CODEX ALIMENTARIUS COMMISSION E

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World Health Organization

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**Agenda Item 3** **CX/MAS 21/41/3**

**March 2021**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING**

**41st Session**
**Virtual**

**17 - 21 and 25 May 2021**

# ENDORSEMENT OF METHODS OF ANALYSIS AND SAMPLING PLANS FOR PROVISIONS IN CODEX STANDARDS

1. This document contains the methods of analysis (Appendix I, II, III, IV and V) proposed by the following Committees:

- FAO/WHO Coordinating Committee for Africa (methods of analysis for provisions in the draft standard for dried meat) (adopted by CAC43 at Step 5)
- FAO/WHO Coordinating Committee for North America and South West Pacific (methods of analysis for provisions in the draft regional standard for fermented noni fruit juice and the regional standard for kava products for use as a beverage when mixed with water) (adopted by CAC43 at Step 5 and Step 5/8, respectively)
- FAO/WHO Coordinating Committee for Near East (methods of analysis for provisions in the draft regional standard for mixed zaatar) (adopted by CAC43 at Step 5)
- Codex Committee on Nutrition and Foods for Special Dietary Uses (methods of analysis for provisions in the *Standard for infant formula and formulas for special medical purposes intended for infants* (CXS 72-1981))
- Codex Committee on Processed Fruits and Vegetables (methods of analysis and sampling plans in the Standard for Gochujang, the Standard for Chili Sauce, the revision to the *Standard for Mango Chutney* (CXS 160-1987), the General Standard for Dried Fruits, and the General Standard for Canned Mixed Fruits) (adopted by CAC43 at Step 5/8)

## FAO/WHO COORDINATING COMMITTEE FOR AFRICA (CCAFRICA23)[1]

***Methods of analysis for provisions in the draft standard for dried meat***

2. The Committee **is invited to endorse** the methods of analysis in Appendix I.

## FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND SOUTH WEST PACIFIC (CCNASWP15)[2]

***Methods of analysis for provisions in the draft regional standard for fermented noni fruit juice and the regional standard for kava products for use as a beverage when mixed with water***

3. The Committee **is invited to endorse** the methods of analysis in Appendix II.

## FAO/WHO COORDINATING COMMITTEE FOR NEAR EAST (CCNE10)[3]

***Methods of analysis for provisions in the draft regional standard for mixed zaatar***

4. The Committee **is invited to endorse** the methods of analysis in Appendix III.

[1] REP20/AFRICA, para. 102 ii) and Appendix V
[2] REP20/NASWP, paras. 83 (ii), 96 (iii) and Appendix II, III
[3] REP20/NE, para. 87 (ii) and Appendix IV

**CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES (CCNFSDU41)[4]**

***Methods of analysis for provisions in the Standard for infant formula and formulas for special medical purposes intended for infants (CXS 72-1981)***

5. The Committee agreed to submit the methods for thiamine, riboflavin, niacin, vitamin $B_6$, choline, carnitine, beta-carotene, lycopene, fructans and biotin to CCMAS for review and endorsement and inclusion in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) and request CCMAS to re-type the existing Type II methods for aforementioned nutrients as Type III in CXS 234-1999; and inform CCMAS that it could include AOAC 2011.14 / ISO 15151 | IDF 229 for calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc as Type III in CXS 234-1999.

6. The Committee **is invited to endorse** the methods of analysis and consider the re-typing of existing methods in Appendix IV.

**CODEX COMMITTEE ON PROCESSED FRUITS AND VEGETABLES (CCPFV29)[5]**

7. The Committee **is invited to endorse or confirm the endorsement of** the methods of analysis and sampling plans for provisions in the Standard for Gochujang, the Standard for Chili Sauce, the *Standard for Mango Chutney* (CXS 160-1987), the General Standard for Dried Fruits, and the General Standard for Canned Mixed Fruits, as detailed in Appendix V.

[4] REP20/NFSDU, para. 197
[5] REP20/PFV paras. 14, 16, 18, 22, 27 and Appendix II, III, IV, V and VI

**APPENDIX I**

**FAO/WHO COORDINATING COMMITTEE FOR AFRICA (CCAFRICA23)**

***Methods of analysis for provisions in the draft standard for dried meat***

| Method | Provision | Principle | Type |
|---|---|---|---|
| AOAC 988.05 | Determination of Moisture Content | Gravimetry | I |
| ISO 1443 (AOAC 960. 39) | Determination of Crude Fat | Gravimetry | I |
| AOAC 928.08 | Determination of Crude Protein | Kjeldhal | II |
| ISO 937 | Determination of Crude Protein | Titrimetry | II |
| ISO 1841-1 and ISO 1841-2 | Determination of Edible Salt | Potentiometric / Volhard method | II |
| AOAC 940.26 | Determination of Ash Content | Gravimetry | I |
| ISO 18787 | Determination of Water Activity | Potentiometric | II |

**APPENDIX II**

**FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND SOUTH WEST PACIFIC (CCNASWP15)**

***Methods of analysis for provisions in the draft regional standard for fermented noni fruit juice***

| Provision | Method | Principle | Type | Notes |
|---|---|---|---|---|
| Brix value | AOAC 983.17 | Refractometry | I | Adopted for fruit juices and nectars |
| pH value | NMKL 179 | Potentiometry | II | Adopted for fruit juices and nectars |
| Ethanol | IFUMA 52 | Enzymatic determination | II | Adopted for fruit juices and nectars |
| Identification of scopoletin | Annex A* | Thin layer chromatography | IV | |
| Identification of deacetylasperulosidic acid | Annex B* | Thin layer chromatography | IV | |

** In compliance with the general criteria for testing laboratories laid down in ISO/IEC Guide 17025:2017*

**ANNEX A**

## IDENTIFICATION OF SCOPOLETIN

### 1. PREPARATION OF SAMPLES

**1.1** Noni fruit is mashed. Two grams of mashed fruit is extracted twice with 125 milliliters methanol. The methanol extract is concentrated by evaporation of the solvent under vacuum. The extract is then re-dissolved in a small quantity of methanol, such as 10 milliliters.

**1.2** Noni juice is filtered through a 0.45 µm membrane filter and then purified by solid-phase extraction (SPE) with Waters OASISS® extraction cartridges, or similar solid-phase extraction cartridge. [SPE cartridges is first equilibrated with water, followed by methanol. The samples are then loaded onto the cartridge and washed with 5% MeOH, followed by 100% MeOH. The MeOH eluate is retained for TLC analysis.]

**1.3** One gram of noni fruit powder is extracted with 5 milliliters of methanol. The methanol extract is filtered and evaporated to dryness under vacuum at 50°C. The extract is dissolved into one milliliter of methanol.

### 2. PREPARATION OF REFERENCE STANDARD

**2.1** A reference standard is prepared by dissolving 1 mg scopoletin in 1 milliliter of methanol.

**2.2** Alternately, certified *Morinda citrifolia* reference plant material may be prepared in the same manner as the samples to be analyzed. The certified *Morinda citrifolia* reference material should be from the same part of the plant as the samples to be analyzed.

### 3. IDENTIFICATION

### 3.1 THIN LAYER CHROMATOGRAPHY

Spot 5 microliters of sample solutions and reference standard solution on a silica gel [60 F254] thin layer chromatography (TLC) plate, previously dried at 110 °C for 15 minutes in a drying oven. [Develop the plate with a lower solution mobile phase of dichloromethane:methanol (19:1, v/v).] View bright fluorescent blue colours on developed plate under UV lamp, 365 nm. Identify scopoletin in samples by comparing Rf values and colours to the standard.

## REFERENCES

**1.** Deng S, West BJ, Jensen J. A Quantitative Comparison of Phytochemical Components in Global Noni Fruits and Their Commercial Products. Food Chemistry 2010, 122 (1): 267-270.

**2.** Potterat O, et al. Identification of TLC markers and quantification by HPLC-MS of various constituents in noni fruit powder and commercial noni-derived products. Journal of Agricultural and Food Chemistry 2007, 55(18):7489–7494.

**3.** Basar S, Westendorf J. Identification of (2E, 4Z, 7Z)-Decatrienoic Acid in Noni Fruit and Its Use in Quality Screening of Commercial Noni Products. Food Analytical Methods 2011, 4(1):57-65. DOI: 10.1007/s12161-010-9125-9.

**4.** Chan-Blanco Y, et al. The ripening and aging of noni fruits (*Morinda citrifolia* L.): microbiological flora and antioxidant compounds. Journal of the Science of Food and Agriculture 2007, 87:1710 – 1716.

**5.** West BJ, Deng S. Thin layer chromatography methods for rapid identity testing of *Morinda citrifolia* L. (noni) fruit and leaf. Advance Journal of Food Science and Technology 2010, 2(5):298-302.

**ANNEX B**

## IDENTIFICATION OF DEACETYLASPERULOSIDIC ACID

### 1. PREPARATION OF SAMPLES

**1.1** Noni fruit is mashed. Two grams of mashed fruit is extracted twice with 125 milliliters methanol. The methanol extract is concentrated by evaporation of the solvent under vacuum. The extract is then re-dissolved in a small quantity of methanol, such as 10 milliliters.

**1.2** Noni juice is filtered through a 0.45 µm membrane filter and then purified by solid-phase extraction (SPE) with Waters OASISS® extraction cartridges, or similar solid-phase extraction cartridge. [SPE cartridges is first equilibrated with water, followed by methanol. The samples are then loaded onto the cartridge and washed with 5% MeOH, followed by 100% MeOH. The MeOH eluate is retained for TLC analysis.]

**1.3** One gram of noni fruit powder is extracted with 5 milliliters of methanol. The methanol extract is filtered and evaporated to dryness under vacuum at 50°C. The extract is dissolved into one milliliter of methanol.

### 2. PREPARATION OF REFERENCE STANDARD

**2.1** A reference standard is prepared by dissolving 1 mg deacetylasperulosidic acid in 1 milliliter of methanol.

**2.2** Alternately, certified *Morinda citrifolia* reference plant material may be prepared in the same manner as the samples to be analyzed. The certified *Morinda citrifolia* reference material should be from the same part of the plant as the samples to be analyzed.

### 3. IDENTIFICATION

### 3.1 THIN LAYER CHROMATOGRAPHY

Spot 5 microliters of sample solutions and reference standard solution on a silica gel [60 F254] thin layer chromatography (TLC) plate, previously dried at 110 °C for 15 minutes in a drying oven. [Develop the plate with a lower solution mobile phase of dichloromethane: methanol: water (13:6:1, v/v/v).] Spray developed plate with 2% anisaldehyde, 10% sulfuric acid-EtOH solution then heat in oven at 110 °C for 1 minute to reveal blue colour. Identify deacetylasperulosidic in samples by comparing Rf values and colours to the standard.

### REFERENCES

**1.** Potterat O, et al. Identification of TLC markers and quantification by HPLC-MS of various constituents in noni fruit powder and commercial noni-derived products. Journal of Agricultural and Food Chemistry 2007, 55(18):7489–7494.

**2.** Deng S, et al. Determination and comparative analysis of major iridoids in different parts and cultivation sources of *Morinda citrifolia*. Phytochemical Analysis 2011, 22(1):26-30.

**3.** West BJ, Deng S. Thin layer chromatography methods for rapid identity testing of *Morinda citrifolia* L. (noni) fruit and leaf. Advance Journal of Food Science and Technology 2010, 2(5):298-302.

***Methods of analysis for provisions in the regional standard for kava products for use as a beverage when mixed with water***

| Provision | Method | Principle | Type |
|---|---|---|---|
| Noble kava varieties | Lebot V, Legendre L (2016), Comparison of kava (Piper methysticum Forst.) varieties by UV absorbance of acetonic extracts and high-performance thin-layer chromatography. Journal of Food Composition and Analysis 48:25-33. http://dx.doi.org/10.1016/j.jfca.2016.01.009<br>and<br>Lebot V, Michalet S, Legendre L. (2019). Kavalactones and flavokavins profiles contribute to quality assessment of kava (Piper methysticumG.Forst.), the traditional beverage of the Pacific. Beverages 2019, 5, 34; https://doi.org/10.3390/beverages5020034 | High performance thin layer chromatography<br>and/or UV absorbance of acetonic extracts measured at 440 nm (less or equal to 0.9) | IV |
| Moisture | *The Fiji Kava Standard 2017*. Section 8.1 | Gravimetry | I |
| [Flavokavins | Lebot V, Legendre L (2016), Comparison of kava (Piper methysticumForst.) varieties by UV absorbance of acetonic extracts and high-performance thin-layer chromatography. Journal of Food Composition and Analysis 48:25-33. http://dx.doi.org/10.1016/j.jfca.2016.01.009<br>and<br>Lebot V, Michalet S, Legendre L. (2019). Kavalactones and flavokavins profiles contribute to quality assessment of kava (Piper methysticumG.Forst.), the traditional beverage of the Pacific. Beverages 2019, 5, 34; https://doi.org/10.3390/beverages5020034 | High performance thin layer chromatography<br>and/or UV absorbance of acetonic extracts measured at 440 nm (less or equal to 0.9)] | IV |

**APPENDIX III**

**FAO/WHO COORDINATING COMMITTEE FOR NEAR EAST (CCNE10)**

***Methods of analysis for provisions in the draft regional standard for mixed zaatar***

| Provision | Method | Principle | Type* |
|---|---|---|---|
| Sodium chloride | AOAC 960.29 | Titrimetry (Mohr: determination of chloride, expressed as sodium chloride) | |
| Moisture | AOAC 925.10 | Gravimetry, drying at 130°C | |
| Acid-insoluble ash | AOAC 941.12 | Gravimetry, Furnace, 550°C (for the HCl insoluble ignited residue) | |
| Extraneous Matter | ISO 927 | Visual Examination, followed by Volumetry | I |
| Foreign Matter | ISO 927 | Visual Examination, followed by Volumetry | I |
| Insects/Excreta/Insect Fragments | Method appropriate for particular spice from AOAC Chapter 16, subchapter 14<br>[ISPM 08 Determination of Pest Status in an area] | Visual Examination | IV |
| Mould damage | Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual, FDA, Technical Bulletin Number 5) | Visual examination (for whole) | IV |
| Excreta Mammalian,<br><br><br><br>Excreta Other | Macroanalytical Procedure Manual, USFDA, Technical Bulletin V.39 B<br>(For whole)<br><br>AOAC 993.27 (For Ground) | Visual Examination<br><br><br>Enzymatic Detection Method | IV<br><br><br>IV |

**APPENDIX IV**

**COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES (CCNFSDU41)**

***Methods of analysis for infant formula***

| Commodity | Provision | Method | Principle | Proposed Type |
|---|---|---|---|---|
| Infant Formula | Thiamine | **AOAC 2015.14 / ISO DIS 21470** | **Enzymatic digestion and LC-MS/MS** | **II** |
| | | EN 14122 | HPLC with pre- or post-column derivatization to thiochrom | ~~II~~ **III** |
| | | AOAC 986.27 | Fluorimetry | III |
| | Riboflavin | **AOAC 2015.14 / ISO DIS 21470** | **Enzymatic digestion and LC-MS/MS** | **II** |
| | | EN 14152 | HPLC | ~~II~~ **III** |
| | | AOAC 985.31 | Fluorimetry | III |
| | Niacin | **AOAC 2015.14 / ISO DIS 21470** | **Enzymatic digestion and LC-MS/MS** | **II** |
| | | EN 15652 | HPLC | ~~II~~ **III** |
| | | AOAC 985.34 | Microbioassay and turbidimetry | III |
| | Vitamin $B_6$ | **AOAC 2015.14 / ISO DIS 21470** | **Enzymatic digestion and LC-MS/MS** | **II** |
| | | AOAC 2004.07 / EN 14164 | HPLC | ~~II~~ **III** |
| | | AOAC 985.32 | Microbioassay | III |
| | | EN 14166 | Microbioassay | III |
| | Choline | **AOAC 2015.10 / ISO DIS 21468** | **LC-MS/MS** | **II** |
| | | AOAC 999.14 | Enzymatic Colorimetric Method with limitations on applicability due to choline and ascorbate concentration | ~~II~~ **III** |
| | Carnitine | **AOAC 2015.10 / ISO DIS 21468** | **LC-MS/MS** | **II** |
| | Fructans | **AOAC 2016.14 / ISO DIS 22579 \| IDF 241** | **Enzymatic digestion with HPAEC-PAD** | **II** |
| | Beta Carotene | **AOAC 2016.13 / ISO DIS 23443** | **UHPLC** | **II** |
| | Lycopene | **AOAC 2016.13 / ISO DIS 23443** | **UHPLC** | **II** |
| | Biotin | AOAC 2016.02 **/ ISO 23305** | HPLC-UV | II |
| | | EN 15607 | HPLC-fluorescence | III |

**APPENDIX V**

**COMMITTEE ON PROCESSED FRUITS AND VEGETABLES (CCPFV29)**

***Methods of analysis for provisions in the Standard for Gochujang***

***Note:*** The *Regional Standard for Gochujan*g (CXS 294R-2009) has been converted to a worldwide standard

Consequentially, the *Regional Standard for Gochujang* (CXS 294R-2009) was revoked.

Methods of analysis provisions in *Standard for Gochujang* have been endorsed previously and are included in the CXS 234.

| Provision | Method | Principle | Type |
|---|---|---|---|
| Capsaicin | AOAC 995.03 | HPLC | II |
| Capsaicin | Described in the Standard (Annex I) | Gas chromatography | IV |
| Crude protein | AOAC 984.13 (Nitrogen conversion facto 6.25) | Kjeldahl | I |
| Moisture | AOAC 934.01 (≤ 70°C, ≤ 50 mm Hg)) | Gravimetry | I |

Sampling plan

SAMPLING PLAN 1

(Inspection Level I, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 6 | 1 |
| 4,801 - 24,000 | 13 | 2 |
| 24,001 - 48,000 | 21 | 3 |
| 48,001 - 84,000 | 29 | 4 |
| 84,001 - 144,000 | 38 | 5 |
| 144,001 - 240,000 | 48 | 6 |
| more than 240,000 | 60 | 7 |
| **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 Number (c)** |
| 2,400 or less | 6 | 1 |
| 2,401 - 15,000 | 13 | 2 |
| 15,001 - 24,000 | 21 | 3 |
| 24,001 - 42,000 | 29 | 4 |
| 42,001 - 72,000 | 38 | 5 |
| 72,001 - 120,000 | 48 | 6 |
| more than 120,000 | 60 | 7 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (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 | 38 | 5 |
| 24,001 - 42,000 | 48 | 6 |
| more than 42,000 | 60 | 7 |

SAMPLING PLAN 2

(Inspection Level II, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 13 | 2 |
| 4,801 - 24,000 | 21 | 3 |
| 24,001 - 48,000 | 29 | 4 |
| 48,001 - 84,000 | 38 | 5 |
| 84,001 - 144,000 | 48 | 6 |
| 144,001 - 240,000 | 60 | 7 |
| more than 240,000 | 72 | 8 |
| **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 Number (c)** |
| 2,400 or less | 13 | 2 |
| 2,401 - 15,000 | 21 | 3 |
| 15,001 - 24,000 | 29 | 4 |
| 24,001 - 42,000 | 38 | 5 |
| 42,001 - 72,000 | 48 | 6 |
| 72,001 - 120,000 | 60 | 7 |
| more than 120,000 | 72 | 8 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 600 or less | 13 | 2 |
| 601 - 2,000 | 21 | 3 |
| 2,001 - 7,200 | 29 | 4 |
| 7,201 - 15,000 | 38 | 5 |
| 15,001 - 24,000 | 48 | 6 |
| 24,001 - 42,000 | 60 | 7 |
| more than 42,000 | 72 | 8 |

**Annex A**

## Determination of Capsaicin in *Gochujang* using Gas Chromatography (GC) Detection

### 1. SCOPE

This method is suitable for the determination of capsaicin in *Gochujang* using gas-chromatographic detection. The method uses squalene as an internal standard. The concentration of capsaicin is expressed as µg/mL.

### 2. PRINCIPLE

To extract capsaicin, the mixture is blended to a homogeneous consistency. Capsaicin in *Gochujang* is extracted with 100% methanol, followed by methanol – hexane fractionation to remove hydrophilic and hydrophobic interfering substances by a separating funnel. Capsaicin in methanol layer is extracted with dichloromethane (DCM) and the saturated solution of NaCl, concentrated by a rotary evaporator. A portion of the concentrated sample extract is then taken and completely solved with DCM containing squalene as an internal standard for analysis using gas chromatographic detection.

### 3. REAGENT AND MATERIALS

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade and water of at least grade 3 as defined in ISO 3696.

#### 3.1 Reagents

3.1.1 Capsaicin (99 + %, $C_{18}H_{27}NO_3$, Fw 305.42, CAS 404-86-4)

3.1.2 Squalene (CAS 111-02-4)

3.1.3 Hexane

3.1.4 Methanol

3.1.5 Methanol + Water (80 + 20, v/v**)**

3.1.6 Dichloromethane

3.1.7 Sodium chloride

3.1.8 Sodium sulfate

#### 3.2 Preparation of standard solution

**3.2.1** Capsaicin Stock solution (A)

Weigh approximately 100 mg of capsaicin, making up to 100 mL in a volumetric flask with DCM to give solution (A) of approximate 1000 µg/mL.

**3.2.2** Capsaicin working solution (B)

Prepare 100-mL intermediate solution B by dilution of 10 mL solution A (3.2.1) with 100 mL of DCM to exactly 100 µg/mL in DCM.

**3.2.3** Squalene internal standard working solution (C)

Weigh approximately 100 mg squalene and make up to 250 mL in a volumetric flask with DCM to give a solution (C) of approximately 400 µg/mL in DCM.

#### 3.3 Calibration solutions of capsaicin

Dispense volumes of the 100 µg/mL solution (B, 3.2.2) into 50 mL flat bottom flask, dried up and add 2 mL of internal standard working solution (C, 3.2.3) to give 10.0, 50.0, 100.0, 300.0, 500.0 µg**/**mL capsaicin.

### 4. APPARATUS

**4.1** Gas chromatograph with flame ionization detector (FID)**.** The following conditions have been found to be suitable:

4.1.1 Injector / Detector temperature : 320°C / 350°C

4.1.2 Oven temperature program: 220°C for 1 minute, ramp at 5°C/min to 250°C, hold for 13 minutes and raise to 280°C holding 5 minutes by 20°C/min. Helium carrier gas at 1.5 mL /minute

4.1.3 Make split injection of 1.0 µL with split ratio 1:5

4.2 GC column, 30 m x 0.32 µm, 0.25 µm film thickness, HP-1 or equivalent

4.3 Analytical balance, capable of weighing to 4 decimal places

4.4 Shaker, capable of attaining 2,000 rpm

4.5 Centrifuge, capable of attaining 3,500 rpm

4.6 Filter paper (Whatman No. 2 or equivalent)

## 5. LABORATORY SAMPLES

On receipt, samples are given a unique sample number. *Gochujang* sample is stored at below 4°C. All other samples are stored at room temperature in an air tight container prior to analysis.

## 6. PROCEDURE

### 6.1 Laboratory sample

Samples should be minced or grated to a homogeneous mixture. All samples should be stored in the air-tight container and at room temperature prior to analysis. All samples should be mixed thoroughly to a homogeneous mixture before analysis.

### 6.2 Test sample

6.2.1 Thoroughly mix the sample. Weigh, to the nearest 0.01 g, and 10 g portion of *Gochujang* into a 250 mL centrifuge bottle.

6.2.2 Add 50 mL of methanol and shaking for 2 hours, extracting capsaicin.

6.2.3 Filter the extract with Whatman No. 2 filter paper into a 250-mL flat bottom flask (Ext-A).

6.2.4 Add additional 30 mL of methanol to residue and shaking for 1 hour, extracting capsaicin (Ext-B).

6.2.5 Repeat step 6.2.3 to 6.2.4 (Ext-C)

6.2.6 Combine Ext-A, Ext-B and Ext-C in 250 mL flat bottom flask, concentrating up to approximately 5 mL.

6.2.7 Solve the concentrate with 20 mL of 80% methanol and 20 mL of hexane.

6.2.8 Transfer the solution into a 250 mL separating funnel.

6.2.9 Shake and separate into two layers, methanol layer (M1-layer, upper) and hexane layer (H1-layer, lower)

6.2.10 Reserve H1-layer in 100 mL flask and transfer M1-layer (6.2.9) into a separating funnel and add additional 20 mL of hexane.

6.2.11 Repeat step 6.2.9 to 6.2.10 (M2-layer and H2-layer)

6.2.12 Repeat step 6.2.9 to 6.2.10 (M3-layer and H3-layer)

6.2.13 Combine H1-layer, H2-layer and H3-layer (HC-layer) in the 250 mL separating funnel, adding 20 mL 80% methanol, shaking and separating into two layers, methanol layer (M'1-lower layer) and hexane layer (H'1-upper layer).

6.2.14 Reserve M'1-layer in the new 250 mL flat bottom flask.

6.2.15 Add 20 mL of 80% methanol into the separating funnel containing HC-layer, shaking and separating into two layers (M'2-layer and H'2-layer)

6.2.16 Combine the all M-layer in the new separating funnel (250 mL), adding 20 mL of saturated solution of NaCl and 20 mL of DCM.

6.2.17 Shake and separate into two layers (D1-layer and WM1-layer) in the 250 mL separating funnel.

6.2.18 Transfer D1-layer into the new 250 mL flat bottom flask.

6.2.19 Add additional 20 mL DCM into the separating funnel (6.2.16), shaking and separating into two layers (D2-layer and WM1-layer)

6.2.20 Repeat step 6.2.16 (D3-layer and WM1-layer)

6.2.21 Combine D1-layer, D2-layer and D3-layer into the 250 mL flat bottom flask, concentrating it (C-D)

6.2.22 Transfer the concentrate (C-D, 6.2.21) into a 100 mL flat bottom flask, solving it completely with DCM.

6.2.23 Mount approximate 3 g of sodium sulfate on the filter paper and dehydrate C-D by passing through

sodium sulfate

6.2.24 Collect the dehydrated C-D layer in 50 mL flat bottom flask and concentrate to dryness by the rotary evaporator

6.2.25 Solve the concentrate with 2 mL of DCM containing squalene as the internal standard solution (C, 3.2.3)

6.2.26 Analyze the sample solution by GC

## 7. CALCULATION – INTERNAL STANDARD METHOD

**7.1** Measure the area of the capsaicin and squalene peaks.

**7.2** Calculate the ratio of the capsaicin and squalene peak areas.

**7.3** Construct a calibration graph for the standards by plotting the peak area ratio against the weight in micrograms of capsaicin in the vial.

**7.4** Calculate the slope of the calibration line.

**7.5** Divide the peak area ratio of the unknowns by the value of the slope to give the weight of capsaicin per vial for the unknown samples.

## 8. FINAL PRESENTATION OF RESULTS

Results are expressed as µg/mL and quoted to 2 significant digits.

## REFERENCES

1. W. Hawer and J. Ha et al.: Effective separation and quantitative analysis of major heat principles in red pepper by capillary GC, Food Chemistry, 49, pp.99-103, 1994.

2. J. Jung and S. Kang: A new method for analysis of capsaicinoids content in microcapsule, Korean J. Food Sci. Technol., Vol.32, No. 1, pp.42-49, 2000.

3. C.A. Reilly et al.: Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products, J. of Forensic Science, Vol.43, No. 3, pp.502-509, 2001.

4. Ha et al.: Gas Chromatography Analysis of Capsaicin in Gochujang, Journal of AOAC International Vol. 91. No. 2, 2008.

Table 1. Summary of repeatability test for trial proper samples (μg/mL)

| Test No. | *Gochujang - K* |
|---|---|
| 1 | 64.7 |
| 2 | 69.0 |
| 3 | 70.6 |
| 4 | 71.8 |
| 5 | 70.5 |
| Mean | 69.3 |
| RSD,% | 3.99 |

Table 2. Summary of recovery test for trial proper samples (%)

| Test No. | *Gochujang – K* |
|---|---|
| 1 | 80.47 |
| 2 | 77.29 |
| 3 | 87.97 |
| 4 | 91.00 |
| 5 | 95.18 |
| Mean | 86.38 |
| RSD,% | 8.56 |

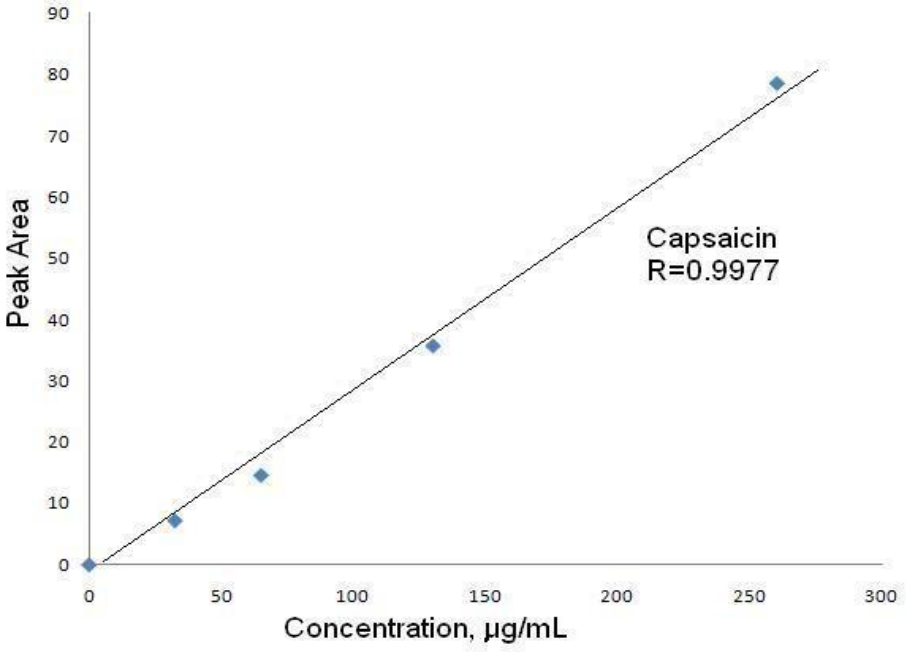


Fig.1. Calibration curve of capsaicin by GC method

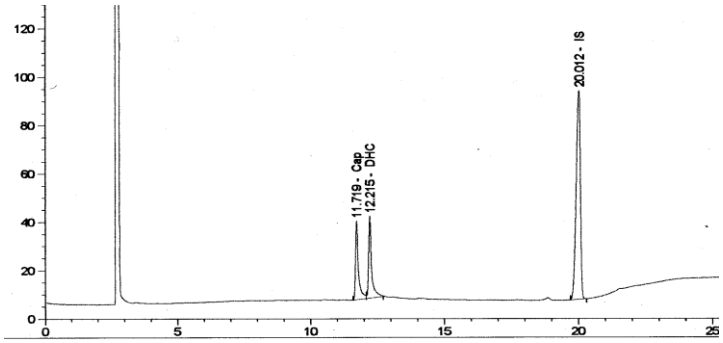


Fig. 2. GC chromatogram of capsaicin standards

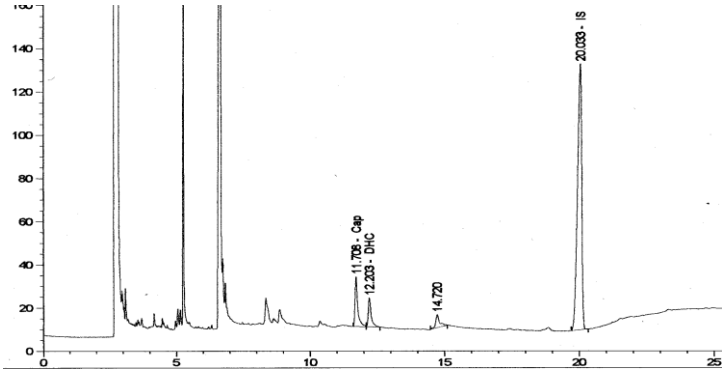


Fig. 3. GC chromatogram of capsaicin in *Gochujang*

***Methods of analysis for provisions in the Standard for Chili sauce***

**Note:** The *Regional Standard for Chili Sauce* (CXS 306R-2011) has been converted to a worldwide standard. Consequently the regional standard has been revoked. CXS234 already contains methods previously endorsed for pH and fill of containers. The sampling plan below is the same as the sampling plan in the CXS 306R.

The appropriate inspection level is selected as follows:
Inspection level I – Normal sampling
Inspection level II – Dispute (Codex referee purpose sample size), enforcement or need for better lot estimate

SAMPLING PLAN 1
(Inspection Level I, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 6 | 1 |
| 4,801 – 24,000 | 13 | 2 |
| 24,001– 48,000 | 21 | 3 |
| 48,001- 84,000 | 29 | 4 |
| 84,001 – 144,000 | 38 | 5 |
| 144,001– 240,000 | 48 | 6 |
| more than 240,000 | 60 | 7 |
| **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 Number (c)** |
| 2,400 or less | 6 | 1 |
| 2,401 – 15,000 | 13 | 2 |
| 15,001– 24,000 | 21 | 3 |
| 24,001– 42,000 | 29 | 4 |
| 42,001– 72,000 | 38 | 5 |
| 72,001 – 120,000 | 48 | 6 |
| more than 120,000 | 60 | 7 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (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 | 38 | 5 |
| 24,001– 42,000 | 48 | 6 |
| more than 42,000 | 60 | 7 |

SAMPLING PLAN 2
(Inspection Level II, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 13 | 2 |
| 4,801 – 24,000 | 21 | 3 |
| 24,001– 48,000 | 29 | 4 |
| 48,001- 84,000 | 38 | 5 |
| 84,001 – 144,000 | 48 | 6 |
| 144,001– 240,000 | 60 | 7 |
| more than 240,000 | 72 | 8 |
| **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 Number (c)** |
| 2,400 or less | 13 | 2 |
| 2,401 – 15,000 | 21 | 3 |
| 15,001– 24,000 | 29 | 4 |
| 24,001– 42,000 | 38 | 5 |
| 42,001– 72,000 | 48 | 6 |
| 72,001 – 120,000 | 60 | 7 |
| more than 120,000 | 72 | 8 |

| NET WEIGHT GREATER THAN 4.5 KG (10 LB) | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 600 or less | 13 | 2 |
| 601 – 2,000 | 21 | 3 |
| 2,001– 7,200 | 29 | 4 |
| 7,201 – 15,000 | 38 | 5 |
| 15,001– 24,000 | 48 | 6 |
| 24,001– 42,000 | 60 | 7 |
| more than 42,000 | 72 | 8 |

***Methods of analysis for provisions in the Standard for Mango Chutney* (CXS 160-1987)**

SAMPLING PLAN 1 (Inspection Level I, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 6 | 1 |
| 4,801 - 24,000 | 13 | 2 |
| 24,001 - 48,000 | 21 | 3 |
| 48,001 - 84,000 | 29 | 4 |
| 84,001 - 144,000 | 38 | 5 |
| 144,001 - 240,000 | 48 | 6 |
| more than 240,000 | 60 | 7 |
| **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 Number (c)** |
| 2,400 or less | 6 | 1 |
| 2,401 - 15,000 | 13 | 2 |
| 15,001 - 24,000 | 21 | 3 |
| 24,001 - 42,000 | 29 | 4 |
| 42,001 - 72,000 | 38 | 5 |
| 72,001 - 120,000 | 48 | 6 |
| more than 120,000 | 60 | 7 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (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 | 38 | 5 |
| 24,001 - 42,000 | 48 | 6 |
| more than 42,000 | 60 | 7 |

SAMPLING PLAN 2 (Inspection Level II, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| Lot Size (N) | Sample Size (n) | Acceptance Number (c) |
| 4,800 or less | 13 | 2 |
| 4,801 - 24,000 | 21 | 3 |
| 24,001- 48,000 | 29 | 4 |
| 48,001 - 84,000 | 38 | 5 |
| 84,001 - 144,000 | 48 | 6 |
| 144,001 -240,000 | 60 | 7 |
| more than 240,000 | 72 | 8 |
| **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 Number (c) |
| 2,400 or less | 13 | 2 |
| 2,401 - 15,000 | 21 | 3 |
| 15,001 - 24,000 | 29 | 4 |
| 24,001 - 42,000 | 38 | 5 |
| 42,001 - 72,000 | 48 | 6 |
| 72,001 - 120,000 | 60 | 7 |
| more than 120,000 | 72 | 8 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| Lot Size (N) | Sample Size (n) | Acceptance Number (c) |
| 600 or less | 13 | 2 |
| 601 - 2,000 | 21 | 3 |
| 2,001 - 7,200 | 29 | 4 |
| 7,201 - 15,000 | 38 | 5 |
| 15,001 - 24,000 | 48 | 6 |
| 24,001 - 42,000 | 60 | 7 |
| more than 42,000 | 72 | 8 |

***Methods of analysis for provision for the General Standard for Dried Fruits***

***Note***

This General standard applies to dried fruits in general, as defined in Section 2 in this standard and also provides specific provisions for products covered in the Annexes (A: Dried appricots, B: Dates, C: Raisins, D:Dried Longans. E: Dried Persimmons)

Consequentially, *Standards for Dried Apricots* (CXS 130-1981), *Dates* (CXS 143-1985), and *Raisins* (CXS 67-1981) were revoked.

Currently CXS 234 contains a method for moisture AOAC 934.06 for dried apricots, dates and the current method for moisture in raisins is AOAC 972.20.

Methods of Analysis

| Commodity | Provision | Method | Principle | Type |
|---|---|---|---|---|
| Dried fruits | Identification of defects | Described in the standard | Visual inspection | I |
| Dried fruits | Moisture | AOAC 934.06 | Gravimetry (vacuum oven | I |

Sampling plans

| The appropriate inspection level is selected as follows: | |
|---|---|
| **Inspection level I** | **- Normal Sampling** |
| **Inspection level II** | **- Disputes, (Codex referee purposes sample size), enforcement or need for better lot estimate** |

SAMPLING PLAN 1 )Inspection Level I, AQL = 6.5(

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 6 | 1 |
| 4,801 – 24,000 | 13 | 2 |
| 24,001 – 48,000 | 21 | 3 |
| 48,001 – 84,000 | 29 | 4 |
| 84,001 – 144,000 | 38 | 5 |
| 144,001 – 240,000 | 48 | 6 |
| more than 240,000 | 60 | 7 |
| **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 Number (c)** |
| 2,400 or less | 6 | 1 |
| 2,401 – 15,000 | 13 | 2 |
| 15,001 – 24,000 | 21 | 3 |
| 24,001– 42,000 | 29 | 4 |
| 42,001 – 72,000 | 38 | 5 |
| 72,001 – 120,000 | 48 | 6 |
| more than 120,000 | 60 | 7 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (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 | 38 | 5 |

| | | |
|---|---|---|
| 24,001 – 42,000 | 48 | 6 |
| more than 42,000 | 60 | 7 |

SAMPLING PLAN 2 )Inspection Level II, AQL = 6.5(

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 13 | 2 |
| 4,801 – 24,000 | 21 | 3 |
| 24,001 – 48,000 | 29 | 4 |
| 48,001 – 84,000 | 38 | 5 |
| 84,001 – 144,000 | 48 | 6 |
| 144,001 – 240,000 | 60 | 7 |
| more than 240,000 | 72 | 8 |
| **NET WEIGHT IS GREATER THAN 1 KG (2.2 LB) BUT NOT MORE THAN 4.5 KG (10 LB)** | | |
| 2,400 or less | 13 | 2 |
| 2,401 – 15,000 | 21 | 3 |
| 15,001 – 24,000 | 29 | 4 |
| 24,001 – 42,000 | 38 | 5 |
| 42,001 – 72,000 | 48 | 6 |
| 72,001 – 120,000 | 60 | 7 |
| more than 120,000 | 72 | 8 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| 600 or less | 13 | 2 |
| 601 – 2,000 | 21 | 3 |
| 2,001 – 7,200 | 29 | 4 |
| 7,201 – 15,000 | 38 | 5 |
| 15,001 – 24,000 | 48 | 6 |
| 24,001 – 42,000 | 60 | 7 |
| more than 42,000 | 72 | 8 |

***Methods of analysis for provision for the General Standard for Canned Mixed Fruits***

***Note:*** This General standard applies to canned mixed fruits in general, as defined in Section 2 in this standard and also provides specific provisions for products covered in the Annexes (A: Canned fruit cocktail, B:Canned tropical fruit salad).
Consequentially, *the standards for Canned Fruit Cocktail* (CXS 78-1981) and *Canned Tropical Fruit Salad* (CXS 99-1981) were revoked.

Sampling Plans

| The appropriate inspection level is selected as follows: | |
|---|---|
| Inspection level I | - Normal Sampling |
| Inspection level II | - Disputes, (Codex referee purposes sample size), enforcement or need for better lot estimate |

SAMPLING PLAN 1 )Inspection Level I, AQL = 6.5(

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 6 | 1 |
| 4,801 – 24,000 | 13 | 2 |
| 24,001 – 48,000 | 21 | 3 |
| 48,001 – 84,000 | 29 | 4 |
| 84,001 – 144,000 | 38 | 5 |
| 144,001 – 240,000 | 48 | 6 |
| more than 240,000 | 60 | 7 |
| **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 Number (c)** |
| 2,400 or less | 6 | 1 |
| 2,401 – 15,000 | 13 | 2 |
| 15,001 – 24,000 | 21 | 3 |
| 24,001– 42,000 | 29 | 4 |
| 42,001 – 72,000 | 38 | 5 |
| 72,001 – 120,000 | 48 | 6 |
| more than 120,000 | 60 | 7 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (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 | 38 | 5 |
| 24,001 – 42,000 | 48 | 6 |
| more than 42,000 | 60 | 7 |

SAMPLING PLAN 2 (Inspection Level II, AQL = 6.5)

| **NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)** | | |
|---|---|---|
| **Lot Size (N)** | **Sample Size (n)** | **Acceptance Number (c)** |
| 4,800 or less | 13 | 2 |
| 4,801 – 24,000 | 21 | 3 |
| 24,001 – 48,000 | 29 | 4 |
| 48,001 – 84,000 | 38 | 5 |
| 84,001 – 144,000 | 48 | 6 |
| 144,001 – 240,000 | 60 | 7 |
| more than 240,000 | 72 | 8 |
| **NET WEIGHT IS GREATER THAN 1 KG (2.2 LB) BUT NOT MORE THAN 4.5 KG (10 LB)** | | |
| 2,400 or less | 13 | 2 |
| 2,401 – 15,000 | 21 | 3 |
| 15,001 – 24,000 | 29 | 4 |
| 24,001 – 42,000 | 38 | 5 |
| 42,001 – 72,000 | 48 | 6 |
| 72,001 – 120,000 | 60 | 7 |
| more than 120,000 | 72 | 8 |
| **NET WEIGHT GREATER THAN 4.5 KG (10 LB)** | | |
| 600 or less | 13 | 2 |
| 601 – 2,000 | 21 | 3 |
| 2,001 – 7,200 | 29 | 4 |
| 7,201 – 15,000 | 38 | 5 |
| 15,001 – 24,000 | 48 | 6 |
| 24,001 – 42,000 | 60 | 7 |
| more than 42,000 | 72 | 8 |