SAMPLING PLAN FOR AFLATOXIN CONTAMINATION IN DRIED FIGS

DEFINITIONS

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	It 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 before being used as an ingredient in foodstuffs, otherwise processed or offered for human consumption.
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 established only for ready-to-eat dried figs.

2. The performance of the 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 are used to calculate operating characteristic (OC) curves that describe the performance of the 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 reflects a reproducibility relative standard deviation of 22%, which is based upon Food Analysis Performance Assessment Scheme (FAPAS) data. 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.

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 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 ng/g 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 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.

10. To assist member countries implement the above 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   = 1 000 kg

c/ Minimum incremental sample size   = laboratory sample size (30 kg)/minimum number of incremental samples,
i.e. for 10 < T ≤ 15 tonnes, 300 g = 30 000/100

19. The suggested minimum weight of the incremental sample is 300 g 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: $SF = (LT \times IS) / (AS \times IP)$

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 (2 400 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 favour mould growth and aflatoxin formation.

HOMOGENISATION - 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. 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.

TEST PORTION

37. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 50 g. 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 with

Criterion	Concentration range (ng/g)	Recommended value	Maximum permitted value
Blanks	All	Negligible	n/a
Recovery	1 to 15	70 to 100%	n/a
	> 15	80 to 110%	n/a
Precision or relative standard deviation RSD_R (Reproducibility)	1 to 120	Equation 4	2 x value derived from Equation 4
	> 120	Equation 5	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

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.

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 dried figs

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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	$= S^2_s + S^2_{sp} + S^2_a$

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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 based upon FAPAS data and considered 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.