CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **CX/MAS 16/37/3**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Thirty-seventh Session

Budapest, Hungary, 22 – 26 February 2016

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

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

- Committee on Contaminants in Foods (sampling plans for fumonisins and deoxynivalenol);
- Committee on Spices and Culinary Herbs (methods of anaylsys for Cumin and Dried Thyme);
- Committee on Fish and Fishery Products (Amendments to methods of analysis for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets – Breaded or in Batter);
- Committee on Nutrition and Foods for Special Dietary Uses (methods of analysis for Infant Formula and Fomulas for Special Medical Purposes Intended for Infants).

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF9)

NOTE: The 37th Session of CAC adopted the MLs at Step 8 subject to endorsement by CCMAS[1].

2. The Committee **is invited to endorse** the proposed sampling plans and performance criteria for method of analysis in Appendix I.

Sampling Plans for Fumonisins in Maize Grain, Maize Flour and Maize Meal[2]

3. The sampling plans had been revised to remove inconsistencies as requested by CCMAS. The performance criteria had been adjusted in accordance with the "Guidelines for establishing numeric values for criteria."

Sampling Plans for Deoxynivalenol (don) in cereal-based foods for infants and young children; in flour, meal, semolina and flakes derived from wheat, maize or barley; and in raw cereal grains (wheat, maize and barley) including sampling plans for raw cereal grains[3]

4. The Committee noted its earlier discussion to have the same sampling plans for all cereals. Therefore, in view of the agreement on the sampling plan for fumonisins, the Committee agreed to align the sampling plan for DON in cereal grains with that for fumonisins. The Committee noted that with the amendments to the sampling plan, i.e. deletion of the aggregate sample, the request for clarification from CCMAS was no longer applicable. The sampling plan was also extended to cereal-based foods for infants and young children and to flour, semolina, meal and flakes derived from wheat, maize or barley.

[1] REP15/CAC, para 36
[2] REP15/CF, para 13, Appendix III
[3] REP15/CF, para 91, Appendix VI

COMMITTEE ON SPICES AND CULINARY HERBS (CCSCH2)

NOTE: The Committee agreed to forward the proposed draft Standards including methods of analysis to the Commission for adoption at Step 5.

Method of Analysis for Cumin[4]

Method of Analysis for Dried Thyme[5]

5. The Committee **is invited to endorse** the proposed sampling plans and performance criteria for method of analysis in Appendix II.

COMMITTEE ON FISH AND FISHERY PRODUCTS (CCFFP34)

Amendments to Section 7.4 of the Standard for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets – Breaded or in Batter[6]

6. CCFFP34 amended Section 7.4 Estimation of Fish Content and with regard to the Chemical Analysis Method (Nitrogen Factor End-product Method) – recognized the importance of the method for verification of the fish content declared on the label and amended it to indicate that it did not require confirmation when used for fully cooked products because AOAC 996.15 (End Product Method) was less precise with these products.

7. The Committee **is invited to endorse** the amended Section 7.4 (CODEX STAN 166-1989) in Appendix III which CCFFP34 agreed to forward for adoption by the CAC39.

COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES (CCNFSDU37)

Methods of analysis in the Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981)[7]

8. The Committee agreed to submit the eight methods for nutrients in infant formula (vitamin B12, myo-inositol, chromium, selenium, molybdenum, nucleotides, vitamins A and E, fatty acid profile, iodine and pantothenic acid) as presented in CX/NFSDU 15/37/10 (Rev) to CCMAS for technical review, typing, endorsement and inclusion in the *Recommended Methods of Analysis and Sampling* (CODEX STAN 234-1999) as these methods reflected the most recent scientific methods of analysis for nutrients in infant formula and were fully validated for these products (Appendix V, Part I).

9. In response to concerns with regard to the typing of some methods, and the inclusion of extremely costly methods, (i.e. those based on inductively coupled plasma-mass spectrometry) as opposed to less expensive atomic absorption spectrometry methods, it was clarified that the methods were for purposes of dispute settlement and that for routine analysis, other methods were available and could be used. It was suggested that the proposed new methods based on the principle ICP-MS were considered as type III, given that some countries may not be able to use these methods in cases of dispute settlement. CCMAS would also be able to further consider the correct typing of the methods.

10. The Committee **is invited to consider/endorse** the methods of analysis in Appendix IV. CCNFSDU37 requested to replace the corresponding methods in the CODEX STAN 234-1999.

[4] REP15/SCH, para 24, Appendix III
[5] REP15/SCH, para 35, Appendix VI
[6] REP16/FFP, paras 57-63, Appendix VII
[7] REP16/NFSDU, paras 96-97, Appendix V

APPENDIX I

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF)

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

Maize grain, unprocessed

Maximum level	4 000 µg/kg FB1 + FB2
Increments	increments of 100 g, depending on the lot weight (≥ 0.5 tonnes)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm - 20 mesh)
Laboratory sample weight	≥ 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
Sample preparation	None
Laboratory sample weight	≥ 1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the fumonisin-sample test result is equal or less than 2000 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 in such a way to ensure that the laboratory sample is still representative of the sublot sampled.

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.

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 (t)	Maximum Weight or minimum number of sub lots	Number of incremental sample	Minimum laboratory Sample Weight (kg)
≥ 1500	500 tonnes	100	1
> 300 and < 1500	3 sublots	100	1
≥ 100 and ≤ 300	100 tonnes	100	1
≥ 50 and < 100	2 sublots	100	1
< 50	-	3-100*	1

* 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 100 grams for lots ≥0.5 tonnes.

4. For lots less than 50 tonnes, the sampling plan must be used with 3 to 100 incremental samples, depending on the lot weight. 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 (t)	Number of incremental sample	Minimum Laboratory Sample Weight (kg)
≤ 0.05	3	1
> 0.05 - ≤ 0.5	5	1
> 0.5 - ≤ 1	10	1
> 1 - ≤ 3	20	1
> 3 - ≤ 10	40	1
> 10 - ≤ 20	60	1
> 20 - < 50	100	1

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 x LT) / (T x 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 x V) / (D x 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 favor mold 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). Utilising this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. Performance criteria for Fumonisin B1+ B2.

Maize Grain

Analyte	**ML** (mg/Kg)	**LOD** (mg/Kg)	**LOQ** (mg/Kg)	**RSD_R**	**Recovery (%)**
FB1 + FB2	4.0	-	-	-	-
FB1		≤ 0.3*	≤ 0.6*	HorRat ≤ 2 (< 27%)	80 - 110
FB2		≤ 0.15*	≤ 0.3*	HorRat ≤ 2 (< 32%)	80 - 110

* - The LOD and LOQ were derived based upon typical B1:B2 ratio of 5:2 in naturally-contaminated samples

Maize Flour/Meal

Analyte	**ML** (mg/Kg)	**LOD** (mg/Kg)	**LOQ** (mg/Kg)	**RSD_R**	**Recovery (%)**
FB1 + FB2	2.0	-	-	-	-
FB1		≤ 0.15*	≤ 0.3*	HorRat ≤ 2 (< 30%)	80 – 110
FB2		≤ 0.06*	≤ 0.15*	HorRat ≤ 2 (< 34%)	80 – 110

* - The LOD and LOQ were derived based upon typical B1:B2 ratio of 5:2 in naturally-contaminated samples

**SAMPLING PLANS FOR DEOXYNIVALENOL (DON) IN CEREAL-BASED FOODS FOR INFANTS AND YOUNG CHILDREN;
IN FLOUR, MEAL, SEMOLINA AND FLAKES DERIVED FROM WHEAT, MAIZE OR BARLEY;
AND IN CEREAL GRAINS (WHEAT, MAIZE AND BARLEY) DESTINED FOR FURTHER PROCESSING**

Cereal grains (wheat, cereal and barley) destined for further processing

Maximum level	2000 µg/kg DON
Increments	increments of 100 g, depending on the lot weight (≥ 0.5 tonnes)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm - 20 mesh)
Laboratory sample weight	≥ 1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the DON-sample test result for the laboratory samples is equal or less than 2000 µg/kg, accept the lot. Otherwise, reject the lot.

Cereal-based foods for infants and young children

Maximum level	200 µg/kg DON
Increments	10 x 100 g
Sample preparation	None
Laboratory sample weight	1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the DON sample test result is equal or less than 200 µg/kg, accept the lot. Otherwise, reject the lot.

Flour, semolina, meal and flakes derived from wheat, cereal or barley

Maximum level	1000 µg/kg DON
Increments	10 x 100 g
Sample preparation	None
Laboratory sample weight	1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the DON sample test result is equal or less than 1000 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 DON test procedure and an accept/reject level. A DON test procedure consists of three steps: sample selection, sample preparation and analysis or DON 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 cereal 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 in such a way to ensure that the laboratory sample is still representative of the sublot sampled.

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 DON for chemical analysis.

SAMPLING PLAN DESIGN CONSIDERATIONS

Material to be sampled

1. Each lot of cereal, which is to be examined for DON, 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 cereal sublots according to lot weight

Lot weight (t)	Maximum Weight or minimum number of sub lots	Number of incremental sample	Minimum laboratory Sample Weight (kg)
≥ 1500	500 tonnes	100	1
> 300 and < 1500	3 sublots	100	1
≥ 100 and ≤ 300	100 tonnes	100	1
≥ 50 and < 100	2 sublots	100	1
< 50	-	3-100*	1

* 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 100 grams for lots ≥ 0.5 tonnes.

4. For lots less than 50 tonnes, the sampling plan must be used with 3 to 100 incremental samples, depending on the lot weight. 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.

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Lot weight (t)	Number of incremental sample	Minimum Laboratory Sample Weight (kg)
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> 0.05 - ≤ 0.5	5	1
> 0.5 - ≤ 1	10	1
> 1 - ≤ 3	20	1
> 3 - ≤ 10	40	1
> 10 - ≤ 20	60	1
> 20 - < 50	100	1

Static Lots

5. A static lot can be defined as a large mass of shelled cereal 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 cereal 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 cereal 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 cereal 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 x LT) / (T x 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 x V) / (D x 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 DON 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 DON formation.

17. As the distribution of DON 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 DON 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). Utilising this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. Proposed method criteria for DON in cereals.

Commodity	ML (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision on HorRat	Minimum applicable range (mg/kg)	Recovery
Cereal grains (wheat, cereal and barley) destined for further processing	2.0	≤ 0.2	≤ 0.4	≤ 2	1-3	80 - 110%
Cereal-based foods for infants and young children	0.2	≤ 0.02	≤ 0.04	≤ 2	0.1 – 0.3	80 – 110%
Flour, semolina, meal and flakes derived from wheat, cereal or barley	1.0	≤ 0.1	≤ 0.2	≤ 2	0.5 – 1.5	80 – 110%

Appendix II

COMMITTEE ON SPICES AND CULINARY HERBS (CCSCH)

METHODS OF ANALYSIS FOR CUMIN

Provision	Method	Principle
Moisture	ISO 938:1980 Alternative: AOAC 2001.12 ASTA 2.0	Distillation
Total ash	ISO 928:1997 Alternative: AOAC 950.49 ASTA 3.0	Gravimetry
Acid-insoluble ash	ISO 930:1997 Alternative: ASTA 4.0	Gravimetry
Volatile oils	ISO 6571:2008 Alternative: AOAC 962.17 ASTA 5.0	Distillation
Extraneous vegetable material	ISO 927:2009 Alternative: ASTA 14.1	Visual examination
Foreign matter	ISO 927:2009	Visual examination
Insect damage	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual, FDA Technical Buletin Number 5)	Visual examination

METHODS OF ANALYSIS FOR DRIED THYME

Provision	Method	Principle
Moisture	ISO 938:1980 Alternative: AOAC 2001.12 ASTA 2.0	Distillation
Total ash	ISO 928:1997 Alternative: AOAC 950.49 ASTA 3.0	Gravimetry
Acid-insoluble ash	ISO 930:1997 Alternative: ASTA 4.0	Gravimetry
Volatile oils	ISO 6571:2008 Alternative: AOAC 962.17 ASTA 5.0	Distillation
Extraneous vegetable material	ISO 927:2009 Alternative: ASTA 14.1	Visual examination
Foreign matter	ISO 927:2009	Visual examination
Insect damage	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual, FDA Technical Buletin Number 5)	Visual examination
Mould damage	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual, FDA Technical Bulletin Number 5)	Visual examination

Appendix III

COMMITTEE ON FISH AND FISHERY PRODUCTS (CCFFP)

AMENDMENTS TO SECTION 7.4 OF THE *STANDARD FOR QUICK FROZEN FISH STICKS (FISH FINGERS), FISH PORTIONS AND FISH FILLETS - BREADED OR IN BATTER* (CODEX STAN 166 – 1989)

7.4 Estimation of Fish Content

AOAC Method 996.15. **(End Product Method)**

Calculation:

% Fish Content = (Wd/Wb) X 100 + Adjustment Factor*

Wd = weight of debattered and/or debreaded test unit

Wb = weight of battered and/or breaded test unit

*Raw Breaded Frozen Coated Fish and Fishery Products: 2.0%

*Batter-dipped Frozen Coated Fish and Fishery Products: 2.0%

*Precooked Frozen Coated Fish and Fishery Products: 4.0%

Reference: J. AOAC Int. 80, 1235(1997)

Other Methods

(1) Chemical Analysis Method (Nitrogen Factor End-Product Method)

Appropriate in cases where there is reason to doubt the composition of the fish core (i.e., appears to contain non-fish ingredients). Except for fully cooked products, this method requires confirmation with the AOAC Method 996.15., or with Method #2 (Determination of Fish Content) in conjunction with investigation at the processing plant when determining product compliance with the labelling provisions in this Standard. This method should trigger in-factory investigation (e.g. raw ingredient recipe checks) when suspect products are identified.

The percentage fish content, corrected for the non-fish flesh nitrogen contributed by the carbohydrate coating, is calculated as follows.

$$\%\text{Fish} = \frac{(\%\ \text{total nitrogen} - \%\ \text{non-fish flesh nitrogen}}{\text{N factor*}}\ x100$$

*appropriate N (nitrogen) factor

The non-fish flesh nitrogen is calculated as follows:

% non-fish flesh nitrogen = % carbohydrate X 0.02

Where the carbohydrate is calculated by difference:

% carbohydrate = 100 – (%water + % fat + % protein + % ash)

References

Determination of nitrogen: ISO 937:1978

Determination of moisture: ISO 1442:1997

Determination of total fat: ISO 1443:1973

Determination of ash: ISO 936:1978

Average nitrogen factors to be used for fish flesh for specific fish species used as raw material for the product can be found at the following website:

http://www.globefish.org/seafood-nitrogen-factors.html

http://www.fao.org/fishery/topic/1514/en

The uncertainty of each nitrogen factor should be taken into account from the statistical data presented with the published nitrogen factor (e.g. 2 standard errors about the mean).

(2) Determination of Fish Content During Production

The fish content of a fish finger (fish stick) is calculated by using the following equation

$$\%\text{Fish Content} = \frac{\text{Weight of ingoing fish}}{\text{Weight of final product}} x100$$

For most products, therefore, the fish ingredient weight is that of the raw ingredient. Any figure placed or declared on a product label would be a typical quantity reflecting the producer's normal manufacturing variations, in accordance with good manufacturing practice."

Appendix IV

PART I. METHODS OF ANALYSIS IN THE *STANDARD FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS* (CODEX STAN 72-1981)

AOAC Official Methods validated in Infant Formula with ISO/IDF References

Commodity	Provision	Method	Principle	Proposed Type
Infant Formula	Vitamin B12	AOAC 2011.10 ISO 20634	High Performance Liquid Chromatography (HPLC)	II
Infant Formula	Myo-Inositol	AOAC 2011.18 ISO 20637	Liquid Chromatography (LC)-pulsed amperometry	II
Infant Formula	Chromium	AOAC 2011.19 ISO 20649 | IDF 235	Inductive Coupled Plasma-Mass Spectro-metry (ICP-MS)	II
Infant Formula	Selenium	AOAC 2011.19 ISO 20649 | IDF 235	ICP-MS	II
Infant Formula	Molybdenum	AOAC 2011.19 ISO 20649 | IDF 235	ICP-MS	II
Infant Formula	5'-Mononucleotides	AOAC 2011.20 ISO 20638	LC	II
Infant Formula	Vitamin A Palmitate (Retinyl Palmiate), Vitamin A Acetate (Retinyl Acetate), Total Vitamin E (dl-α-Tocopherol and dl-α-Tocopherol Acetate)	AOAC 2012.10 ISO 20633	HPLC	II
Infant Formula	Total Fatty Acid Profile	AOAC 2012.13 ISO 16958 | IDF 231	Gas Chromatography	II
Infant Formula	Iodine	AOAC 2012.15 ISO 20647 | IDF 234	ICP-MS	II
Infant Formula	Pantothenic Acid	AOAC 2012.16 ISO 20639	Ultra HPLC-MS/MS	II