# PHILIPPINE NATIONAL STANDARD

PNS/BFAD 09:2007 ICS 67.160.20

Mango beverage products – Specification



BUREAU OF PRODUCT STANDARDS

#### PHILIPPINE NATIONAL STANDARD

#### PNS/BFAD 09:2007

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#### Foreword

This project is a continuation of the development of ethnic food products composed of the Technical Working Group (TWG) of different agencies and industry groups namely the Industrial Technology Development Institute (ITDI) of the Science and Technology (DOST), Bureau of Food & Drugs (BFAD) of the Department of Health (DOH), Bureau of Agriculture and Fisheries Product Standards (BAFPS), Bureau of Product Standards (BPS), Bureau of Export and Trade Promotions (BETP) and Food Products Division of the Department of Trade and Industry (DTI), Philippine Chamber of Food Manufacturers Incorporated (PCFMI) and Integrated Food Manufacturers Association of the Philippines (INFOMAPP).

The Philippine Council for Industry and Energy Research (PCIERD) of the DOST is the financing agency while the Philippine Food Processors and Exporters Organization, Inc. (PHILIFOODEX) signifies as the collaborating agency and the Department of Food Science and Nutrition (FSN) of the College of Home Economics, University of the Philippines as the implementing agency.

The TWG main task is to draft standards and codes of practice for identified ethnic food products which will be later adopted as national standards after a series of reviews and public consultation in coordination with the Bureau of Food and Drugs.

To gather more inputs from the big players of these mango beverage products the public consultation was held in Cebu City of the Center for Health Development – Central Visayas. From the public consultation, concerns of food processors were raised and clarified as to the standards of the product.

In the development of this standard, Codex General Standard for Fruit Juices and Nectars, Codex Stan 247-2005 was considered.

#### 1 Scope

This standard shall apply to mango beverages including ready-to-drink (RTD), concentrated juices in powder and liquid forms, sweetened and unsweetened juices and purees made from sound and mature mango (*Mangifera indica* L.) preserved exclusively by physical means. Preservation by physical means does not include ionizing radiation.

#### 2 Definition of terms

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

#### 2.1

#### acid food

it is any food that has a natural pH of 4.6 or below

# 2.2

#### acidified low-acid food

it is any food that has been treated so as to attain an equilibrium pH of 4.6 or lower after processing

# 2.3

# brix

it is the concentration of sugar in syrup corresponding approximately to concentration of solutes expressed in percentage as measured with a refractometer or hydrometer and expressed in °Brix units

# 2.4

#### container

it is 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

# 2.5

# current Good Manufacturing Practices (cGMP)

it is 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

# 2.6

#### hermetically sealed container

it is container which is airtight sealed to protect the contents against the entry of microorganisms during and after heat processing

# 2.7

# food

it is any substance, whether processed, semi-processed or raw, which is intended for human consumption, and includes drink, chewing gum and any substance which has been used in the manufacture, preparation or treatment of "food" but does not include cosmetics or tobacco or substances used only as drugs

# 2.8

## food additives

it is any substance the intended use of which results or may reasonably be expected to result, 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 of food; and including any source of radiation intended for any such use), if such substance is 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

# 2.9

#### food standard

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

# 2.10

#### ingredient

it is 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

# 2.11

# label

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

# 2.12

# labelling

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

# 2.13

# lot

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

# 2.14

#### mango syrup

it is liquid resulting from the dehydration process of mango using sugar as the osmotic agent. It may contain other food additives

# 2.15

# packaging

it is 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

# 2.16

# pН

it is the intensity or degree of acidity of a food material

# 2.17

# processing aids

are additives that are used in the processing of food to achieve a specified technological purpose and which may or may not result in the presence of residues or derivatives in the final product

# 2.18

# potable water

it is water fit for human consumption and potability determined by health authorities cited in Philippine National Standards for drinking water (PNS 991:1993 Agricultural and Other Food Products – Bottled Drinking Water Specifications)

# 2.19

# refractometer

it is the instrument used to measure the percent soluble solids of sugars referred to as degree Brix (°Bx); concentration of sugars expressed in terms of number of grains of sucrose per 100 g of liquid

# 2.20

# sweetening agent

it includes one or more of the sugars, honey, high intensity sweeteners and artificial sweeteners

# **3** Description of products

# 3.1 **Product definition**

**3.1.1** Mango beverage products are prepared from the flesh or any edible part of sound and mature mango (*Mangifera indica* Linn.) including ready-to-drink (RTD) and concentrated juices in liquid and powdered forms; sweetened and unsweetened juices and purees with or without the addition of food additives packed in any suitable sealed containers.

**3.1.2 Puree** – Mango puree for use in the manufacture of mango nectar, juice, juice drink, flavored drink and powdered drink, is the unfermented product obtained by suitable processes e.g. by sieving, grinding, milling the edible part of the whole or peeled fruit. The inherent aromatic substances and volatile flavor components of the puree may be restored by any suitable physical means and all of which must be recovered from the same kind of

mango. Pulp and mango bits may also be added. The puree shall have a pH of 3.0 - 4.40, titrable acidity (as % citric acid) of 0.20 - 0.60 and total soluble solids of  $13.0^{\circ}$  Brix -  $18.0^{\circ}$ Brix.

Sweetened mango pure shall have a titrable acidity (as % citric acid) of not less than 0.50 and total soluble solids of not less than  $36.0^{\circ}$ Bx.

**3.1.3** Nectar – The unfermented product obtained by adding water with or without the addition of sweetening agent or agents to greater than or equal to 25 % mango puree. Aromatic substances, volatile flavor components, pulp and bits all of which must be recovered from the same kind of mango and obtained by suitable physical means may be added.

**3.1.4 Juice** – The unfermented liquid obtained from the edible part of sound, appropriately mature and fresh fruit. The juice may be cloudy or clear and may have restored aromatic substances and volatile flavor components, all of which must be obtained by suitable physical means, and all of which must be recovered from the same kind of mango. Pulp and mango bits may also be added.

**3.1.5 Juice drink** – A ready-to-drink beverage prepared by mixing water and sweetening agent or agents to not less than 5 % mango puree.

**3.1.6** Flavored juice drink – A ready-to-drink beverage prepared by mixing water and sweetening agent or agents to not less than 1 % mango puree. Flavors that are nature identical or artificial and other permitted food additives may be added.

**3.1.7 Powdered juice/drink** – A mixture of mango powder and dry or almost dry ingredients (may contain flavors, sweetening agent or agents, citric acid and/or malic acid and other food additives) that are readily soluble in water. Ex. Powdered Mango Juice, Powdered Mango Juice Drink.

#### **3.2 Process definition**

The products shall be packed in any suitable sealed containers and shall have received a heat treatment sufficient to ensure quality and shelf life stability at ambient conditions.

#### **3.3 Product forms**

Product form must meet the required fill-in weight and may include liquid, semi-liquid, puree and powdered forms of beverage.

#### 4 Essential composition and quality factors

- 4.1 Raw materials
- 4.1.1 Basic ingredients

## 4.1.1.1 Mango

Fruit to be used shall be fresh, sound, clean and mature from any cultivated variety or type suitable conforming to the characteristics of the fruits of *Mangifera indica* L.

#### 4.1.1.2 Potable water

Water fit for human consumption.

#### 4.1.1.3 Sweetening agent

One or more of the sugars, honey, high intensity sweeteners or artificial sweeteners.

#### 4.1.1.4 Other food ingredients

Other food grade ingredients may be added.

#### 4.2 Quality criteria

#### 4.2.1 General requirements

The mango beverage product shall have the characteristic color, aroma and flavor of the variety of mango from which it is made and should be free from objectionable sensory characteristics.

## 4.2.1.1 Minimum content of fruit ingredient

The minimum content of mango pure ingredient shall be 100% m/m for pure, greater than or equal to 25% m/m for nectar and not less than 5% for juice drink and not less than 1% for flavored juice drink.

#### 4.2.1.2 Soluble solids

The soluble solids content of the product shall be not more than 20% m/m, as determined by refractometer at 20 °C, uncorrected for acidity and read as °Brix on the International Sucrose Scales.

# 4.2.1.3 Ethanol content

The ethanol content shall not exceed 3g/kg.

# 4.2.1.4 Sensory properties

The product shall have the characteristic color, aroma and flavor of mango, taking into consideration the addition of honey as sweetening agent.

# 4.2.1.5 Microbiological limits

- 1. Standard plate count: 1,000 cfu/ml, maximum
- 2. Yeast and mold count: 50 cfu/ml, maximum

- 3. Coliform count: 10 cfu/ml, maximum
- 4. *Escherichia coli*: Negative

# 4.2.2 Types of defects

## 4.2.2.1 Foreign matter

The presence in the sample unit of any matter, which has not been derived from mango, 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.

# 4.2.2.2 Odor/flavor/color

A sample unit affected by objectionable odors or flavors indicative of decomposition and unacceptable discoloration due to product deterioration.

#### 4.2.3 Classification of "defectives"

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

#### 4.2.4 Lot acceptance

A lot will be considered as meeting the applicable quality requirements when the number of "defectives", as defined in 4.2.3, does not exceed the acceptance number of the appropriate sampling plan.

#### 5 Food additives

Food additives when used shall be in accordance with the regulations established by the Bureau of Food and Drugs (BFAD) (Bureau Circular No. 016 s.2006. Updated List of Food Additives), the Codex Alimentarius Commission and/or authority for these products.

The following food additives listed in, but not limited to, Table 1, may be used for the manufacture of mango beverage products.

# 6 Contaminants

# 6.1 **Pesticide residues**

Amount of residue shall comply with those maximum residue limits for pesticides established by the Codex Alimentarius Commission and/or authority for these products.

# 6.2 Heavy metal contaminants

The mango beverage products covered by the provisions of this standard shall comply with those maximum residue levels for heavy metal contaminants established by the Codex Alimentarius Commission and/or authority for these products.

Function	Food additive	Function	Food additive			
A. Acidity	1. Citric acid	F. Processing aids	a. Antifoaming agent -			
regulator	2. Malic acid	_	Polydimethylsiloxane			
	3. Calcium carbonate		b. Clarifying agents/Filtration			
B. Anticaking	1. Calcium aluminum		aids/Flocculating agents -			
agent	silicate (Synthetic)		Adsorbent clays, Adsorbent			
-	2. Microcrystalline cellulose		resins, Activated carbon(only			
	3. Aluminum silicate		from plants),Bentonite,			
	4. Carnauba wax		Cellulose, Chitosan, Colloidal			
C. Antioxidant	1. Ascorbic acid		silica, Diatomaceous earth,			
	2. Calcium ascorbate		Gelatin (from skin collagen),			
	3. Erythorbic acid		Ion exchange resins (cation			
	4. Potassium ascorbate		and anion), Kaolin, Perlite,			
	5. Sodium ascorbate		Polyvinylpolypyrrolidone,			
	6. Sodium erythorbate		Rice hulls, Silicasol, Tannin			
D. Colour	1. Carotenoids		c. Enzyme preparations –			
	2. Chlorophylls, Copper		Pectinases (for breakdown of			
	complexes		pectin), Proteinases (for			
	3. Curcumin		breakdown of proteins),			
	4. Riboflavin		Amylases (for breakdown of			
	5. Sunset yellow		starch), Cellulases (limited use			
	6. Tartrazine		to facilitate disruption of cell			
			walls)			
			d. Packing gas – nitrogen, carbon			
ED (	1.D.					
E. Preservative	1. Benzoates	G. Stabilizer/	1. Calcium chioride			
	2. Hydroxybenzoates	Inickener	2. Carob bean gum			
	5. Solution		5. Callan aven			
	4. Sulphiles		4. Genan gum			
	6. Phosphatos		6 Cum grabia			
	0. Fliosphates		7 Karaya gum			
			7. Karaya guin 8. Lactic and fatty acid esters of			
			Glycerol			
			9 Pectins			
			10 Potassium alginate			
			11 Sodium alginate			
			12 Tara gum			
			13 Tragacanth gum			
			14 Xanthan gum			
			15. Agar			
			16. Konjac flour			
			17. Sodium			
			Carboxymethylcellulose			
		H. Sweetener	1. Acesulfame potassium			
			2. Aspartame			
			3. Saccharin			
			4. Sucralose			
* Based on the Food Category System: 14.1.2.1 canned or bottled (pasteurized) fruit juice, 14.1.2.3						
Concentrates (liquid or solid) for fruit juice, 14.1.3.1 Canned or bottled (pasteurized) fruit nectar and						
14.1.3.3 Concentrates (liquid or solid) for fruit nectar.						

Table 1 – Food additives for fruit juices\*. (BFAD B.C. No. 016 s.2006. Updated List of Food Additives)

# 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) and/or the BFAD A.O. No. 153 s. 2004 - Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food and processed according to the Recommended Code of Practice for the Processing and Handling of Mango Beverage Products (PNS/BFAD 102007).

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

**7.2.1** Shall be free from filth that may pose a hazard to health,

7.2.2 Shall be free from parasites which may represent a hazard to health,

**7.2.3** Shall not contain any substance originating from microorganisms in amounts which may represent a hazard to health,

**7.2.4** Shall be free from microorganisms capable of development under normal conditions of storage, and

**7.2.5** Shall be free from container integrity defects which may compromise the hermetic seal.

# 8 Weight and measures

# 8.1 Fill of container

# 8.1.1 Minimum fill

The mango beverage product shall occupy not less than 90 % of the water capacity of the container. The water capacity of the container is the volume of distilled water at 20 °C, which the sealed container will hold when completely filled. A container that fails to meet the requirement for minimum fill (90 % container capacity) shall be considered "slack filled".

# 8.1.2 Lot acceptance

A lot will be considered as meeting the requirement of 8.1.1 when the number of "slack filled" containers does not exceed the acceptance number of the appropriate sampling plan.

# 9 Labelling

**9.1** Each container shall be labeled and marked with the following information in accordance with current BFAD's Labeling Regulation:

**9.1.1** The name of the product shall be "[Mango + Type of beverage product]" (ex. Mango puree, Mango puree powder).

**9.1.2** Products using artificial sweetener/s shall have statement/s referring to its low and/or reduced caloric value and the possibility of hypersensitivity to some of its components.

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

**9.1.4** 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.

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

9.1.6 Open date marking

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

9.1.7 Lot or code number identifying product lot.

9.1.8. The words "Product of the Philippines", or the country of origin if imported.

9.1.9 Additional requirements

A pictorial representation of fruit(s) on the label should not mislead the consumer with respect to the fruit so illustrated.

#### **9.1.10** Directions for use

Directions for use should be indicated in the label.

#### **9.1.11** Storage instructions

Where mango beverage product requires to be kept under conditions of refrigeration, there shall be information for storage and, if necessary, thawing of the product. Where practicable, storage instructions should be in close proximity to the open date marking.

#### 9.2 Nutrition labelling

Nutrition labelling shall conform to current BFAD regulations.

#### 10 Method of analysis and sampling

#### **10.1** Determination of total soluble solids

According to the AOAC Official Methods of Analysis, Method No. 932.14C, 16<sup>th</sup> ed., 1995 (Annex 1).

#### **10.2** Determination of alcohol in fruit products

According to the AOAC Official Methods of Analysis, Method No. 920.150, 16<sup>th</sup> ed., 1995 (Annex 2).

#### 10.3 Microbiological examination of mango beverage product

According to the AOAC Official Methods of Analysis, Method No. 972.44, 16<sup>th</sup> ed., 1995 (Annex 3).

## 10.4 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.

#### **10.5** Determination of lead using atomic absorption spectrophotometer

According to the AOAC Official Methods of Analysis, Method No. 972.25, 16<sup>th</sup> ed., 1995.

#### 10.6 Determination of tin using atomic absorption spectrophotometer

According to the AOAC Official Methods of Analysis, Method No. 985.16, 16<sup>th</sup> ed., 1995.

#### Annex 1

#### Determination of total soluble solids

#### 1 Apparatus

- a) Balance. With capacity of  $\leq 2$  kg and sensitivity of 0.1 g
- b) High speed blender
- c) Hand refractometer. With scale reading of 0 ° Brix 35 ° Brix

#### 2 Standardization of refractometer

Adjust instrument to read n of 1.3330 of 0 % sucrose with H<sub>2</sub>O at 20 °.

#### **3 Preparation of sample**

Mix representative aliquots of liquid and solid materials at same liquid-to-liquid ratio as original sample, and blend to workable paste.

Accurately weigh ca 10 g prepared paste, dissolve in equal amount of  $H_2O$  at 20 °C. Mix thoroughly.

#### 4 Determination

Place sufficient amount of sample on the prism of the instrument, and determine by direct reading in terms of °Brix.

Calculation is simplified by multiplying Brix of solution by 2.

## Annex 2

#### Determination of alcohol in fruit products (By Volume from specific gravity)

#### A Distillation of sample

Measure 100 mL original material into 300 mL - 500 mL distillation flask, noting temperature, and add 50 mL water. Attach flask to vertical condenser by means of bent tube, distill almost 100 mL, and dilute to 100 mL at same temperature before proceeding with distillation.

#### **B** Calibration

Fill thoroughly cleaned pycnometer with recently distilled water, stopper, and immerse in constant temperature water bath with bath level above graduation mark on pycnometer. After 30 min, remove stopper and with capillary tube adjust until bottom of meniscus is tangent to graduation mark. With small roll of filter paper, dry inside neck of pycnometer, stopper, and immerse in water at room temperature for 15 min. Remove pycnometer, dry, let stand 15 min, and weigh. Empty pycnometer, rinse with acetone, and dry thoroughly in air with suction. Let empty flask come to room temperature, stopper, and weigh. Weight of water = weight of filled pycnometer – weight of empty pycnometer

#### **C** Determination of Specific Gravity at Room Temperature

1. Determine weight of sample as in B.

Weight of sample = weight of filled pycnometer – weight of empty pycnometer

2. Calculate specific gravity as follows:

Specific gravity = SW,

where

S is the weight of sample, and

W is the weight of water.

#### **D** Determination of alcohol

Obtain corresponding % alcohol by volume from Appendix C: Reference Volumes 913.02. AOAC Manual.  $16^{th}$  ed.

# Annex 3

#### Microbiological examinations of mango beverage products

## 1 Media and reagents

- (a) Tryptone broth (Aerobic medium) Dissolve 10.0 g tryptone or Trypticase, 5.0 g glucose, 1.25 g K<sub>2</sub>HPO<sub>4</sub>, 1.0 g yeast extract, and 2.0 mL 2 % alcoholic solution of bromcresol purple in 1 L H<sub>2</sub>O with gentle heat, if necessary. Dispense 10 mL portions into 20 mm x 150 mm screw-cap test tubes and autoclave 20 min at 121 °C. Do not exhaust before using.
- (b) Modified PE-2 medium (Anaerobic medium) Dissolve 20.0 g peptone, 3.0 g yeast extract and 2.0 mL 2 % alcoholic solution of bromcresol purple in 1 L H<sub>2</sub>O with gentle heat, if necessary. Dispense 19 mL portions into 20 mm x 150 mm screw-cap test tubes containing 8-10 untreated Alaska seed peas (hardware store). Autoclave 30 min at 121 °C. If not freshly prepared, heat to 100 °C and cool to 55 °C before using.
- (c) Glucose starch agar (Aerobic medium; Difco dehydrated [No.0001], or equivalent) Dissolve 15.0 g proteose peptone No.3, 2.0 g glucose, 10.0 g soluble starch, 5.0 g NaCl, 3.0 g Na<sub>2</sub>HPO<sub>4</sub>, 20.0 g gelatin and 10.0 g agar in 1 L H<sub>2</sub>O, heat to boiling point, and autoclave 15 min at 121°C in Erlenmeyer. Aseptically pour into sterile Petri dishes and allow to solidify.
- (d) Nutrient agar (Aerobic medium for spore production; Difco dehydrated [No.0001], or equivalent) Dissolve 3.0 g beef extract, 5.0 g peptone, and 15.0 g agar in 1 L H<sub>2</sub>O, heat to boiling point, and autoclave 30 min at 121 °C.
- (e) Detergent sanitizer solution. pHisoHex (3 % hexachlorophene), or equivalent.

# 2 Apparatus

- (a) Can opener. Bacti-Disc Cutter (Wilkens-Anderson Co., 4525 W Division St., Chicago, IL 60651, No. 10810-01), bacteriological can opener (Marmora Machine Co., 1956 N Latrobe Ave., Chicago, IL 60639), or equivalent.
- (b) Caps. Disposable, operating room-type (Baxter Hospital Supply Div., 1450 Waukegan Rd., McGaw Park, IL 60085), or equivalent.
- (c) Pipets. Straight wall, 200mm 250 mm long x 7 mm id, 9 mm od (Scientific Products Inc., cut and fire polished, or equivalent).

# 3 Sampling

Conduct test in clean room. (If necessary, open room may be used but outside windows must be closed and direct drafts across work area must be eliminated.) If available, use laminar flow cabinet. Strip labels from cans, examine cans for external defects and record descriptions. Wash cans with soap (or detergent sanitizer solution) and  $H_2O$ , and dry with clean paper towels. Wipe counter top with100 ppm Cl solution (e.g., Clorox or diluted NaOCl solution) immediately before placing washed and dried can on it. Place code end of can in down position and number cans in ink or with CuSO<sub>4</sub> marking solution to right of side seam.

Wash hands and face with soap, and rewash hands and face with detergent sanitizer solution. Completely cover hair with clean disposable operating room cap.

Hold non-coded end of can over large Meker burner, just above blue portion of flame. Heat this end of can until all condensation is evaporated; then return can to table in former position. Clean handle and blade of special can opener, (a), with paper towel moistened with 70 % alcohol, flame metal portion enough to destroy all microorganisms, and use it to make 4 cm (1.5 in) diameter hole in non-coded, heated end of can. Immediately and without moving can, use straight-wall sterile glass pipet, (c), transfer ca 2 g food to separate tubes, 2 each of aerobic and 2 of anaerobic media (4 total) (No other transferring tool may be substituted). Pre-loosen screw cap and hold it between little and ring fingers while transfer is being made. Flame lips of media tubes both before and after addition of food. When transferring food to anaerobic tubes, food must be inoculated into lower portion of medium. Tighten screw caps after inoculation, incubate tubes 72 h at 35 °C, and observe daily. Record results for each tube separately.

Remove additional  $\geq 10$  g food sample from each container with sterile pipet and place in sterile 25 mm x 200 mm screw-cap test tube. Use pipet-like spatula, if necessary, for this operation (thermophilic contamination unlikely). Number tube to correspond to can and refrigerate for later testing, if necessary.

#### 4 Contamination control

Use sterile loop or glass rod to streak plate of glucose starch agar, (c). On table, open plate of glucose starch agar for time equal to longest duration that any medium tube or plate is exposed. Incubate plates at 72 h at 35  $^{\circ}$ C, and observe daily.

#### 5 Microscopic examination

With pair of metal cutting shears, enlarge hole in can and record odor. Microscopically (oil immersion) examine heat-fixed thin smear of food, stained 10 s with 1 % gentian (or crystal) violet and wash in running tap  $H_2O$ , or, alternatively, examine wet mounts with phase contrast microscope. If food contains appreciable fat, xylol should be dripped across food smear while it is still hot from heat fixing. Compare stained smear with one made from normal product, if possible.

#### 6 pH determination

Determine pH with pH meter, using reference buffer near normal pH of food. Record both reference buffer pH and sample pH. Compare to normal can of food, if available.

# 7 Confirmation of results

If there is any abnormal odor, abnormal appearance, abnormal pH, numbers of bacteria on microscopic examination, and/or growth in media from any can of food, subculture corresponding refrigerated tube as follows: Flame lip of tube and, with straightwall sterile glass pipet, (c), transfer ca 2 g food to 2 tubes each of aerobic and anaerobic media (4 total). Flame lips of media tubes both before and after addition of food. Tighten caps after inoculation, incubate tubes 72 h at 55 °C, and observe daily. Record results for each tube separately.

Any organisms isolated from normal cans having obvious vacuum which produce gas in anaerobic medium at 35 °C should immediately be suspected as being from laboratory

contamination. Aseptically inoculate growing organism into another normal can, close hole with solder, and incubate 14 days at 35 °C. Any swelling of container indicates that organism was not in original sample. Record as laboratory contamination and review results of additional cans to verify finding of contamination.

Growth in aerobic medium at 35 °C from normal cans indicates either noncommercial sterility or laboratory contamination. Unless there is abnormal odor, abnormal appearance, abnormal pH, and/or numbers of bacteria on microscopic examination from product in original can, record results as laboratory contamination and review results of additional cans to verify finding of contamination. Otherwise, observe subculture results at 55 °C. Growth at 35 °C and absence of growth at 55 °C confirm non-sterility of original container. Check growth under aerobic conditions on nutrient agar plates, (d), at 55 °C and confirm for spores after 72 h. Confirmation indicates non-sterility due to flat sour spoilage. Record growth at 55 °C under anaerobic conditions with gas production as commercially sterile. Growth is caused by dormant spores incapable of growth at normal temperatures of storage and distribution.

If only one duplicate tube is positive after incubation and streaked glucose starch agar is also negative, record as laboratory contamination. Growth on air control plate of glucose starch agar also indicates potential laboratory contamination.

#### References

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

A.O. No. 153 s. 2004. Guidelines, Current Good Manufacturing Practice in Manufacturing, Packing, Repacking or Holding Food. Bureau of Food and Drugs. Department of Health. Alabang, Muntinlupa City, Philippines.

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# FORMULATING BODY Development of Standards for Selected Ethnic Food Products

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