall apply to ethnic milk-based confectioneries, specifically pastillas (milk candy) and yema (custard candy), in suitable packaging materials or containers.

**2 References**

The titles of the standards publications referred to in this standard are listed on the inside back cover.

**3 Definition of terms**

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

**3.1 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)

**3.2 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

**3.3 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

**3.4 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)

**3.4.1 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

**3.4.2**

**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

**3.4.3**

**artificial flavoring substances**

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

**3.5**

**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)

**3.6**

**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)

**3.7**

**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 Act of 2009)

**3.8**

**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

**3.9**

**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.

**3.10**

**label**

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

**3.11**

**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

**3.12**

**lot**

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

**3.13**

**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)

**3.14**

**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)

**3.15**

**packaging**

the process of packing that is part of the production cycle applied to a bulk product to obtain the finished product. 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

**3.16**

**rancidity**

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

**3.17**

**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)

## 4 Description of products

### 4.1 Product definition

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

**4.1.1 Pastillas** – This is also known as milk candy, or milk fudge. The product is made from milk or milk products, and sugar. In place of fresh milk and sugar, a combination of powdered milk and condensed milk could also be used. Other ingredients such as fruits and nuts could be added. The product mixture is formed into thin cylinders, sticks, or balls, and may be rolled in sugar.

**4.1.2 Yema** – The product may also be called custard candy. Yema is traditionally made from egg yolks cooked with sugar and milk. It is now commonly made from condensed milk, and eggs. Since condensed milk is already sweetened, the formulation usually does not require additional sugar. Other ingredients such as root crops, fruits, and nuts could also be added. The product mixture could be formed into pyramid-like shapes/forms. It could also be formed into balls and dipped in caramel glaze.

### 4.2 Process description

The product is prepared by mixing the ingredients together and cooking over low heat to form a thick paste. In the case of pastillas, milk products and sugar could be mixed together without cooking. 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 container.

## 5 Essential composition and quality factors

### 5.1 Raw materials

#### 5.1.1 Basic ingredients

**5.1.1.1 Milk and milk products** – 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.

The preparation of pastillas and yema may utilize two forms of milk or milk product, namely:

- (1) Liquid milk – This may be as fresh, evaporated, condensed milk, and other suitable types of liquid milk.
- (2) Powdered milk – This may be as full cream, skimmed powdered milk, and other suitable types of powdered milk.

**5.1.1.2 Sugar and other sweeteners** – May include table sugar, corn syrup, and other similar food items, and must conform to all applicable standards. In some cases, condensed milk also acts as the source of sugar and/or sweetener since it is already sweetened.

**5.1.1.3 Eggs** – Applicable for yema only. Must come from 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.

**5.1.2 Optional ingredients**

**5.1.2.1 Butter, margarine, and other similar food items** – Must comply with FDA A.O. No. 243 s. 1975 (Regulation: B-4 Definition and Standards of Food; B-4.18 Margarine), and other applicable food standards.

**5.1.2.2 Fruit, vegetables, nuts, and root crops** – May include fresh items or preserves and must conform to all applicable food standards.

**5.1.2.3 Potable water** – Water fit for human consumption.

**5.1.2.4 Flavor and flavoring substances** – All flavor/flavoring substances as defined in 2.4 shall be certified as food grade by the FDA.

**5.1.2.5 Other ingredients** – May include starch, cocoa powder, and other ingredients. All other ingredients to be used shall be of food grade quality and conform to all applicable food standards.

**5.2 Quality criteria**

**5.2.1 General requirements**

**5.2.1.1 Water activity**

The water activity ( $a_w$ ) shall not be greater than 0.85 at 25 °C.

**5.2.1.2 Microbiological limits**

The microbiological limits of ethnic milk-based confectioneries (pastillas and yema) shall conform to the standards set for Processed Foods, under the food description Chocolate products (Bureau Circular No. 01-A, s. 2004; Guidelines for the Assessment of Microbiological Quality of Processed Foods,). The microbiological limits of ethnic milk-based confectioneries (pastillas and yema) shall be as follows\*.

**Table 1 – Microbiological limits of ethnic milk-based confectioneries (pastillas and yema)\***

Test microorganism	n	c	M	M
Yeast and Molds, cfu/g	5	2	10 <sup>2</sup>	10 <sup>4</sup>
Salmonella/ 25g	10	0	0	
Coliforms, MPN/g	5	2	<1.8	10 <sup>2</sup>
SPC/APC, cfu/g	5	2	10 <sup>4</sup>	10 <sup>6</sup>
* Based on Bureau Circular No. 1-A, 2004; Guidelines for the Assessment of Microbiological Quality of Processed Foods; under the Food Description, Chocolate Products				

Legend:

- n – the number of sample units selected from a lot of food to be examined.
- m – the acceptable level of microorganism determined by a specified method; the values are generally based on levels that are achievable under GMP.
- M – the level which when exceeded in one or more samples would cause the lot to be rejected as this indicates potential health hazard or imminent spoilage.
- c – the maximum allowable number of defective or marginally acceptable units.

## **5.2.2 Types of defects**

### **5.2.2.1 Odor/flavor/color**

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

### **5.2.2.2 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.

### **5.2.2.3 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.

## **5.2.3 Classification of “defectives”**

A container that has any of the type of defects set in 5.2.2 shall be considered as “defective”.

## **5.2.4 Lot acceptance**

A lot shall be considered as meeting the applicable quality requirements when the number of “defectives”, as defined in 5.2.3, does not exceed the acceptance number of the appropriate sampling plan.

## **6 Food additives**

**6.1** Food additives when used shall be in accordance with the regulations established by the Food and Drug 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 milk-based confectioneries (pastillas and yema).

**6.2** 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 Milk-based Confectioneries (Pastillas and Yema)\* (B.C. No.016 s. 2006. Updated List of Food Additives)**

Function	Food additive	Maximum level of usage
Anti-caking agent	Polydimethylsiloxane	10 mg/kg**
		50 mg/kg***
Antioxidant	BHA	100 mg/kg (Fat or oil basis)**
		200 mg/kg (Fat or oil basis)**
		2 mg/kg***
	BHT	200 mg/kg (Fat or oil basis)**
		90 mg/kg (On dry ingredient, dry weight, dry mix or concentrate basis)***
	Gallate, propyl	200 mg/kg (Fat or oil basis)**
		90 mg/kg (On dry ingredient, dry weight, dry mix or concentrate basis)***
Tertiary butylhydroquinone	200 mg/kg (Fat or oil basis)**	
Tocopherols	500 mg/kg (Fat or oil basis)**	
	150 mg/kg***	
Color	Allura red AC	348 mg/kg**
		300 mg/kg***
	Amaranth	100 mg/kg**
		300 mg/kg***
	Annatto extracts	25 mg/kg (As total bixin or norbixin)**
		10 mg/kg***
	Brilliant blue FCF	300 mg/kg**
		150 mg/kg***
	Caramel colour, Class III	GMP**
		GMP***
	Caramel colour, Class IV	GMP**
GMP***		
Fast green FCF	100 mg/kg**	
	100 mg/kg***	
Sunset yellow FCF	400 mg/kg**	
	300 mg/kg***	
Preservative	Benzoates	1500 mg/kg (As benzoic acid)**
		1000 mg/kg (As benzoic acid)***
	Hydroxybenzoates, p-	2000 mg/kg (As p-hydroxybenzoic acid)**
Stabilizer	Polysorbates	10000 mg/kg**
		5000 mg/kg***
	Sorbates	2000 mg/kg (As sorbic acid)**
		1000 mg/kg (As sorbic acid)***
	Sorbitan esters of fatty acids	20000 mg/kg*
5000 mg/kg***		
Sweetener	Alitame	300 mg/kg*
	Acesulfame Potassium	3500 mg/kg**
		350 mg/kg***
	Aspartame	10000 mg/kg**
		1000 mg/kg***
	Saccharin	3000 mg/kg**
100 mg/kg***		
Sucralose	1500 mg/kg**	
	250 mg/kg***	
<p>* Based on the Food Category System: 5.0 Confectionery;</p> <p>** 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;</p> <p>*** Based on the Food Category System:10.4 Egg-based desserts (e.g. custards)</p>		

## **7 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.

## **8 Hygiene**

**8.1** 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 BFAD A.O. No. 153 s. 2004 - Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food

**8.2** 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.

## **9 Packaging and labeling**

### **9.1 Packaging**

The packaging used for ethnic milk-based confectioneries (pastillas and yema) 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. Primary packaging used for pastillas and yema may include uncolored cellophane paper, and wax paper.

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.

### **9.2 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):

**9.2.1** The name of the product shall be “Pastillas” (Milk Candy) or “Yema” (Custard Candy). Additional descriptors pertaining to the ingredients used or the product form may also be included (e.g. “Pastillas de Leche”, “Ube Pastillas”, “Yema balls”). Other local or regional names referring to products similar to those defined in 3.1 may also be included, provided that these names are acceptable in the area of distribution.

**9.2.2** The complete list of ingredients and food additives used in the preparation of the product in descending order of proportion.

**9.2.3** 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.

**9.2.4** The name and address of the manufacturer, packer and/or distributor of the food.

**9.2.5** Open date marking

The words “Consume Before” or “Expiry Date” indicating end of period at which the product shall retain its optimum quality attributes at defined storage conditions.

**9.2.6** Lot or code number identifying product lot.

**9.2.7** The words “Product of the Philippines”, or the country of origin if imported.

**9.2.8** 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.

**9.2.9** Optional information

Storage instructions may also be indicated on the label.

### **9.3 Nutrition labeling**

Nutrition labeling shall conform to the established regulations of FDA.

## **10 Methods of analysis and sampling**

### **10.1 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.

### **10.2 Determination of water activity**

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

**10.3 Enumeration of yeast and mold count**

According to the USFDA Bacteriological Analytical Manual (2001).

**10.4 Isolation of *salmonella***

According to the USFDA Bacteriological Analytical Manual (2001).

**10.5 Enumeration of coliform and E.coli**

According to the USFDA Bacteriological Analytical Manual (2001).

**10.6 Enumeration of Standard Plate Count**

According to the USFDA Bacteriological Analytical Manual (2001)

## 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)

<b>Lot size (N)</b>	<b>Sample size (n)</b>	<b>Acceptance number (c)</b>
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)

<b>Lot size (N)</b>	<b>Sample size (n)</b>	<b>Acceptance number (c)</b>
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 is greater than 4.5 kg (10 lb)

<b>Lot size (N)</b>	<b>Sample size (n)</b>	<b>Acceptance number (c)</b>
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)

<b>Lot size (N)</b>	<b>Sample size (n)</b>	<b>Acceptance number (c)</b>
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)

<b>Lot size (N)</b>	<b>Sample size (n)</b>	<b>Acceptance Number (c)</b>
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 is greater than 4.5 kg (10 lb)

<b>Lot size (N)</b>	<b>Sample size (n)</b>	<b>Acceptance number (c)</b>
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 H<sub>2</sub>O in product to vapor pressure of pure H<sub>2</sub>O 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, Andrastrasse 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 CH<sub>3</sub>COOH.
- (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 H<sub>2</sub>O.
- (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 H<sub>2</sub>O 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  $0.1^\circ$  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  $25^\circ$**

Salt	$a_w$	Salt	$a_w$
MgCl <sub>2</sub>	0.328	KBr	0.809
K <sub>2</sub> CO <sub>3</sub>	0.432	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.810
Mg(NO <sub>3</sub> ) <sub>2</sub>	0.529	KCl	0.843
NaBr	0.576	Sr(NO <sub>3</sub> ) <sub>2</sub>	0.851
CoCl <sub>2</sub>	0.649	BaCl <sub>2</sub>	0.902
SrCl <sub>2</sub>	0.709	KNO <sub>3</sub>	0.936
NaNO <sub>3</sub>	0.743	K <sub>2</sub> SO <sub>4</sub>	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 H<sub>2</sub>O in ca 2 mL increments, stirring well with spatula after each addition, until salt can absorb no more H<sub>2</sub>O as evidenced by free liquid. Keep free liquid to minimum needed to establish saturation of salt with H<sub>2</sub>O. 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  $\geq 5$  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 H<sub>2</sub>O bath, (f), until temperature is stabilized at 25±1°. 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<sub>w</sub> unit are evidence of adequately close approach to equilibrium. Continue readings at 60-min intervals, if necessary. Convert last reading to a<sub>w</sub> 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 prelabeled 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**  
**Isolation of *Salmonella* (USFDA, 2001)**

**A. Sample preparation**

**For candy and candy coating (including chocolate)** – Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile, [reconstituted nonfat dry milk](#) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand  $60 \pm 5$  min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to  $6.8 \pm 0.2$ . Add 0.45 ml 1 % aqueous brilliant green dye solution and mix well. Loosen jar caps 1/4 turn and incubate  $24 \pm 2$  h at  $35^\circ\text{C}$ . Continue as in B., below.

**For egg-containing products (noodles, egg rolls, macaroni, spaghetti), cheese, dough, prepared salads (ham, egg, chicken, tuna, turkey), fresh, frozen, or dried fruits and vegetables, nut meats, crustaceans (shrimp, crab, crayfish, langostinos, lobster), and fish** – Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw below  $45^\circ\text{C}$  for  $<15$  min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at  $2^\circ\text{C} - 5^\circ\text{C}$ .

Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile [lactose broth](#) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand  $60 \pm 5$  min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to  $6.8 \pm 0.2$ . Mix well and loosen jar cap about 1/4 turn. Incubate  $24 \pm 2$  h at  $35^\circ\text{C}$ . Continue as in B., below.

**B. Isolation of *Salmonella***

1. Tighten lid and gently shake incubated sample.

**Guar gum and foods suspected to be contaminated with *S. Typhi*** – Transfer 1 ml mixture to 10 ml [selenite cystine \(SC\) broth](#) and another 1 ml mixture to 10 ml [TT broth](#). Vortex.

**All other foods** – Transfer 0.1 ml mixture to 10 ml [Rappaport-Vassiliadis \(RV\) medium](#) and another 1 ml mixture to 10 ml [tetrathionate \(TT\) broth](#). Vortex.

2. Incubate selective enrichment media as follows:

**Foods with a high microbial load** – Incubate RV medium  $24 \pm 2$  h at  $42 \pm 0.2^\circ\text{C}$  (circulating, thermostatically-controlled, water bath). Incubate TT broth  $24 \pm 2$  h at  $43 \pm 0.2^\circ\text{C}$  (circulating, thermostatically-controlled, water bath).

**Foods with a low microbial load (except guar gum and foods suspected to be contaminated with *S. Typhi*)** – Incubate RV medium  $24 \pm 2$  h at  $42 \pm 0.2^\circ\text{C}$  (circulating, thermostatically controlled, water bath). Incubate TT broth  $24 \pm 2$  h at  $35 \pm 2.0^\circ\text{C}$ .

**Guar gum and foods suspected to be contaminated with *S. Typhi*** – Incubate SC and TT broths  $24 \pm 2$  h at  $35^\circ\text{C}$ .

3. Mix (vortex, if tube) and streak 3 mm loopful (10  $\mu\text{l}$ ) incubated TT broth on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. **Prepare BS plates the day before streaking and store in dark at room temperature until streaked.**
4. Repeat with 3 mm loopful (10  $\mu\text{l}$ ) of RV medium (for samples of high and low microbial load foods) and of SC broth (for guar gum).
5. Refer to 994.04 in *Official Methods of Analysis* (1) for option of refrigerating incubated sample preenrichments and incubated sample selective enrichments (SC and TT broths only) of low moisture foods. This option allows sample analyses to be initiated as late as Thursday while still avoiding weekend work.
6. Incubate plates  $24 \pm 2$  h at  $35^\circ\text{C}$ .
7. Examine plates for presence of colonies that may be *Salmonella*.
8. Lightly touch the very center of the colony to be picked with sterile inoculating needle and inoculate TSI slant by streaking slant and stabbing butt. Without flaming, inoculate LIA slant by stabbing butt twice and then streaking slant. Since lysine decarboxylation reaction is strictly anaerobic, the LIA slants must have deep butt (4 cm). Store picked selective agar plates at  $5^\circ\text{C} - 8^\circ\text{C}$ .
9. Incubate TSI and LIA slants at  $35^\circ\text{C}$  for  $24 \pm 2$  h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive  $\text{H}_2\text{S}$  production. *Salmonella* in culture typically produces alkaline (red) slant and acid (yellow) butt, with or without production of  $\text{H}_2\text{S}$  (blackening of agar) in TSI. In LIA, *Salmonella* typically produces alkaline (purple) reaction in butt of tube. Consider only distinct yellow in butt of tube as acidic (negative) reaction. Do not eliminate cultures that produce discoloration in butt of tube solely on this basis. Most *Salmonella* cultures produce  $\text{H}_2\text{S}$  in LIA. Some non- *Salmonella* cultures produce a brick-red reaction in LIA slants.
10. All cultures that give an alkaline butt in LIA, regardless of TSI reaction, should be retained as potential *Salmonella* isolates and submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an alkaline slant and acid butt in TSI should also be considered potential *Salmonella* isolates and should be submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an acid slant and acid butt in TSI may be discarded as not being *Salmonella*. Test retained, presumed-positive TSI cultures as directed in D-11, below, to determine if they are *Salmonella* species, including *S. arizonae*. If TSI cultures fail to give typical reactions for *Salmonella* (alkaline slant and acid

butt) pick additional suspicious colonies from selective medium plate not giving presumed-positive culture and inoculate TSI and LIA slants as described, above.

11. Apply biochemical and serological identification tests to:
  - a. Three presumptive TSI cultures recovered from set of plates streaked from RV medium (or SC broth for guar gum), if present, and 3 presumptive TSI agar cultures recovered from plates streaked from TT broth, if present.
  - b. If 3 presumptive-positive TSI cultures are not isolated from one set of agar plates, test other presumptive-positive TSI agar cultures, if isolated, by biochemical and serological tests. Examine a minimum of 6 TSI cultures for each 25 g analytical unit or each 375 g composite.

## Annex 5

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  $45.5 \pm 0.2$  °C. Water level should be above the medium in immersed tubes.
2. Immersion-type thermometer, 1-55 °C, about 55 cm long, with 0.1 °C subdivisions, certified by National Institute of Standards and Technology (NIST), or equivalent
3. Incubator,  $35 \pm 1.0$  °C
4. Balance with capacity of  $\geq 2$  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 [ $\sim 365$  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  $\leq 18$  h at 2-5 °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  $24 \pm 2$  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  $48 \pm 2$  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  $48 \pm 2$  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  $24 \pm 2$  h at 45.5 °C and examine for gas production. If negative, re-incubate and examine again at  $48 \pm 2$  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  $45.5 \pm 0.2$  °C for all foods, except for water testing and in shellfish and shellfish harvest water analysis, which uses an incubation temperature of  $44.5 \pm 0.2$  °C.

## Annex 6

### Enumeration of standard plate count Conventional plate count method (USFDA, 2001)

#### A. Equipment and materials

1. Work area, level table with ample surface in room that is clean, well-lighted (100 foot-candles at working surface) and well-ventilated, and reasonably free of dust and drafts. The microbial density of air in working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies/plate during 15 min exposure.
2. Storage space, free of dust and insects and adequate for protection of equipment and supplies
3. Petri dishes, glass or plastic (at least 15 x 90 mm)
4. Pipets with pipet aids (no mouth pipetting) or pipettors, 1, 5, and 10 ml, graduated in 0.1 ml units
5. Dilution bottles, 160 ml (6 oz), borosilicate-resistant glass, with rubber stoppers or plastic screw caps
6. Pipet and petri dish containers, adequate for protection
7. Circulating water bath, for tempering agar, thermostatically controlled to  $45 \pm 1^\circ\text{C}$
8. Incubator,  $35 \pm 1^\circ\text{C}$ ; milk,  $32 \pm 1^\circ\text{C}$
9. Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate
10. Tally register
11. Dilution blanks,  $90 \pm 1$  ml Butterfield's phosphate-buffered dilution water (R11); milk,  $99 \pm 2$  ml
12. Plate count agar (standard methods) (M124)
13. Refrigerator, to cool and maintain samples at  $0-5^\circ\text{C}$ ; milk,  $0-4.4^\circ\text{C}$
14. Freezer, to maintain frozen samples from  $-15$  to  $-20^\circ\text{C}$
15. Thermometers (mercury) appropriate range; accuracy checked with a thermometer certified by the National Institute of Standards and Technology (NIST)

#### B. Procedure for analysis of frozen, chilled, precooked, or prepared foods

Using separate sterile pipets, prepare decimal dilutions of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and others as appropriate, of food homogenate (see Chapter 1 for sample preparation) by transferring 10 ml of previous dilution to 90 ml of diluent. Avoid sampling foam. Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish. Add 12-15 ml plate count agar (cooled to  $45 \pm 1^\circ\text{C}$ ) to each plate within 15 min of original dilution. Pour agar and dilution water control plates for each series of samples. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify. Invert solidified petri dishes, and incubate promptly for  $48 \pm 2$  h at  $35^\circ\text{C}$ . Do not stack plates when pouring agar or when agar is solidifying.

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PNS/FDA 34:2011

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**FORMULATING BODY**  
**Development of Standards for Ethnic Milk-Based Confectioneries**  
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