Standard Operating Procedure

Sampling instructions for assessing microbiology and/or toxin of stacked 'flour' foods (i.e. flours, meal, and FBF)

Date: 13/02/2007, Rev 04/06/2010

Version: V2.0

1. Reference:

- INTERNATIONAL STANDARD ISO 24333 (First edition: 2009-12-15) Cereals and cereal products — Sampling
- European Commission Regulation (EC) No 401/2006

2. Scope

This International Standard specifies requirements for the dynamic or static sampling, by manual or mechanical means, of cereals and cereal products, for assessment of their quality and condition. It is applicable to sampling for the determination of heterogeneously distributed contaminants, undesirable substances, and parameters usually homogeneously distributed like those used to assess quality or compliance with specification.

The procedure was adapted for the sampling of stacked food.

3. Sampling of stacked commodity

- For each and every side of the stack and the top (i.e. 5 sides) prepare one bulk sample (i.e. 5 samples in all).
- Sterilization of the equipment (e.g. with alcohol)



Sterilization of the probe, and plate with alcohol





To clean the bag before sampling

Sampling from the top of the stack

- Probe sampling for bagged free flowing commodity: At each side of the stack use a sampling probe (the thinner the better and the use of an alcohol-sterilized hand-held stainless steel sampling probe is compulsory for microbiology analysis) to extract a sample of ____ g (i.e. quantity to sample divided by the number of unit to sample) from each of ____ bags (i.e. predetermined number of unit to sample)
- Clean the bag with a clean cloth prior to sampling

 For paper-bags from the top stack layer, remove the paper bag before sampling to assess if the inner-layer of the bag was damaged (and then the product possibly infested)



WRONG: contaminant will be sampled too



WRONG: Damaged inner layer, infested product



RIGHT: Sound bags ready for sampling

Introduced up-side down into the bag and to collect the same quantity per bag, this is an *increment*



Introduce the probe up-side down





Empty the probe in a clean container

 In doing this, select at least one bag from each layer of the stack, or bags going across the entire top of the stack.

the bag

 Make sure that you seal the holes made in the bags using tape (brown parcel tape works well for this).



Hole made by the probe



Sealed hole with brown tape

- When the job is done you will have 5 samples each of about _____ kg (i.e. sample size).
- To put the each collected food sample in one container (clean) ONE CONTAINER PER SIDE.
- To mix evenly the collected sample or increments
- To divide it as follow: FOR EACH SIDE OF THE STACK

4. Sample preparation

For aflatoxin determination in powdered products: 1 kg minimum per lot is necessary

4.1. Sample division

Either use of a sample divider on a flat surface, or follow the coning or quartering method as described hereunder:

- The aggregate sample (i.e. all increment samples) shall be thoroughly homogenized prior to any division procedure intended to obtain the *laboratory sample* and the *retention sample*.
- to make one cone (on a clean table),
- to flatten the cone for quartering,
- to dive into two parts,
- to divide into two parts perpendicularly to the first division,
- to take the opposite quarters for mixing and forming a new cone (roughly _____ kg, i.e. half of the sample size) - to repeat the process until you reach roughly 1 kg (minimum – if only one sample is needed).

Grain mixed and coned	Cone flattened for quartering
First division	Second division
Opposite quarters taken	The reduced sample
for missing and forming	

- To take 1 kg of this mix (i.e. the reduced sample), to divide into 4 parts (4 x 250g).
- To send 250g x 5 (one per side) to the laboratory and to keep 250g x 5 (one per side) at your office. To discard the rest or to keep for another party who might want to have a sample (e.g. public health authorities, supplier, etc).
- Each sample should be placed (safely do not touch the product with unclean bare hands) in two plastic bags, closed tightly and securely.
- Between the inner and outer plastic bags place a label indicating the SI number and any other information that is needed by you to identify the sample.
- Pack the samples so they will not be damaged in transit and send them by courier to the lab.

The laboratory will reconstitute a composite sample by mixing an equal quantity of each of the 5 samples (each sample representing one side or the top of the stack), prior to analyze the sample for its microbiological content.

4.2. Packaging of samples

Laboratory samples shall be placed in clean containers. The containers shall be suitable for the 1kg of the laboratory samples. The containers shall also preserve the initial characteristics of laboratory samples. These containers shall be completely full and shall be sealed to avoid any change in their contents. Seals shall be tamper-proof and identifiable.

4.3. Labels for samples

The information listed below shall be marked indelibly and legibly. The information on the labels on the laboratory sample shall include the instructions required under the terms of the contract, for example:

- a) Product name;
- b) 1 kg;
- c) the lot identifier;
- d) the contract number;
- e) the sampling date;
- f) the location and point of sampling;
- g) the name of the person who carried out the sampling.

4.4. Shipment of samples

Samples should be sent to the laboratory as quickly as possible. The samples should be stored and transported in conditions appropriate to the preservation of their integrity.

4.5. Sampling report

The sampling report may contain some or all of the following information:

- a) the date of sampling;
- h) the name and signature of the persons authorized to carry out sampling;
- i) if necessary:
 - the name and signature of the seller,
 - the name and signature of the buyer,
 - the name and signature of the deliverer;
- j) the description of the product, including:
 - sample reference,
 - sample mass,
 - lot size,
 - sample origin (e.g. vertical silo, lorry);
- k) the description of the sampling operation, including:
 - the location and point of sampling,
 - the number of increments per lot,
 - the number of laboratory samples per lot,
 - the sampling procedure used (equipment, static/flowing, etc.),
 - the destination of the sample, e.g. the name and address to which the samples are to be shipped,
 - comments if any;
- 1) the transportation and storage conditions.

5. Testing

5.1. Reference

The *European Commission Regulation (EC) No 401/2006* must be followed to analyse the 1 kg sample.

The method implies to mix the flour thoroughly and then, only then, to extract the quantity needed for the analysis.

The method is available on line and the link should be provided to the laboratory. http://www.icc.or.at/task/EC401-2006.pdf

5.2. Existing methods for detection of mycotoxins in foods

- Thin Layer Chromatography (TLC),
- High Performance Liquid Chromatography (HPLC),
- Minicolumns, Immunoassays such as Enzyme Linked Immunosorbent Assay (ELISA)
- Immunoaffinity Columns (IAC).

QUALITATIVE Test kits, such as Aflacard T20, AgraStrip, AgriScreen, QuickTox, ReVeal, Rosa-P/N, Diachemix FPA, MycoAflatox, ReVealSQ can be used

QUANTITATIVE Test kits, such as Aflatest, Fluoroquant, Myco, RidascreenFastAflatoxin, Rosa-Quantitative, or VeratoxAST can be used for maize, sorghum, and maize meal.