CODEX ALIMENTARIUS COMMISSION E

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World Health Organization

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Agenda Item 3 **CX/MAS 15/36/3**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Thirty-sixth Session

Budapest, Hungary, 23 – 27 February 2015

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

1. This document contains the methods of analysis and/or sampling (Appendix I) proposed by the following Committees:

- Committee on Processed Fruits and Vegetables (methods of analysis for canned fruits and ginseng and their accompanying sampling plans);
- Committee on Contaminants in Foods (sampling plans for fumonisins in maize and maize products) .

COMMITTEE ON PROCESSED FRUITS AND VEGETABLES (CCPFV)

Methods for canned fruits

2. The Committee is invited to note that the methods of analysis for canned fruits are those methods previously endorsed as general methods for processed fruits and vegetables.

Methods for ginseng

3. The Committee is invited to note that the methods in the *Regional Standard for Ginseng Products* (CODEX STAN 295R-2009) were previously endorsed by CCMAS.[1] The methods are resubmitted to CCMAS following the finalization of its conversion to a worldwide standard by CCPFV.

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF)

Sampling plans for fumonisins in maize and maize products[2]

4. The Committee noted that the sampling plans were based on OC curves derived for MLs of 2 000 and 5 000 µg/kg, but that the sampling plan for raw maize was not expected to change with the change in ML for these products, and agreed to the sampling plans as proposed for both the raw maize grains and the maize flour and maize meal. It was noted that the issues raised by CCMAS on the sampling plans for DON did not apply to these sampling plans.

5. The Committee is **invited to endorse** the proposed the sampling plans in Appendix I.

[1] ALINORM 08/31/23, para. 57

[2] REP14/CF, para. 70

APPENDIX I

COMMITTEE ON PROCESSED FRUITS AND VEGETABLES (CCPFV)

METHODS OF ANALYSIS AND SAMPLING FOR CANNED FRUITS

Provision	Method	Principle	Type
Drained weight	AOAC 968.30 (Codex general method for processed fruits and vegetables)	Sieving Gravimetry	I
Fill of containers	CAC/RM 46-1972 (for glass containers) (Codex general method for processed fruit and vegetables) and ISO 90.1:1999 (for metal containers) (Codex general method for processed fruit and vegetables)	Weighing	I
Soluble solids	ISO 2173:2003 (Codex general method for processed fruit and vegetables) AOAC 932.14C	Refractometry	I

DETERMINATION OF WATER CAPACITY OF CONTAINERS
(CAC/RM 46-1972)

1. **SCOPE**

This method applies to glass containers.

2. **DEFINITION**

The water capacity of a container is the volume of distilled water at 20°C which the sealed container will hold when completely filled.

3. **PROCEDURE**

3.1 Select a container which is undamaged in all respects.

3.2 Wash, dry and weigh the empty container.

3.3 Fill the container with distilled water at 20°C to the level of the top thereof, and weigh the container thus filled.

4. **CALCULATION AND EXPRESSION OF RESULTS**

Subtract the weight found in 3.2 from the weight found in 3.3. The difference shall be considered to be the weight of water required to fill the container. Results are expressed as ml of water.

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 (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 AND SAMPLING FOR GINSENG

PREPARATION OF TEST SAMPLE

Dried ginseng is pulverized using a grinder to make approximately 3 mm-sized particles for the analysis. Ginseng extract is used in the analysis as is.

METHODS OF ANALYSIS

PROVISION	METHOD	PRINCIPLE	TYPE
Moisture	AOAC 925.45 B (Dried ginseng) Quantity of sample: 2 g AOAC 925.45 D (Ginseng extract) Quantity of sample: 1.5 g (mixing with 20 g of sea sand)	Gravimetry	IV
Solids	AOAC 925.45 B (Dried ginseng) - calculated by subtracting the content of moisture from 100% Quantity of sample: 2 g AOAC 925.45 D (Ginseng extract) - calculated by subtracting the content of moisture from 100% Quantity of sample: 1.5 g (mixing with 20 g of sea sand)	Calculation	IV
Ash	AOAC 923.03	Gravimetry	IV
Water-insoluble solids	described in Annex III	Gravimetry	IV
Water-saturated n-butanol extracts	described in Annex IV	Gravimetry	IV
Identification of ginsenosides Rb1, and Rf	described in Annex V	TLC or HPLC	IV

References

1. Standard Operation Procedure (SOP) for Determination of Moisture (*attached to the Standard*)

2. Standard Operation Procedure (SOP) for Determination of Ash (*attached to the Standard*)

ANNEX 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

ANNEX II

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 III

Determination of water-insoluble solid content

1. **Scope of application**

This method can be applied for the analysis of ginseng extract (liquid and powder form).

2. **Principles**

Samples are dissolved in distilled water and centrifuged. The supernatant is removed, and the remaining solid is precipitated and dried. Its weight is determined to be the water-insoluble solid content.

3. **Equipment & Apparatus**

3.1 Centrifuge (temperature controllable).

3.2 Centrifuge tubes for centrifugation.

3.3 Serum separation tube or micro-pipette.

3.4 Drying oven with a thermostat (±1℃ temperature control).

3.5 Electronic balance (measurable down to 0.1 mg).

3.6 Desiccator (silica gel).

3.7 Tongs.

4. **Experimental procedures**

4.1 Dry a centrifuge tube in a drying oven at 105℃ for 3 hours. After drying, place the centrifuge tube in a desiccator, let it stand at room temperature for 30 minutes, and then record its weight.

4.2 Repeat procedure step 4.1 until a constant weight is obtained for the centrifuge tube. Note, however, that the drying time should be 1-2 hours.

4.3 Precisely weigh out approximately 1 g of sample and place it in the centrifuge tube with known constant weight[3].

4.4 Add 15 ml of distilled water to the centrifuge tube containing the sample to dissolve the sample.

4.5 Centrifuge the tube at room temperature at 1,000×g[4] for 15 minutes and then remove the supernatant immediately using a serum separation tube while trying not to touch the separated precipitate. The supernatant may not be able to be completely removed due to the necessity of leaving a small amount of the supernatant to prevent the loss of suspended solids.

4.6 Repeat procedural steps 4.4 and 4.5 two more times with the solid that remains in the centrifuge tube.

4.7 Dry the centrifuge tube with the remaining sample in a drying oven at 105℃ for 5 hours.

4.8 After drying, place the centrifuge tube in a desiccator, let it stand at room temperature for 30 minutes, and then measure its weight.

4.9 Repeat procedures step 4.7 and 4.8 until a constant weight is obtained for the centrifuge tube containing the sample. Note, however, that the drying time should be 1-2 hours.

4.10 The water-insoluble solid content is calculated as follows:

$$\text{Water-insoluble solid content (\%)} = \frac{W_1 - W_0}{S} \times 100$$

W_0: Weight of the centrifuge tube (g)

W_1: Weight of the centrifuge tube with the solid residue after drying (g)

S: Weight of the sample (g)

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[3] The constant weight is the smaller value among weights measured successively when the weight difference between the current weight measurement and the previous weight measurement is less than 2 mg.

[4] $g = G\frac{M}{R^2}$ (g: gravity acceleration, G: gravity constant, R: radius, M: mass)

ANNEX IV

Determination of water-saturated n-butanol extracts

1. **Scope of application**

This method can be applied for the analysis of dried ginseng and ginseng extracts (liquid and powder forms).

2. **Principles**

Crude saponin is extracted from ginseng products using water-saturated n-butanol as the solvent after the removal of the nonpolar lipids and carbohydrates using diethyl ether and distilled water.

3. **Equipment & Apparatus**

3.1 Separatory funnel (250 ml).

3.2 Round flat flask (200-300 ml).

3.3 Erlenmeyer flask (200-300 ml).

3.4 Standard sieve (No. 80).

3.5 Filter paper (No. 2).

3.6 Glass funnel.

3.7 Funnel Shaker.

3.8 Rotary evaporator.

3.9 Constant-temperature water bath.

3.10 Electronic balance (measurable down to 0.1 mg).

3.11 Drying oven with a thermostat (±1℃ temperature control).

3.12 Desiccator (silica gel).

3.13 Grinder.

3.14 Tongs.

4. **Reagents**

4.1 n-butanol (over EP grade).

4.2 Diethyl ether (over EP grade).

4.3 Distilled water.

5. **Preparation of the water-saturated n-butanol solution**

5.1 Mix n-butanol and distilled water at a ratio of 70:30.

5.2 Shake the mixture sufficiently and let it stand so that the upper layer (water-saturated n-butanol layer) and the lower layer (water layer) separate completely.

5.3 After complete separation is achieved, the water-saturated n-butanol layer is stored in a container and capped until further use.

6. **Pretreatment of samples**

Dried ginseng samples are pulverized using a grinder and sifted through an 80-mesh sieve for experimental use. The ginseng extract is used in the experiment as is.

7. **Experimental procedures for dried ginseng**

7.1 Precisely weigh out approximately 5 g of sample and place it in a round flat flask (A). Then, add 50 ml of the water-saturated n-butanol solution. Perform reflux extraction in a constant-temperature water bath at 75-80℃ for 1 hour and then let it stand for 30 minutes.

7.2 Transfer the solution obtained in step 7.1 into a separatory funnel after filtering it through filter paper.

7.3 Repeat procedures step 7.1 and 7.2 two more times for the solid remains in the round flat flask (A).

7.4 Add 50 ml of distilled water to the mixed solution obtained in step 7.2-7.3 and then shake the solution using a funnel shaker (approximately 15 minutes). Let it stand until the upper layer (water-saturated n-butanol layer) and the lower layer (water layer) are completely separated.

7.5 Transfer the upper layer (water-saturated n-butanol layer) into a previously weighed flat bottom flask (B) and vacuum-concentrate and dry (60℃) the sample until the liquid is completely removed.

7.6 Add 50 ml of diethyl ether to the round flat flask (B) containing the precipitates and reflux the sample again in a constant-temperature water bath at 46℃ for 30 minutes.

7.7 Discard the diethyl ether in the flat bottom flask (B) by filtering the sample through filter paper and then collect the precipitates on the filter paper in a flat bottom flask (B) by dissolving them with methanol.

7.8 Concentrate the contents in the round flat flask (B) until the odors of diethyl ether and methanol disappear.

7.9 After drying the round flat flask (B) in a drying oven at 105℃ for 1 hour, place it in a desiccator at room temperature, let it stand for 1 hour, and then measure its weight.

7.10 The water-saturated n-butanol content of dried ginseng is calculated as follows:

$$\text{Water-saturated n-butanol extract (mg/g)} = \frac{W_1 - W_0}{S}$$

W_0: Weight of the flask (mg)

W_1: Weight of the flask after concentration and drying (mg)

S: Weight of the sample (g)

8. **Experimental procedures for ginseng extracts**

8.1 Precisely weigh out approximately 2 g of sample in an Erlenmeyer flask, add 60 ml of distilled water to dissolve the sample, and then transfer it to a separatory funnel (A).

8.2 Add 60 ml of diethyl ether, shake the funnel several times, and then remove the gas by opening the cork. Repeat the above procedure step 8.2, 2-3 times.

8.3 Shake the separatory funnel sufficiently in a funnel shaker (approximately 15 minutes) and then let it stand until the upper layer (diethyl ether layer) and the lower layer (water layer) are completely separated.

8.4 Transfer the lower portion (water layer) to a different separatory funnel (B), add 60 ml of the water-saturated n-butanol solution, shake the funnel under the same conditions as described in step 8.3, and let it stand until the layers are completely separated. The supernatant (water-saturated n-butanol layer) is collected (collected from above of the boundary surface) and transferred to another flask.

* At this time, the lower layer (water layer) is considered the emulsion layer in the next two separation stages but not in the final separation stage.

8.5 Repeat procedure step 8.4 two more times on the lower layer (water layer) left in the separatory funnel (B). At the final separation stage, the supernatant including the emulsion is slowly removed, leaving only the upper layer, by opening the spout of the separatory funnel.

8.6 Collect the solution (supernatants from each separation stage) obtained from procedures step 8.4-8.6 into the separatory funnel (B), add 50 ml of distilled water, and shake the funnel under the same conditions as described in (c). Then, let it stand_until the upper layer (n-butanol layer) and the lower layer (water layer) are completely separated.

8.7 Transfer the supernatant (n-butanol layer) into the previously weighed flat bottom flask and vacuum-concentrate (60℃) it until the liquid is completely removed.

8.8 Dry the flat-bottomed flask in a drying oven at 105℃ for 1 hour and then place in a desiccator at room temperature. Let it stand for 1 hour and then measure its weight.

8.9 Calculate the water-saturated n-butanol content in the ginseng extract using the same method as described in step 7.10.

ANNEX V

Identification of ginsenosides Rb_1, and Rf

Ginsenosides in ginseng products can be identified by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC).

1. **Sample solution preparation**

The dried 1-butanol extract obtained according to the method for the measurement of the water-saturated n-butanol extract in Annex IV is completely dissolved in 10 ml of methanol and then filtered through a 0.45-μm membrane filter.

2. **Standard solution preparation**

Reference substances for ginsenoside Rb_1 and ginsenoside Rf are dissolved in methanol to concentrations of 0.2%, and then the solutions are filtered through a 0.45-μm membrane filter.

3. **Identification**

3.1 **Thin-Layer Chromatography (TLC)**

3.1.1 **Preparation of the developing solvent**

(a) Mix n-butanol: ethyl acetate:water at a ratio of 50:10:40 (A), or chloroform:methanol:water at a ratio of 65:35:10 (B) in a separatory funnel.

(b) Shake the funnel sufficiently and let it stand until the solvent is completely separated.

(c) Collect only the upper layer when using solvent (A) as the developing solvent and only the lower layer when using solvent (B) and store the layers for further use. Collect from above (A) or below (B) the boundary surface of the relevant solvent when each solvent is separated and stored to increase the purity of the developing solvent.

3.1.2 **Developing chamber**

(a) Use a developing chamber with a cover (the developing chamber is completely sealed by applying glycerin, etc.).

(b) Attach filter paper to the sides and back of the inside of the developing chamber and soak them with the developing solvent.

(c) Place the developing solvent slowly into the developing chamber (approximately halfway up to the starting line of the TLC plate).

(d) Place the cover on and let it stand until the inside of the developing chamber is sufficiently saturated (30 minutes).

3.1.3 **TLC preparation**

(a) The TLC plate is cut into appropriate pieces over 10 cm in length and wide enough to accommodate the number of samples needed for identifying the ginsenosides.

(b) Place the plate in a clean drying oven and dry it at 110℃ for 10-15 minutes before use.

(c) Draw a line (starting line) 1 cm from the bottom of the TLC plate and mark the spots for dropping the samples. Then, draw a line (ending line) at exactly 8 cm from the starting line.

3.1.4 **TLC identification**

(a) Five-microliter samples of the ginsenoside references and the sample solutions prepared as described above are dropped while drying using a dryer. Each 5-μl sample is dropped by dividing it into several drops carefully without scraping off the silica gel of the TLC plate and not by using one drop.

(b) After the dropping is completed, dry the TLC plate with a dryer.

(c) Place the TLC plate in the developing chamber with its starting line at the bottom and develop the samples.

(d) When the developing solvent reaches the ending line, the TLC plate is taken out and dried with a dryer.

(e) Spray a 10% sulfuric acid solution evenly on the TLC plate.

(f) Place the plate in a dryer at 110℃ for 5-10 minutes for the development of the colors.

(g) Compare the R_f values and colors of the substances separated from the sample with those of the ginsenoside references to identify the relevant ginsenosides in the ginseng products.

$$R_f = \frac{distance\ sample\ solution\ migrated}{distance\ developing\ solvent\ migrated}$$

3.2 **High-Performance Liquid Chromatography (HPLC)**

The sample solution prepared according to the description above and the ginsenoside references are analyzed using HPLC under the conditions described below. Ginsenosides in the sample solutions can be identified by comparing their retention times with the peaks shown by the ginsenosides in the reference substances.

<Operating conditions>

(a) Column: ODS column

(b) Detector: UV (203 nm) or ELSD

(c) Eluent

- UV: acetonitrile:water (30:70, v/v)-
- ELSD: acetonitrile:water:isopropanol (94.9:5.0:0.1, v/v/v)

(d) Flow rate: 1.0 ml/min~2.0 ml/min

※ The analytical conditions can be adjusted depending on the laboratory conditions, but the peaks of Rb_1, and Rf in the chromatogram should NOT be located in the first 5 minutes NOR in the last 5 minutes of the retention time.

Reference 1

Standard Operation Procedure for Determination of Moisture

1. **Scope of application**

This method can be applied for the analysis of dried ginseng and ginseng extract.

2. **Principles**

It is assumed that the moisture is the only volatile component in food. When the pressure of the water vapor in food is increased by heating, that of the surroundings is reduced relative to that of the food. The moisture in a food sample can be completely evaporated during heating at 105℃ without the occurrence of any chemical change.

3. **Equipment & Apparatus**

3.1 Weighing bottle with a lid.

3.2 Glass rod (It should protrude at least 1.5 cm from the surface of the sea sand when inserted at a 45° angle into a weighing bottle containing 20 g of sea sand.).

3.3 Drying oven with a thermostat (±1℃ temperature control).

3.4 Electronic balance (measurable down to 0.1 mg).

3.5 Sea sand (20-35 mesh).

3.6 Desiccator (silica gel).

3.7 Grinder.

3.8 Tongs.

4. **Pre-treatment of samples**

Dried ginseng samples are pulverized using a grinder to make approximately 3-mm-sized particles for the experiment. The ginseng extract is used in the experiment as is.

5. **Experimental procedures - dried ginseng and ginseng extract (powder form)**

5.1 Dry a weighing bottle and a lid separately in a drying oven at 105℃ for 5 hours. Afterwards, place the weighing bottle capped tightly with the lid in a desiccator, let it stand at room temperature for 30 minutes, and then measure its weight.

5.2 Repeat procedure step 5.1 until a constant weight is obtained for the bottle and lid. Note, however, that the drying time should be 1-2 hours.

5.3 Precisely weigh out approximately 2 g of sample, and place it into the weighing bottle with known constant weight.

5.4 Dry the weighing bottle containing the sample in a drying oven at 105℃ for 3 hours. The lid is placed slightly ajar to dry the sample in the weighing bottle.

5.5 Place the weighing bottle capped tightly with the lid in a desiccator, let it stand at room temperature for 30 minutes, and then measure its weight.

5.6 Repeat procedures 5.4 and 5.5 until a constant weight is obtained for the bottle containing the sample. Note, however, that the drying time should be 1-2 hours.

5.7 The moisture content is calculated as follows:

$$\text{Moisture content in the sample (\%)} = \frac{S-(W_1-W_0)}{S} \times 100$$

W_0: Weight of the weighing bottle (g)

W_1: Weight of the weighing bottle with the sample after drying (g)

S: Weight of the sample (g)

6. **Experimental procedures - ginseng extract (liquid form)**

6.1 Dry the weighing bottle containing 20 g of sea sand and a glass rod in a drying oven at 105℃ for 5 hours.

6.2 After drying, place the weighing bottle in a desiccator, let it stand at room temperature for 30 minutes, and then measure its weight.

6.3 Repeat procedures 6.1 and 6.2 until a constant weight is obtained for the bottle containing the sea salt and the glass rod. Note, however, that the drying time should be 1-2 hours.

6.4 Precisely weigh out approximately 1.5 g of sample and place it into the weighing bottle with a known constant weight. Then, mix the sample well with the sea sand and evenly spread the mixture on the surfaces of the weighing bottle walls using the glass rod.

6.5 The remaining analytical steps and calculations are the same as for step 5.4 and 5.5 of Section 5 above.

Reference 2

Standard Operation Procedure for Determination of Ash

1. **Scope of application**

This method can be applied for the analysis of dried ginseng samples.

2. **Principles**

Samples are collected in a container (crucible) for ash analysis and burned at 525-600℃ to remove the organic substances. The total mineral weight of the remaining sample is considered the ash content.

3. **Equipment & Apparatus**

3.1 Porcelain crucible with a lid.

3.2 Electric heating plate.

3.3 Electric furnace with a thermostat (±1℃ temperature control).

3.4 Electronic balance (measurable down to 0.1 mg).

3.5 Desiccator (silica gel).

3.6 Grinder.

3.7 Tongs.

4. **Pretreatment of samples**

Dried ginseng samples are pulverized using a grinder to make approximately 3-mm-sized particles for the experiment.

5. **Experimental procedures**

5.1 Heat a clean porcelain crucible in an electric furnace at 550℃ for 3 hours. Let it stand at room temperature for 1 hour, and then measure its weight.

5.2 Repeat procedure step 5.1 until a constant weight is obtained. Note, however, that the ashing time should be 1-2 hours.

5.3 Precisely weigh out approximately 3 g of sample in the porcelain crucible with known constant weight.

5.4 Place the porcelain crucible containing the sample in an electric furnace at 550℃ and ash the sample by heating the crucible with the lid on it until white or bright grayish white ash is formed.

5.5 After ashing is complete, place the porcelain crucible containing the sample in a desiccator, let it stand at room temperature for 1 hour, and then measure its weight.

5.6 Repeat procedures step 5.4 to 5.5 until a constant weight is obtained for the crucible containing the sample. Note, however, that the ashing time should be 1-2 hours.

5.7 The ash content is calculated as follows:

$$\text{Ash content in the sample (\%)} = \frac{W_2 - W_1}{S} \times 100$$

W_1: Weight of the porcelain crucible before ashing (g)

W_2: Weight of the porcelain crucible after ashing (g)

S: Weight of the sample (g)

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF)

SAMPLING PLAN FOR FUMONISINS (FB1 + FB2) IN MAIZE GRAIN AND MAIZE FLOUR AND MAIZE MEAL

Raw Maize Grain

Maximum level	4 000 µg/kg FB1 + FB2
Increments	increments of 100 g, depending on the lot weight (≥ 50 tonnes)
Aggregate sample size	5 kg (lot ≥ 50 tonnes)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample size	1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the fumonisin-sample test result for the laboratory samples is equal or less than 4 000 µg/kg, accept the lot. Otherwise, reject the lot.

Maize Flour and Maize Meal

Maximum level	2 000 µg/kg FB1 + FB2
Increments	10 x 100 g
Aggregate sample size	1 kg
Sample preparation	None
Laboratory sample size	25 g test portion
Number of laboratory samples	1
Test portion	same as laboratory sample
Method	HPLC
Decision rule	If the fumonisin-sample test result is equal or less than 2 000 µg/kg, accept the lot. Otherwise, reject the lot.

DEFINITION

Lot - an identifiable quantity of a food commodity delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor, or markings.

Sublot - designated part of a larger lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.

Sampling plan - is defined by a fumonisin test procedure and an accept/reject level. A fumonisin test procedure consists of three steps: sample selection, sample preparation and analysis or fumonisin quantification. The accept/reject level is a tolerance usually equal to the Codex maximum level (ML).

Incremental sample – the quantity of material taken from a single random place in the lot or sublot.

Aggregate sample - the combined total of all the incremental samples that is taken from the lot or sublot. The aggregate sample has to be at least as large as the laboratory sample or samples combined.

Laboratory sample – the smallest quantity of shelled maize comminuted in a mill. The laboratory sample may be a portion of or the entire aggregate sample. If the aggregate sample is larger than the laboratory sample(s), the laboratory sample(s) should be removed in a random manner from the aggregate sample.

Test portion – a portion of the comminuted laboratory sample. The entire laboratory sample should be comminuted in a mill. A portion of the comminuted laboratory sample is randomly removed for the extraction of the fumonisin for chemical analysis.

Operating characteristic (OC) curve – a plot of the probability of a accepting a lot versus lot concentration for a specific sampling plan design. The OC curve provides an estimate of the chances of rejecting a good lot (exporter's risk) and the chances of accepting a bad lot accepted (importer's risk) by a specific fumonisin sampling plan design. A good lot is defined as having a fumonisin concentration below the ML; a bad lot is defined as having a fumonisin concentration above the ML.

SAMPLING PLAN DESIGN CONSIDERATIONS

Material to be sampled

1. Each lot of maize, which is to be examined for fumonisin, must be sampled separately. Lots larger than 50 tonnes should be subdivided into sublots to be sampled separately. If a lot is greater than 50 tonnes, the lot should be subdivided into sublots according to Table 1.

Table 1. Subdivision of maize sublots according to lot weight

Lot weight (ton)	Weight or number of lots	Number of incremental sample	Aggregate sample weight
≥ 1 500	500	100	5
> 300 and < 1 500	3 sublots	100	5
≥ 50 and ≤ 300	100 tonnes	100	5
< 50	-	3 - 100*	1 - 5

* see Table 2

2. Taking into account that the weight of the lot is not always an exact multiple of the weight of sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 20%.

Incremental Sample

3. The suggested minimum weight of the incremental sample should be approximately 100 g for lots of 50 metric tonnes (50 000 kg) or higher

4. For lots less than 50 tonnes, the sampling plan must be used with 10 to 100 incremental samples, depending on the lot weight, resulting in an aggregate sample of 1 to 5 kg. For very small lots (≤ 0.5 tonnes) a lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall be also in that case at least 1 kg. Table 2 may be used to determine the number of incremental samples to be taken.

Table 2. Number of incremental samples to be taken depending on the weight of the lot

Lot weight (ton)	Number of incremental sample
≥ 0.05	3
> 0.05 - ≤ 0.5	5
> 0.5 - ≤ 1	10
> 1 - ≤ 3	20
> 3 - ≤ 10	40
> 10 - ≤ 20	60
> 20 - ≤ 50	100

Static Lots

5. A static lot can be defined as a large mass of shelled maize contained either in a large single container such as a wagon, truck or railcar or in many small containers such as sacks or boxes and the maize is stationary at the time a sample is selected. Selecting a truly random sample from a static lot can be difficult because all containers in the lot or sublot may not be accessible.

6. Taking incremental samples from a static lot usually requires the use of probing devices to select product from the lot. The probing devices should be specifically designed for the commodity and type of container. The probe should (1) be long enough to reach all products, (2) not restrict any item in the lot from being selected, and (3) not alter the items in the lot. As mentioned above, the aggregate sample should be a composite from many small incremental samples of product taken from many different locations throughout the lot.

7. For lots traded in individual packages, the sampling frequency (SF), or number of packages that incremental samples are taken from, is a function of the lot weight (LT), incremental sample weight (IS), aggregate sample weight (AS) and the individual packing weight (IP), as follows:

   SF = (LT x IS) / (AS x IP)

8. The sampling frequency (SF) is the number of packages sampled. All weights should be in the same mass units such as kg.

Dynamic Lots

9. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of shelled maize as the lot is transferred from one location to another. When sampling from a moving stream, take small incremental samples of product from the entire length of the moving stream; composite the incremental samples to obtain an aggregate sample; if the aggregate sample is larger than the required laboratory sample(s), then blend and subdivide the aggregate sample to obtain the desired size laboratory sample(s).

10. Automatic sampling equipment such as a cross-cut sampler is commercially available with timers that automatically pass a diverter cup through the moving stream at predetermined and uniform intervals. When automatic sampling equipment is not available, a person can be assigned to manually pass a cup through the stream at periodic intervals to collect incremental samples. Whether using automatic or manual methods, incremental samples should be collected and composited at frequent and uniform intervals throughout the entire time the maize flow past the sampling point.

11. Cross-cut samplers should be installed in the following manner: (1) the plane of the opening of the diverter cup should be perpendicular to the direction of the flow; (2) the diverter cup should pass through the entire cross sectional area of the stream; and (3) the opening of the diverter cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the diverter cup opening should be about two to three times the largest dimensions of items in the lot.

12. The size of the aggregate sample (S) in kg, taken from a lot by a cross cut sampler is:

    $S = (D \times LT) / (T \times V)$,

    where D is the width of the diverter cup opening (cm), LT is the lot size (kg), T is interval or time between cup movement through the stream (seconds), and V is cup velocity (cm/sec).

13. If the mass flow rate of the moving stream, MR (kg/sec), is known, then the sampling frequency (SF), or number of cuts made by the automatic sampler cup can be computed as a function of S, V, D, and MR.

    $SF = (S \times V) / (D \times MR)$

Packaging and Transportation of Samples

14. Each laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination, sunlight, and against damage in transit. All necessary precautions shall be taken to avoid any change in composition of the laboratory sample, which might arise during transportation or storage. Samples should be stored in a cool dark place.

15. Each laboratory sample taken for official use shall be sealed at the place of sampling and identified. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

SAMPLE PREPARATION

16. Sunlight should be excluded as much as possible during sample preparation, since fumonisin may gradually break down under the influence of ultra-violet light. Also, environmental temperature and relative humidity should be controlled and not favour mould growth and fumonisin formation.

17. As the distribution of fumonisin is extremely non-homogeneous, laboratory samples should be homogenised by grinding the entire laboratory sample received by the laboratory. Homogenisation is a procedure that reduces particle size and disperses the contaminated particles evenly throughout the comminuted laboratory sample.

18. The laboratory sample should be finely ground and mixed thoroughly using a process that approaches as complete homogenisation as possible. Complete homogenisation implies that particle size is extremely small and the variability associated with sample preparation approaches zero. After grinding, the grinder should be cleaned to prevent fumonisin cross-contamination.

Test portion

19. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 25 g.

20. Procedures for selecting the test portion from the comminuted laboratory sample should be a random process. If mixing occurred during or after the comminuting process, the test portion can be selected from any location throughout the comminuted laboratory sample. Otherwise, the test portion should be the accumulation of several small portions selected throughout the laboratory sample.

21. It is suggested that three test portions be selected from each comminuted laboratory sample. The three test portions will be used for enforcement, appeal, and confirmation if needed.

ANALYTICAL METHODS

22. A criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate. The criteria-based approach has the advantage that, by avoiding setting down specific details of the method used, developments in methodology can be exploited without having to reconsider or modify the specific method. A list of possible criteria and performance levels are shown in Table 3 (EC Regulation No 401/2006). Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. Performance criteria for Fumonisin B1 and B2

Level (µg/kg)	Precision		Recovery (%)
	RSDr (%)	RSDR (%)	
≤ 500	≤ 30	≤ 60	60 to 120
> 500	≤ 20	≤ 30	70 to 110