CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Agenda Item 3 **CX/MAS 13/34/3**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Thirty-fourth Session
Budapest, Hungary, 4 - 8 March 2013

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

1. This document contains the methods of analysis and/or sampling proposed by the following Committees in draft standards and related texts under elaboration or as update of current methods:

PART I Methods of Analysis
- A. Committee on Fish and Fishery Products
- B. FAO/WHO Coordinating Committee for Asia
- C. Committee on Contaminants in Foods
- D. Committee on Processed Fruits and Vegetables

PART II Methods of Sampling
- A. Committee on Contaminants in Foods
- B. Committee on Fish and Fishery Products
- C. Committee on Processed Fruits and Vegetables

PART I METHODS OF ANALYSIS

A. COMMITTEE ON FISH AND FISHERY PRODUCTS (CCFFP)

1. See Table section A for the complete list of the proposed methods of analysis. Discussion at the Committee was as follows:

Standard for Smoked Fish, Smoke-Flavoured Fish and Smoke-Dried Fish[1]

2. The Committee agreed to advance the draft Standard to Step 8 for adoption by the 36th Session of the Commission and to return to Step 6 the additives for which further consideration was required as indicated above. The provisions on food additives, food labelling and methods of analysis and sampling will be sent to the relevant committees for endorsement.

Standard for Live Abalone and for Raw Fresh Chilled or Frozen Abalone for Direct Consumption or for Further Processing[2]

Part I- Live Abalone

I-8 Sampling, Examination and Analysis

3. The Committee agreed to refer to "sample unit" in this section and throughout the standard where relevant. The Committee made some changes to the text for clarification purposes, and agreed that the "sample unit shall be a minimum of 20 individual abalones" as there was no need to specify the weight of the sample and, taking into account a rate of defectives of 5%, this would correspond to rejection when two or more units were defective.

1 REP13/FFP para. 40 and Appendix III
2 REP13/FFP paras 73, 74, 80, 83 and Appendix IV

4. In section I-8.4 Determination of Biotoxins, it was agreed to refer to the methods specified in the Standard for Live and Raw Bivalve Molluscs in order to ensure consistency with the contaminants section, and the current text in square brackets and the Table were deleted. The proposed text in the Standard is as follows:

> Where a risk exists, the marine biotoxins of concern shall be determined according to the methods specified in the *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN 292-2008).

Part II-Raw, Fresh Chilled or Frozen Abalone

II-8.6 Determination of Biotoxins

5. The Committee agreed to use the same text as in section I-8.4 (See para. 5 of the document).

6. The Committee agreed to advance the draft Standard to Step 8 for adoption by the 36th Session of the Commission (Appendix IV). The provisions on food labelling and methods of analysis and sampling will be sent to the relevant committees for endorsement.

Standard for Live and Raw Bivalve Molluscs[3]

7. A delegation recalled that the development of methods for biotoxin determination was evolving and that the criteria approach had been followed to take this into account. The Delegation noted the criteria had been crafted in such a way to allow flexibility for the inclusion of biological methods such as the widely used mouse bioassay as well as multi-analogue HPLC methods. It was also noted that there was a need for the development of better and more accurate methods and that in future such methods could be listed in the Standard.

8. The Committee agreed to amend the paragraph immediately below the first table to ensure that international scientifically validated toxicity equivalent factors were used to calculate total toxicity for methods that do not measure total toxicity directly.

9. The last sentence in square brackets was deleted since it was difficult to have certified reference materials for each analyte. The requirement for certified reference materials would mean that some analogues in Table 2 would have to be deleted.

10. The Committee agreed to advance the proposed draft Section to the 36th Session of the Commission for adoption at Step 5 and to CCMAS for endorsement.

B. FAO/WHO COORDINATING COMMITTEE FOR ASIA (CCASIA)

11. See Table section B for the complete list of the proposed methods of analysis. Discussion at the Committee was as follows:

Regional Standard for Tempe[4]

12. The Coordinating Committee agreed that the method of analysis for lipid content should be AOAC 983.23 as it was more appropriate for the commodity. It was also agreed to propose the method for protein content (AOAC 955.04D) as type I.

13. The Coordinating Committee agreed to forward the proposed draft Regional Standard for Tempe to the 36th Commission for adoption at Step 5/8, with the recommendation to omit Steps 6 and 7 (Appendix II) and to forward the relevant sections to CCFA, CCMAS and CCFL for endorsement.

Regional Standard for Non-Fermented Soybean Products[5]

14. The Coordinating Committee agreed to remove the section on sampling as it did not contain any specific sampling plan.

15. The Coordinating Committee replaced AOAC 2001.11.F with AOAC 955.04D for the method of analysis for determination of protein content as it was more appropriate.

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3 REP13/FFP paras 95 – 99, Appendix VII
4 REP13/ASIA paras 115, 117 and Appendix II
5 REP13/ASIA paras 105, 106, 109 and Appendix III

16. The Coordinating Committee agreed to forward the proposed draft regional Standard to the Commission for adoption at Step 5 (Appendix III) and to forward the relevant sections to CCMAS and CCFL for endorsement.

C. COMMITTEE ON PROCESSED FRUITS AND VEGETABLES (CCPFV)

17. See Table section C for the complete list of the proposed methods of analysis. Discussion at the Committee was as follows:

Standard for Canned Applesauce[6]

18. The Committee noted that revised Codex standards for processed fruits and vegetables listed and/or displayed the relevant methods of analysis and sampling in the corresponding section of the standards in view of the discontinuation of the publication of Volume 13 on methods of analysis and sampling.

19. In this regard, the Committee had noted that there were no provisions for methods of analysis for canned applesauce and, in order to keep consistency with the approach taken on methods of analysis and sampling in Codex standards for processed fruits and vegetables, it had agreed to request comments on relevant methods of analysis for inclusion in the Standard for Canned Applesauce (CODEX STAN 17-1981).

20. The Committee noted that comments submitted in reply to CL 2010/52-PFV indicated that Codex's general methods for processed fruits and vegetables for soluble solids and minimum fill were relevant to canned applesauce and should therefore be included in the Standard.

21. The Committee agreed to include methods of analysis for soluble solids and minimum fill in the Standard for Canned Applesauce and to forward this editorial amendment to the 36th Session of the Codex Alimentarius Commission for adoption.

Standard for Table Olives[7]

22. The method for acidity of brine was deleted, as there was no provision for acidity of brine in the Standard.

23. The Committee agreed to forward the proposed draft Standard for Table Olives (Revision of CODEX STAN 66-1981) to Step 5/8 with omission of Steps 6 and 7 for adoption by the 36th Session of the Commission.

PART II METHODS OF SAMPLING

A. COMMITTEE ON CONTAMINANTS IN FOODS

Draft Maximum Level for Total Aflatoxins in Dried Figs including Sampling Plans[8]

24. The Committee agreed to forward the proposed draft ML of 10 µg/kg and associated revised sampling plan to the 35th Session of the Commission for adoption at Step 5/8 and they were adopted as proposed (see Annex I for the sampling plan).

B. COMMITTEE ON FISH AND FISHERY PRODUCTS (CCFFP)

Standard for Smoked Fish, Smoke-Flavoured Fish and Smoke-Dried Fish

25. See Section A of Part I for the background.

26. The proposed sampling plan is as follows:

> 8.1 Sampling
>
> Sampling of lots for examination of the product shall be in accordance with the *General Guidelines on Sampling* (CAC/GL 50-2004).
>
> A sample unit is the individually packed product or a 1 kg portion from bulk containers.
>
> The number of samples to be taken for the determination of the levels of histamine in a lot shall be determined by the Competent Authority having jurisdiction.

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6 REP13/PFV paras 125 - 128 and Appendix VII
7 REP13/PFV paras 37, 38 and Appendix II
8 REP12/CF paras 79 – 82 and Appendix VI

Standard for Live Abalone and for Raw Fresh Chilled or Frozen Abalone for Direct Consumption or for Further Processing

27. See Section A of Part I for the background.

28. The proposed sampling plan is as follows:

PART I – LIVE ABALONE

I-8.1 Sampling

(i) Sampling of lots for examination of the product shall be in accordance with the *General Guidelines on Sampling* (CAC/GL 50-2004).

(ii) The sample shall include a sufficient number of sample units selected throughout the lot to ensure that the sample is representative of the lot. The sample unit shall be a minimum of 20 individual abalones.

(iii) The portion of the abalone to be analysed shall be the part to be consumed.

PART II – RAW FRESH CHILLED OR FROZEN ABALONE

II-8.1 Sampling

Refer to I-8.1

C. COMMITTEE ON PROCESSED FRUITS AND VEGETABLES (CCPFV)

Standard for Table Olives

29. See Section C of Part I for the background and Annex II for the proposed sampling plan.

A. COMMITTEE ON FISH AND FISHERY PRODUCTS

Standard for Smoked Fish, Smoke-Flavoured Fish and Smoke-Dried Fish

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Smoked Fish, Smoke-Flavoured fish and Smoke-dried fish	Water phase salt	AOAC 952.08 AOAC 937.09 Described in standard[9]	 Calculation	
Smoked Fish, Smoke-Flavoured fish and Smoke-dried fish	Water activity	Described in standard[10]		
Smoked Fish, Smoke-Flavoured fish and Smoke-dried fish	histamine	AOAC 977.13 or other scientifically equivalent validated method		

Standard for Live Abalone and for Raw Fresh Chilled or Frozen Abalone for Direct Consumption or for Further Processing

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Live abalone	biotoxins	Described in standard[11]		
Raw fresh chilled or frozen abalone	biotoxins	Described in standard[12]		
frozen abalone (covered by glaze)	Net weight	AOAC 963.18		

[9] % salt x 100 / (%water + %salt)

[10] Water activity measurement is performed with a water activity meter that is properly calibrated with reference standards, and operated and maintained in accordance with the manufacturer's instructions.

[11] Where a risk exists, the marine biotoxins of concern shall be determined according to the methods specified in the *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN 292-2008).

[12] Where a risk exists, the marine biotoxins of concern shall be determined according to the methods specified in the *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN 292-2008).

Standard for Live and Raw Bivalve Molluscs

Determination of Biotoxins

Type II and Type III methods shall be selected in accordance with the "General Criteria for the Selection of Methods of Analysis" and "General Criteria for the Selection of Single-Laboratory Validated Methods of Analysis" in the *Codex Procedural Manual.*

The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use.

Methods shall meet the numerical criteria listed in Table 1 and may either meet the minimum applicable range, or LOD and[13] LOQ criteria listed.

Multi-analogue method total toxicity criteria are estimated for toxin profiles encountered using validation study data.

I-8.6.1 Numerical Criteria Values for Biotoxins in Bivalve Molluscs

Table 1

Group	**Toxin**	**Maximum level /kg of mollusc flesh**	**Minimum applicable range**	**LOD**	**LOQ**	**Precision (RSD_R)**	**Recovery percent**
Saxitoxin (STX) group	Total Toxicity	≤ 0.8 milligrams (2HCL) of saxitoxin equivalent	0.4 – 1.2	0.08	0.16	33%	70 – 120
Okadaic acid (OA) group	Total Toxicity	≤ 0.16 milligrams of okadaic equivalent	0.05 – 0.27	0.016	0.032	44%	70 - 120
Domoic acid (DA) group	Domoic Acid (DA)	≤ 20 milligrams domoic acid	13.2 – 26.8	2	4	22%	85 - 110
Brevetoxin (BTX) group	Total Toxicity	≤ 200 Mouse Units or (0.8 milligrams BTX2 equivalent)	74 – 326 MU (0.26 – 1.34 mg BTX2 eq.)	20 (0.08)	40 (0.16)	44%	70 - 120
Azaspiracid (AZA) group	Total Toxicity	≤ 0.16 milligrams AZA1 equivalent	0.05 – 0.27	0.016	0.032	44%	70 - 120

Internationally scientifically validated toxicity equivalent factors (TEFs) must be used to calculate total toxicity for methods that do not measure total toxicity directly.

[13] It might be replaced by "or", depending on the discussion under Agenda Item 2 (See CX/MAS 13/34/2 para. 9).

Methods that do not measure total toxicity directly should be validated and used for the relevant toxin analogues that may contribute to total toxicity. Currently known toxin analogues to consider are listed in Table 2.

Table 2. Toxin analogues to consider

Group	**Toxin**
Saxitoxin (STX) group	Saxitoxin (STX)
	Neosaxitoxin (NEO)
	Decarbamoyl-saxitoxin (dcSTX)
	Decarbamoyl-neosaxitoxin (dcNEO)
	Gonyautoxin-1 (GTX1)
	Gonyautoxin-2 (GTX2)
	Gonyautoxin-3 (GTX3)
	Gonyautoxin-4 (GTX4)
	Gonyautoxin-5 (B1)
	Gonyautoxin-6 (B2)
	Decarbamoyl-gonyautoxin-2 (dcGTX2)
	Decarbamoyl-gonyautoxin-3 (dcGTX3)
	N-sulfocarbamoyl-gonyautoxin-1 (C3)
	N-sulfocarbamoyl-gonyautoxin-2 (C1)
	N-sulfocarbamoyl-gonyautoxin-3 (C2)
	N-sulfocarbamoyl-gonyautoxin-4 (C4)
Okadaic acid (OA) group	Okadaic acid (OA)
	Dinophysistoxin-1 (DTX1)
	Dinophysistoxin-2 (DTX2)
	Esters of OA, DTX1 and DTX2 (FA-ESTERS)
Domoic acid (DA) group	Domoic Acid (DA)
Brevetoxin (BTX) group	Brevetoxin-1 (BTX1)
	Brevetoxin-2 (BTX2)
	Brevetoxin-1 derivatives (devBTX1)
	Brevetoxin-2 derivatives (devBTX2)
Azaspiracid (AZA) group	Azaspiracid-1 (AZA1)
	Azaspiracid-2 (AZA2)
	Azaspiracid-3 (AZA3)

B. FAO/WHO COORDINATING COMMITTEE FOR ASIA

Regional Standard for Tempe

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Tempe	Moisture content	AOAC 925.09	Gravimetry (vacuum oven)	**type I**
Tempe	Protein content	AOAC 955.04D (Nitrogen factor 5.71)	Titrimetry, Kjeldahl digestion	**type I**
Tempe	Lipid Content	AOAC 983.23	Gravimetry (Roese-Gottlieb)	**type I**
Tempe	Crude fibre	ISO 5498:1981	Ceramic fibre filteration	**type I**

Regional Standard for Non-Fermented Soybean Products

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Non-fermented soybean products	Moisture content	AOAC 925.09	Gravimetry (vacuum oven)	**type I**
Non-fermented soybean products	Protein content	AOAC 955.04D (Nitrogen factor 5.71)	Titrimetry, Kjeldahl digestion	**type I**

C. COMMITTEE ON PROCESSED FRUITS AND VEGETABLES

Standard for Canned Apple Sauce

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Canned Apple Sauce	Fill of containers	CAC/RM 46-1972* (for glass containers) (Codex general method for processed fruits and vegetables) and ISO 90.1:1999 (for metal containers) (Codex general method for processed fruits and vegetables)	Weighing	**Type I**
Canned Apple Sauce	Soluble solids	AOAC 932.12 ISO 2173:2003 (Codex general method for processed fruits and vegetables)	Refractometry	**Type I**

Standard for Table Olives

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Table olives	Drained weight	AOAC 968.30 (Codex general method for processed fruits and vegetables)	Sieving Gravimetry	**Type I**
Table olives	Fill of containers	CAC/RM 46-1972* (for glass containers) (Codex general method for processed fruits and vegetables) and ISO 90.1:1999 (for metal containers) (Codex general method for processed fruits and vegetables)	Weighing	**Type I**
Table olives	pH of brine	NMKL 179:2005 (Codex general method for processed fruits and vegetables)	Potentiometry	**type II**
Table olives		AOAC 981.12 (Codex general method for processed fruits and vegetables)		**Type III**
Table olives		ISO 1852:1991		**Type IV**
Table olives	Salt in brine	AOAC 971.27 (Codex general method)	Potentiometry	**Type II**
Table olives		ISO 3634:1979 "chloride expressed as sodium chloride" (Codex general method for processed fruits and vegetables)		**Type III**
Table olives	Lead	AOAC 972.25 (Codex general method)	AAS (Flame absorption)	**Type III**
Table olives	Tin	AOAC 980.19 (Codex general method)	AAS	**Type II**

*** 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.

Annex I

SAMPLING PLAN FOR AFLATOXIN CONTAMINATION IN DRIED FIGS

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 an aflatoxin test procedure and an accept/reject level. An aflatoxin test procedure consists of three steps: sample selection of sample(s) of a given size, sample preparation and aflatoxin quantification. The accept/reject level is a tolerance usually equal to the Codex maximum level.

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 dried figs 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 aflatoxin for chemical analysis.

Ready-to-eat dried figs – dried figs, which are not intended to undergo an additional processing/treatment that have proven to reduce levels of aflatoxin.

Operating Characteristic (OC) Curve – a plot of the probability of accepting a lot versus lot concentration when using a specific sampling plan design. The OC curve also provides an estimate of good lots rejected (exporter's risk) and bad lots accepted (importer's risk) by a specific aflatoxin sampling plan design.

SAMPLING PLAN DESIGN CONSIDERATIONS

1. Importers commercially classify dried figs mostly as "ready-to-eat" (RTE). As a result, maximum levels and sampling plans are proposed for only ready-to-eat dried figs.

2. The performance of the proposed draft sampling plan was computed using the variability and aflatoxin distribution among laboratory samples of dried figs taken from contaminated lots. Because the dried fig count per kg is different for different varieties of dried figs, the laboratory sample size is expressed in number of dried figs for statistical purposes. However, the dried fig count per kg for each variety of dried figs can be used to convert laboratory sample size from number of dried figs to mass and vice versa.

3. Uncertainty estimates (variances) associated with sampling, sample preparation, and analysis and the negative binomial distribution[14] are used to calculate operating characteristic (OC) curves that describe the performance of the proposed aflatoxin-sampling plans for dried figs.

4. The analytical variance measured in the sampling study reflects within laboratory variance and was replaced with an estimate of analytical variance that reflects a reproducibility relative standard deviation of 22%, which is suggested by Thompson and is based upon Food Analysis Performance Assessment Scheme (FAPAS) data[15]. A relative standard deviation of 22% is considered by FAPAS as an appropriate measure of the best agreement that can be reliably obtained between laboratories. An analytical uncertainty of 22% is larger than the within laboratory variation measured in the sampling studies for dried figs.

5. The issue of correcting the analytical test result for recovery is not addressed in this document. However, Table 2 specifies several performance criteria for analytical methods including suggestions for the range of acceptable recovery rates.

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[14] Whitaker, T., Dickens, J., Monroe, R., and Wiser, E. 1972. Comparison of the negative binomial distribution of aflatoxin in shelled peanuts to the negative binomial distribution. J. American Oil Chemists' Society, 49:590-593.

[15] Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. J. Royal Society of Chemistry, 125:385-386.

AFLATOXIN TEST PROCEDURE AND MAXIMUM LEVELS

6. An aflatoxin-sampling plan is defined by an aflatoxin test procedure and a maximum level. A value for the proposed maximum level and the aflatoxin test procedure are given below in this section.

7. The maximum level for "ready-to-eat" dried figs is 10 µg/kg total aflatoxins.

8. Choice of the number and size of the laboratory sample is a compromise between minimizing risks (false positives and false negatives) and costs related to sampling and restricting trade. For simplicity, it is recommended that the proposed aflatoxin sampling plan uses three 10 kg aggregate samples of dried figs.

9. The RTE sampling plan has been designed for enforcement and controls concerning total aflatoxins in bulk consignments (lots) of dried figs traded in the export market.

Maximum level – 10 µg/kg total aflatoxins

Number of laboratory samples – 3

Laboratory sample size - 10 kg

Sample preparation – water-slurry grind and a test portion that represents 55 g mass of dried figs

Analytical method – performance based (see Table 2)

Decision rule – If the aflatoxin test result is less than or equal to 10 µg/kg total aflatoxins for all three 10 kg laboratory samples, then accept the lot. Otherwise, reject the lot.

The operating characteristic curve describing the performance of the sampling plan for the ready-to-eat dried figs is shown in paragraph 46 at the end of this Annex.

10. To assist member countries implement the above Codex sampling plan, sample selection methods, sample preparation methods, and analytical methods required to quantify aflatoxin in laboratory samples taken from bulk dried fig lots are described in the following sections.

SAMPLE SELECTION

Material to be sampled

11. Each lot, which is to be examined for aflatoxin, must be sampled separately. Lots larger than 15 tonnes should be subdivided into sublots to be sampled separately. If a lot is greater than 15 tonnes, the number of sublots is equal to the lot weight in tonnes divided by 15 tonnes. It is recommended that a lot or a sublot should not exceed 15 tonnes.

12. Taking into account that the weight of the lot is not always an exact multiple of 15 tonnes, the weight of the sublot may exceed the mentioned weight by a maximum of 25%.

13. Samples should be taken from the same lot, i.e. they should have the same batch code or at the very least the same best before date. Any changes which would affect the mycotoxin content, the analytical determination or make the aggregate samples collected unrepresentative should be avoided. For example do not open packaging in adverse weather conditions or expose samples to excessive moisture or sunlight. Avoid cross-contamination from other potentially contaminated consignments nearby.

14. In most cases any truck or container will have to be unloaded to allow representative sampling to be carried out.

Incremental Sample Selection

15. Procedures used to take incremental samples from a dried fig lot are extremely important. Every individual fig in the lot should have an equal chance of being chosen. Biases will be introduced by sample selection methods if equipment and procedures used to select the incremental samples prohibit or reduce the chances of any item in the lot from being chosen.

16. Since there is no way to know if the contaminated figs are uniformly dispersed throughout the lot, it is essential that the aggregate sample be the accumulation of many small incremental samples of product selected from different locations throughout the lot. If the aggregate sample is larger than desired, it should be blended and subdivided until the desired laboratory sample size is achieved.

17. For lots less than 10 tonnes, the size of the aggregate sample is reduced so that the aggregate sample size doesn't exceed a significant portion of the lot or sublot size.

Number and Size of Incremental Samples for Lots of varying weight

18. The number of incremental samples to be taken from a lot (sublot) depends on the weight of the lot. Table 1 shall be used to determine the number of incremental samples to be taken from lots or sublots of various sizes. The number of incremental samples varies from 10 to 100 for lots or sublots of various sizes.

Table 1.Number and size of incremental samples composited for an aggregate sample of 30 kg[a] as a function of lot (or sublot) weight.

Lot or Sublot Weight [b] (T in Tonnes)	Minimum Number of Incremental Samples	Minimum Incremental Sample Size [c] (g)	Minimum Aggregate Sample Size (kg)	Laboratory Sample Size (kg)	Number of Laboratory Samples
15.0 ≥ T > 10.0	100	300	30	10	3
10.0 ≥ T > 5.0	80	300	24	8	3
5.0 ≥ T > 2.0	60	300	18	9	2
2.0 ≥ T > 1.0	40	300	12	6	2
1.0 ≥ T > 0.5	30	300	9	9	1
0.5 ≥ T > 0.2	20	300	6	6	1
0.2 ≥ T > 0.1	15	300	4.5	4.5	1
0.1 ≥ T	10	300	3	3	1

a/ Minimum aggregate sample size = laboratory sample size of 30 kg for lots above 10 tonnes

b/ 1 Tonne = 1000 kg

c/ Minimum incremental sample size = laboratory sample size (30 kg)/minimum number of incremental samples, i.e. for $10 < T \leq 15$ tonne, 300 g = 30000 g/100

19. The suggested minimum weight of the incremental sample is 300 grams for lots and sublots of various sizes.

Static Lots

20. A static lot can be defined as a large mass of dried figs 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 dried figs are 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.

21. 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.

22. 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:

Equation 1:SF=(LT x IS)/(AS x IP).

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

Dynamic Lots

24. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of dried figs 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).

25. 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 figs flow past the sampling point.

26. 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.

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

Equation 2: $S = (D \times LT) / (T \times V)$,

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).

28. 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 from Equation 3 as a function of S, V, D, and MR.

Equation 3: $SF = (S \times V) / (D \times MR)$.

29. Equations 2 and 3 can also be used to compute other terms of interest such as the time between cuts (T). For example, the time (T) required between cuts of the diverter cup to obtain a 30 kg aggregate sample from a 20,000 kg lot where the diverter cup width is 5.0 cm and the cup velocity through the stream 20 cm/sec. Solving for T in Equation 2,

$T = (5.0 \text{ cm} \times 20{,}000 \text{ kg})/(30 \text{ kg} \times 20 \text{ cm/sec}) = 167 \text{ sec}$.

30. If the lot is moving at 500 kg per minute, the entire lot will pass through the sampler in 40 minutes (2400 sec) and only 14.4 cuts (14 incremental samples) will be made by the cup through the lot (Equation 3). This may be considered too infrequent, in that too much product (1,388.9 kg) passes through the sampler between the time the cup cuts through the stream.

Packaging and Transportation of Samples

31. 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.

Sealing and Labelling of Samples

32. 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

Precautions

33. Sunlight should be excluded as much as possible during sample preparation, since aflatoxin gradually breaks down under the influence of ultra-violet light. Also, environmental temperature and relative humidity should be controlled and not favor mold growth and aflatoxin formation.

Homogenization - Grinding

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

35. The laboratory sample should be finely ground and mixed thoroughly using a process that approaches as complete homogenization as possible. Complete homogenization 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 aflatoxin cross-contamination.

36. The use of vertical cutter mixer type grinders that mix and comminute the laboratory sample into a paste represent a compromise in terms of cost and fineness of grind or particle size reduction[16]. A better homogenization (finer grind), such as a liquid slurry, can be obtained by more sophisticated equipment and should provide the lowest sample preparation variance[17].

Test portion

37. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 50 grams. If the laboratory sample is prepared using a liquid slurry, the slurry should contain 50 g of fig mass.

38. Procedures for selecting the 50 g test portion from the comminuted laboratory sample should be a random process. If mixing occurred during or after the comminution process, the 50 g test portion can be selected from any location throughout the comminuted laboratory sample. Otherwise, the 50 g test portion should be the accumulation of several small portions selected throughout the laboratory sample.

39. 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

Background

40. 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 analytical method. The performance criteria established for analytical methods should include all the parameters that need to be addressed by each laboratory such as the detection limit, repeatability coefficient of variation (within lab), reproducibility coefficient of variation (among lab), and the percent recovery necessary for various statutory limits. Analytical methods that are accepted by chemists internationally (such as AOAC) may be used. These methods are regularly monitored and improved depending upon technology.

Performance Criteria for Methods of Analysis

41. A list of criteria and performance levels are shown in Table 2. Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 2: Specific Requirements with which Methods of Analysis Should Comply

Criterion	Concentration Range (ng/g)	Recommended Value	Maximum Permitted Value
Blanks	All	Negligible	n/a
Recovery	1 to 15	70 to 110%	n/a
	>15	80 to 110%	n/a
Precision or Relative Standard Deviation RSD_R (Reproducibility)	1 to 120	Equation 4 by Thompson	2 x value derived from Equation 4
	>20	Equation 5 by Horwitz	2 x value derived from Equation 5
Precision or Relative Standard Deviation RSD_r (Repeatability)	1 to 120	Calculated as 0.66 times Precision RSD_R	n/a
	>120	Calculated as 0.66 times Precision RSD_r	n/a

n/a = not applicable

[16] Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T., Slate, A., and Giesbrecht, F. 2006. Sampling hazelnuts for aflatoxin: Uncertainty associated with sampling, sample preparation, and analysis. J. Association Official Analytical Chemists, Int., 89:1004-1011.

[17] Spanjer, M., Scholten, J., Kastrup, S., Jorissen, U., Schatzki, T., Toyofuku, N. 2006. Sample comminution for mycotoxin analysis: Dry milling or slurry mixing?, Food Additives and Contaminants, 23:73-83.

42. The detection limits of the methods used are not stated. Only the precision values are given at the concentrations of interest. The precision values (expressed as a %) are calculated from equations 4 and 5 developed by Thompson[2] and Horwitz and Albert[18], respectively.

Equation 4: $RSD_R = 22.0$

Equation 5: $RSD_R = 45.25C^{-0.15}$

where:

- RSD_R = the relative standard deviation calculated from results generated
- under reproducibility conditions
- RSD_r = the relative standard deviation calculated from results generated under repeatability conditions = $0.66RSD_R$
- C = aflatoxin concentration or mass of aflatoxin to mass of dried figs (i.e. ng/g)

43. Equations 4 and 5 are generalized precision equations, which have been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

44. Results should be reported on the sample.

UNCERTAINTY, AS MEASURED BY THE VARIANCE, ASSOCIATED WITH THE SAMPLING, SAMPLE PREPARATION, AND ANALYTICAL STEPS OF THE AFLATOXIN TEST PROCEDURE USED TO DETECT AFLATOXIN IN DRIED FIGS

45. The sampling, sample preparation, and analytical variances associated with the aflatoxin test procedure for dried figs are shown in Table 3.

Table 3.Variances[a] associated with the aflatoxin test procedure for each dried figs

Test Procedure	Variances for Dried Figs
Sampling[b,c]	$S^2_s = (590/ns)2.219C^{1.433}$
Sample Prep[d]	$S^2_{sp} = (55/nss)0.01170C^{1.465}$
Analytical[e]	$S^2_a = (1/na)0.0484C^{2.0}$
Total	$S^2_t = S^2_s + S^2_{sp} + S^2_a$

a/ Variance = S^2 (t, s, sp, and a denote total, sampling, sample preparation, and analytical steps, respectively, of aflatoxin test procedure)

b/ ns = laboratory sample size in number of dried figs, nss =test portion size in grams of fig mass, na = number of aliquots quantified by HPLC, and C = aflatoxin concentration in ng/g total aflatoxins.

c/ Count/kg for dried figs averaged 59/kg.

d/ Sample preparation variance reflects a water-slurry method and a test portion that reflects 55 g fig mass.

e/ Analytical variances reflect FAPAS recommendation for upper limit of analytical reproducibility uncertainty. A relative standard deviation of 22% is considered by Thompson[2] (based upon FAPAS data) as an appropriate measure of the best agreement that can be obtained between laboratories. An analytical uncertainty of 22% is larger than the within laboratory uncertainty measured in the sampling studies for the three dried figs.

[18] Horwitz, W. and Albert, R. 2006. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J. Association of Official Analytical Chemists, Int., 89:1095-1109.

OPERATING CHARACTERISTIC CURVE DESCRIBING THE PERFORMANCE OF THE DRAFT AFLATOXIN SAMPLING PLAN FOR READY-TO-EAT DRIED FIGS

46. The operating characteristic curve describing the performance of draft aflatoxin sampling plan for ready-to-eat dried figs is shown in Figure 1.

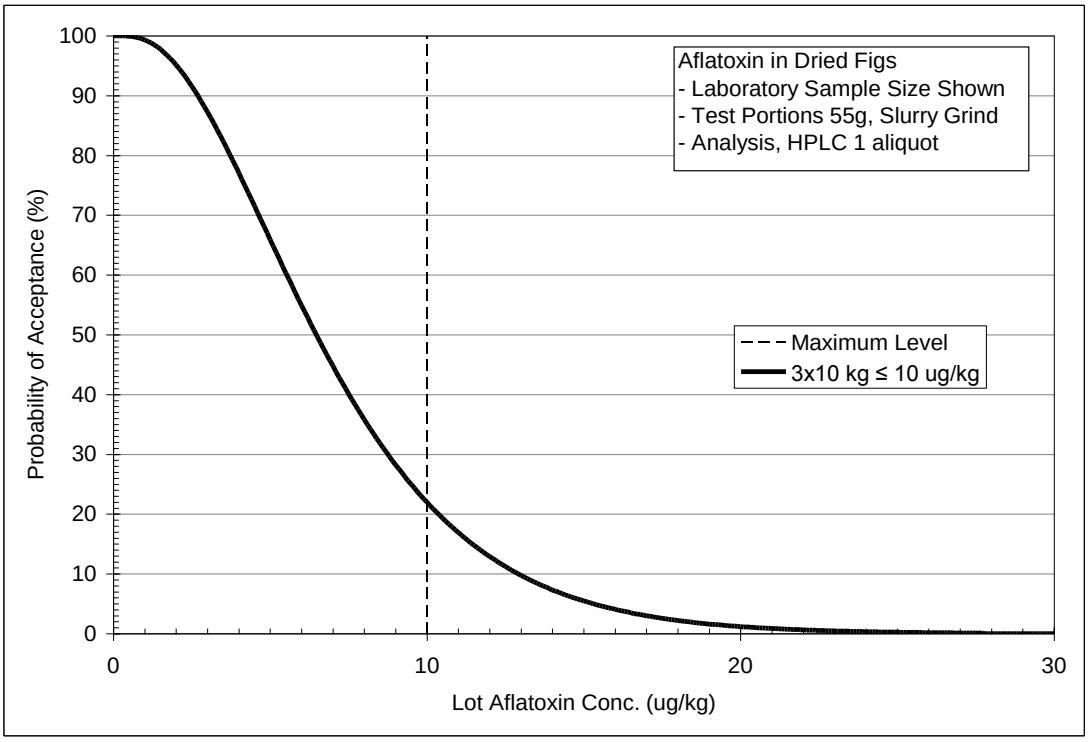

Figure 1. Operating characteristic (OC) curve describing the performance of the aflatoxin sampling plan for ready-to-eat dried figs using three laboratory samples of 10 kg each and a maximum level of 10 µg/kg total aflatoxins, water-slurry comminution method, test portion that reflects 55 g fig mass, and quantification of aflatoxin in a the test portion by HPLC.

Annex II

SAMPLING PLAN FOR TABLE OLIVES

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)		
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