DRAFT TANZANIA STANDARD

Sampling procedures for aflatoxin analysis in groundnuts and its products

TANZANIA BUREAU OF STANDARDS

Sampling procedures for aflatoxin analysis in groundnuts and its products

0 Foreword

Aflatoxins are secondary metabolites produced in food crops by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are potent hepatotoxic and carcinogenic to humans and animals. There are four major types of aflatoxins which naturally contaminate agricultural commodities i.e. Aflatoxin B1, B2, G1 and G2. Aflatoxin formation can occur while the crop is in the field or after harvesting. Improper postharvest handling of the commodity, i.e. insufficient drying and improper storage conditions can result in increased levels of aflatoxins.

The primary commodities associated with aflatoxin contamination are maize, groundnuts, tree nuts, cottonseed, rice, and dried fruits, with groundnuts being the most contaminated. Groundnuts are among the commodities widely consumed in Tanzania and frequently used as ingredient for complementary food.

Aflatoxin contaminated units are not homogeneously distributed throughout a lot. A few units are likely to be highly contaminated (aflatoxin clusters), while most of the kernels are aflatoxin-free. Collecting samples only from the highly contaminated kernels or from the aflatoxin-free ones will provide inaccurate final results. Therefore, proper sampling is one of the most crucial elements of addressing and managing aflatoxin contamination of food.

Good sampling provides reliable samples for analysis that can represent the basis for ‘‘fit for purpose’’ investigations. Samples should be randomly gathered from a number of sufficient incremental samples from groundnuts in bulk in order for the analysis to be representative of the whole lot.

This standard for sampling procedures has been designed to ensure that a representative sample is obtained for reliable analytical data of aflatoxins in groundnuts and its products.

In preparation of this standard considerable help was derived from CXS 193-1995(Rev.2019) - *General standard for contaminants and toxins in Food and Feed published by Codex Alimentarius Commission and EAS 900:2017, Cereals and pulses – sampling published by East African Community.*

1 Scope

This Tanzania Standard specifies methods of sampling groundnuts and its products for aflatoxins analysis in bulk consignment, bagged kernels and processed groundnuts in packed units aimed for human and animal consumption.

2 Normative references

There is no normative reference in this document.

3 Terms and definitions

For the purposes of this Tanzania Standard, the following terms and definitions shall apply:

3.1 lot

an identifiable quantity of a groundnuts or its products determined officially to have common characteristics, such as origin, variety, type of packaging, packer, consignor or marking

3.2 sublot

designated part of a large lot in order to apply the sampling method on that designated part.

3.3 sampling

drawing or constituting a sample

3.4 sampling procedures

detailed outline on how a sample will be taken at what times, on which material, in what manner

3.5 dynamic sampling

drawing or constituting a sample from a flowing lot

3.6 static sampling

drawing or constituting a sample from a stationary lot

3.7 incremental sample
quantity of material taken from a single point in the lot or sublot to be combined to produce the aggregate sample

3.8 aggregate sample

combined total of all incremental samples taken from a lot or sublot

3.9 laboratory sample

minimum quantity/portion of comminuted sample which is the representative of the aggregate sample

4.0 General requirements

4.1 For the purpose of this standard, sampling includes the following stages:

a) taking a defined number of increments to constitute an aggregate sample;

b) homogenization of the aggregate sample; and

c) reduction of the homogenized aggregate sample into the laboratory sample(s).

4.2 Since aflatoxin contamination in a lot of groundnuts or their products is rarely homogeneously distributed, a sufficient number of increments shall be taken and carefully mixed to constitute an aggregate sample from which it will subsequently be possible to obtain one or several laboratory sample(s). For non-flowing commodities (static), particular care shall be taken to ensure that these increments are distributed regularly throughout the groundnut mass, both at the surface and deep down.

4.3 Groundnuts and their products which are damaged on transit or out of condition shall be kept away from the sound groundnuts. Sampling shall be done separately and avoid mixing of sound sample with the unsound one.

4.4 In the event of arbitration, samples shall be taken jointly by representatives of both the purchaser and the vendor (seller), or by a third party nominated by common accord.

4.5 Precautions shall be taken to guarantee the integrity of all samples between the moment they are taken and the moment they are used in the laboratory.

5.0 Equipment and devices

5.1 Make sure that the most suitable sampling equipment are chosen taking into account the product to be sampled, the quantity required and the containers to be used.

5.2 Make sure that all sampling equipment used are clean and dry. The sampling procedures shall be carried out in such a way that the sampled material is protected from any source of accidental contamination.

5.3 Annex A describes the general types of mechanical sampling devices used on flowing groundnut, and shows illustrations of examples of such devices. Annex B gives examples of instruments used to sample static products, and examples of instruments used to divide samples.

6.0 Sampling

6.1 Material to be sampled

Each lot to be examined shall be sampled separately. Large lots should be subdivided into sub-lots and each sublot to be sampled separately. The subdivision can be done following provisions laid down in Table 1. Considering that the weight of the lot is not always an exact multiple of the weight of the sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 20 %. If the mass of the laboratory sample cannot be complied with, the number of increments shall be increased.

NOTE: One laboratory sample is required by lot or sub-lot of 500 tonnes maximum e.g. For a lot of 6 000 tonnes, analyse at least four laboratory samples.

Table 1. Subdivision of large lots into sub lots for sampling

<table>
<tr><th rowspan="2">Lot weight –ton(T)</th><th rowspan="2">Weight or numbers of sublots</th><th rowspan="2">Minimum number of incremental samples</th><th colspan="2">Laboratory sample* weight(kg)</th></tr>
<tr><th>shelled</th><th>unshelled</th></tr>
<tr><td>≥ 500</td><td>100 tons</td><td rowspan="4">100</td><td rowspan="4">20</td><td rowspan="4">27</td></tr>
<tr><td>>100 and <500</td><td>5 sublots</td></tr>
<tr><td>≥ 25 and ≤100</td><td>25 tones</td></tr>
<tr><td>< 25</td><td>1 lot</td></tr>
</table>

*If the aggregate sample is larger than 20 kg for shelled and 27 kg for unshelled, a 20 kg and 27 kg laboratory sample respectively should be removed in a random manner from the aggregate sample.

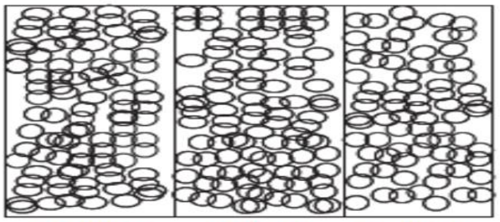


Figure 1: Example of diving large lot into sublots

6.2 Sampling of bulk consignment

The bulk consignment can be static or dynamic. For dynamic lot, it is preferred that the sampling should be carried out when the products are flowing (for example, during loading or unloading) so that all the constituent parts of the lot have the same probability of being sampled. When mechanical means are not available, implement a manual sampling plan.

The methods (mechanical or manual) of taking samples from flowing lots shall be adapted to the speed at which the products are flowing. For static groundnut, whichever method of sampling is used, the increments should be taken at regular intervals over the entire width and depth, up to 9 m.

Lots of bulk groundnut which are more than 9 m deep should be sampled when they are flowing. For lorries and trailers, it is recommended that samples be taken statically.

In order for the aggregate sample to be representative, the number of increments shall be as high as possible.

6.2.1 Sampling of dynamic bulk products

6.2.1.1 General

Considering that the characteristics and make-up of the lot can vary, the increments shall be taken from the whole lot, that is, as long as the material is dynamic.

6.2.1.2 Mechanical sampling

Adjust the equipment so that the size of the increments or the frequency of sampling is varied over a wide range.

A series of fixed-size increments shall be taken at pre-determined intervals according to the flow and in such a way that each part of the lot has the same chance of entering the sampling device intake.

EXAMPLE: Crosscut sampling devices meet this requirement irrespective of the type of flow. The minimum numbers of increments to be drawn are specified in Table 1.

6.2.1.3 Manual sampling

Take increments at regular intervals. The minimum numbers of increments to be drawn are specified in Table 2.

Table 2 — Sampling of flowing groundnut by mechanical or manual means

Method	**Range of mass of increment** (g)	**Minimum number of increment** [a]	**Minimum mass of laboratory sample for aflatoxin**
Mechanical sampling	300 - 1900	• 20 per lot or sub-lot of 500 tonnes • 25 per lot or sub-lot of one 500 tonnes for large batches of size greater than one 500 tonnes	20kg for shelled and 27 kg for unshelled
Manual sampling	300 - 1 900	• 20 per lot or sub-lot of 500 tonnes • 25 per lot or sub-lot of 1 500 tonnes for large batches of size greater than 1 500 tonnes	
a Frequency according to groundnut flow			

6.2.2 Sampling of static bulk products

The sampling procedure to obtain the minimum mass of laboratory sample for static consignment shall be as given in Table 3

Table 3 — Sampling of static bulk groundnuts (mechanical sampling systems recommended) in trailers or lorries, wagons, ships or bulk tankers, silos or warehouses

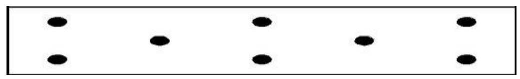
Size of lot or sub-lot	**Range of mass of increment**[a] **g**	**Minimum number of increment**[b]	**Minimum mass of laboratory sample for aflatoxins**
≤15 t	400 - 3 000	3 sampling points	20 kg for shelled and 27 kg for unshelled
15< *and* ≤ 30 t		8 sampling points	
30<*and* ≤ 45 t		11 sampling points	
45< *and* ≤ 100 t		15 sampling points	
100< *and* ≤ 300 t		18 sampling points	
300< *and* ≤ 500 t		20 sampling points	
500< *and* ≤ 1500 t		25 sampling points	

Per lot or sub-lot of 1 500 t		25 sampling points	

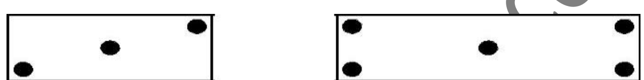
a If taken mechanically, the mass of the sample can be appropriate to the equipment.

b Frequency according to groundnut flow

Figure 2 shows examples of the distribution of eight sampling points and Figure 3 for 25 sampling points.



a) For lorries “in one piece” (for example, dumper truck, semi-trailer)



b) For lorries distributed in chassis and trailer

Figure 2 — Examples of the distribution of sampling points for eight points

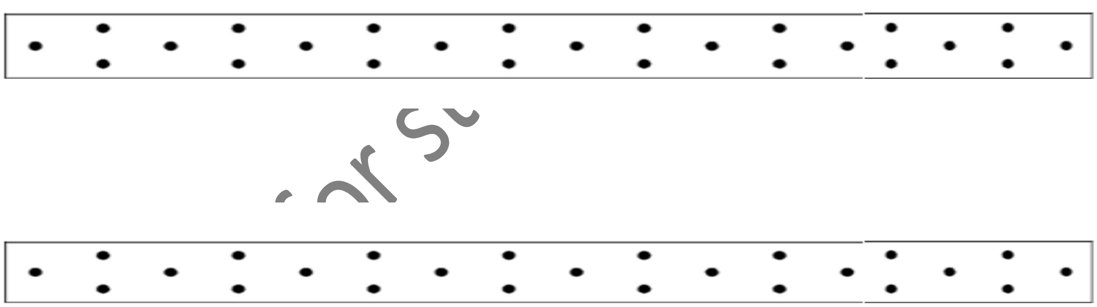


Figure 3 — Examples of the distribution of sampling points for 25 points

6.3 Sampling from bagged kernels

6.3.1 General

6.3.1.1 Increment

Samples shall be drawn from original bags selected at random from the consignment which are clearly identifiable with the appropriate markings. The minimum number of bags to be sampled in each consignment shall be in accordance with the scheme in Table 4.

Table 4 — Minimum number of bags to be sampled in each consignment

Number of bags	
In consignment	**To be sampled (min)**
≤ 20 bags	a sample will be drawn from every bag
>20 and ≤ 100 bags	Not less than 20 of the bags shall be sampled
>100 and ≤ 1 000 bags	Not less than 50 of the bags shall be sampled
> 1 000 bags	Not less than 0.5 % (1 in 200 bags) with a minimum of 50 of the bags shall be sampled

6.3.1.2 Increment samples shall be drawn uniformly by a piercing spear from the top, middle and bottom of each bag. If it is not possible to draw a sample by spear efficiently, then the original bags may be opened to sample by a hand scoop. As far as possible samples shall be drawn from the ends and middle of the bags in rotation (see Figure 4)

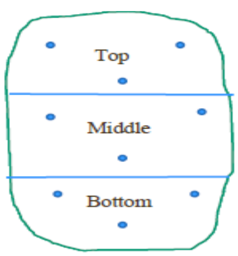


Figure 4: Examples of the distribution of sampling points in sack

6.4 Sampling of processed groundnut in packed units

6.4.1 Bales, “crates boxes” or pallets to be sampled

The packed units are generally stored/ transported in crates, boxes or pallets which contain an appropriate number of basic units. The method applicable to sacks shall be used to define the appropriate number of crates boxes or pallets that are to be sampled (see Table 5).

6.4.2 Increments of packed units

The packed unit shall be the increment sampled at random from the overall contents of the lot selected for sampling.

IMPORTANT — Avoid selecting packed units which occupy the same position in several crates, boxes or pallets.

6.4.3 Number and mass of increments

The number of incremental samples to be taken from a lot (sublot) depends on the weight of the lot. Table 5 shall be used to determine the number of incremental samples to be taken from lots or sublots of various sizes below 25 tons. The number of incremental samples varies from a minimum of 10 and to a maximum of 100.

Table 5. Number and size of incremental samples composited for an aggregate sample of 1 kg as a function of lot (or sublot) weight

Lot or sublot weight [b] (T in tons)	Minimum number of incremental samples	Minimum incremental sample size [c] (g)	Minimum aggregate sample size (Kg)
T < 1	10	100	1
1 ≤T < 5	25	40	1
5 ≤ T < 10	50	20	1
10 ≤ T < 15	75	13.3	1
15 ≤T	100	10	1

a) Minimum aggregate sample size = laboratory sample size of 1 kg
b) 1 Ton = 1 000 kg
c) Minimum incremental sample size = laboratory sample size (1 kg) / minimum number of incremental samples, i.e. for 0.5 < T < 1 ton, 2 000 g = 20 000/10

6.4.4 The increments shall be taken from the centre and over the whole length of each crate, box or pallet to be sampled using a tapered sampling probe specially designed for crates, boxes or pallets and shall be taken as specified in Table 6.

6.5 Sampling intervals

The following equation shall be used as a guide for determining the frequency of sampling per lot, *F*(*n*), for lots marketed in individual packages (sacks, retail packages, etc.).

$$F(n) = \frac{m_B m_I}{m_A m_P}$$

where

m_B is the mass, in kilograms, of the lot;

m_I is the mass, in kilograms, of the increments (approximately 0.100 kg);

m_A is the mass, in kilograms, of the aggregate sample

m_P is the mass, in kilograms, of the individual package.

Take an incremental sample from every nth sack or bag (decimal figures should be rounded to the nearest whole number), where n is the number of packed units between two increments.

NOTE: For analyses of aflatoxins, m_A is approximately 1 kg for groundnut flour and peanut butter and 20 kg for shelled and 27 kg for unshelled groundnut

EXAMPLE: The frequency of sampling per lot calculated with a mass of increment of 0.100 kg corresponds to a minimum number of increments to be taken. If the mass of the increment is greater than 0.100 kg, the frequency of sampling calculated with 0.100 kg shall be applied.

Practical examples of a calculated frequency of sampling for lots of 25 t, 50 t and 100 t are given in Table 6.

Table 6 — Sampling procedure to obtain the minimum mass of laboratory sample for processed products in sack/crate: practical examples for lots of 25tonnes

Lot size Kg	**Mass of individual sacks** kg	**Mass of increment** kg	**Calculated frequency of sampling for aflatoxin analysis** 1 increment every *n*th sack/crate	**Equivalent per sack/crate** 1 sample shall be all *n* sack/crate
25 000	1	0.100	833	1 sample every833 sack/crate ,
25 000	5	0.100	167	1 sample every 167 unit sack/crate,
25 000	25	0.100	33	1 sample every 33 unit sack/crate, or 1 sample per pallet
25 000	40	0.100	21	1 sample every 21 unit sack/crate
25 000	50	0.100	17	1 sample every 17 unit sack/crate

NOTE: Laboratory samples of approximately 1 kg shall be randomly taken from the aggregate samples**.**

7.0 Packaging, labelling and transportation of samples

7.1 Packaging

Each laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination 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.

7.2 Labelling

The following information shall be legibly and indelibly labelled on the laboratory sample and shall include the instructions required under the terms of the contract. For example:

a) nature of the product;
b) mass represented;
c) lot identifier (number);

d) contract number (if necessary);
e) sampling date; and
f) location and point of sampling.

7.3. Transportation
Samples shall be sent to the laboratory immediately after sampling. The samples shall be stored and transported in conditions appropriate to the preservation of their integrity.

8.0 Sampling report

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

a) date of sampling;
b) name and signature of the persons authorized to carry out sampling;
c) if necessary:
   i. name and signature of the vendor/seller;
   ii. name and signature of the purchaser/buyer; and
   iii. name and signature of the deliverer;
d) description of the product, including:
   i. sample reference;
   ii. sample mass;
   iii. lot size; and
   iv. sample origin (for example, flat silo, vertical silo, lorry);
e) the description of the sampling operation, including:
   i. location and point of sampling;
   ii. number of increments per lot;
   iii. number of laboratory samples per lot;
   iv. sampling procedure used (equipment, static/flowing, etc.);
   v. destination of the sample, e.g. the name and address to which the samples are to be shipped; and
   vi. comments if any; and
f) transportation and storage conditions

9 Hygiene and safety

The sampling device and containers used shall comply with applicable hygiene and safety requirements and, in particular, have sufficient lighting for inspection, manoeuvring and maintenance. A suitable mask should be worn if the device is used in a potentially dusty environment.

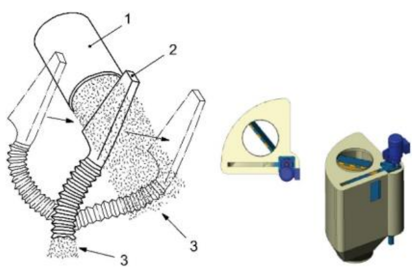
WARNING — Walking on groundnuts stored in storage bins, ships' holds, silos and lorries is dangerous. In some cases, the atmosphere in silos may be asphyxiating or toxic as a result of the accumulation of gas caused by the metabolism of the groundnuts and fungi/mould. Local legislation and industrial safety standards shall be observed.

Annex A
(informative)

Examples of mechanical sampling devices used on flowing groundnuts

A.1 Crosscut sampling devices

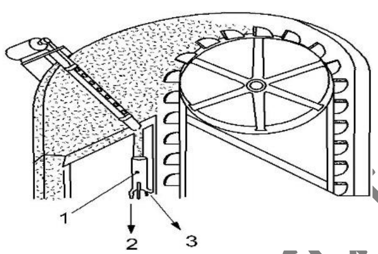
Crosscut sampling devices allow a complete cross- section of a freely falling flow of groundnuts to be taken. They may be open-nozzle sampling devices (see Figure A.1), tubular sampling devices with adjustable apertures (see Figure A.2) or tubular sampling devices with a worm screw (see Figure A.3).



Key

1 nozzle
2 sampling device
3 groundnuts

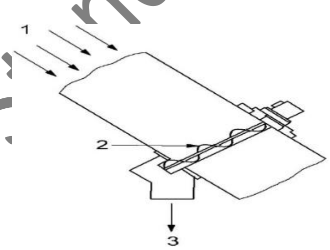
Figure A.1 — Open-nozzle crosscut sampling device, ensuring intermittent, repeated sampling



Key

1 sample divider
2 sampling flow
3 return of excess groundnuts into system

Figure A.2 — Tubular crosscut sampling device with adjustable apertures



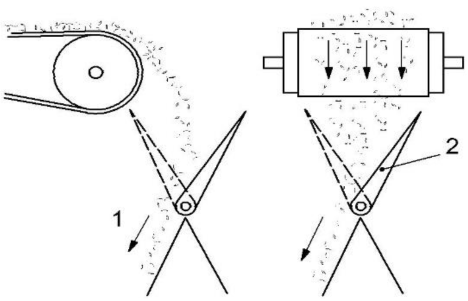
Key

1 groundnuts flow
2 worm screw
3 sample flow

Figure A.3 — Tubular sampling device with worm screw

A.2 Full-flow diverter-type sampling devices

In this type of sampling device, a flap or shutter intermittently diverts the flow of groundnuts (see Figure A.4).



Key

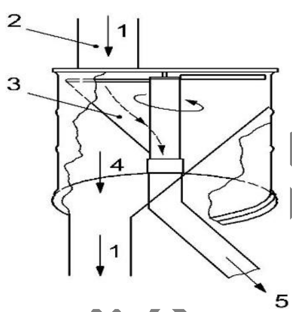
1 sample flow

2 flap or shutter

Figure A.4 — Full-flow diverter-type sampling device

A.3 Rotating cup sampling devices

The freely falling flow of groundnuts is intermittently sampled by a cup which rotates around a central vertical axis (see Figure A.5).



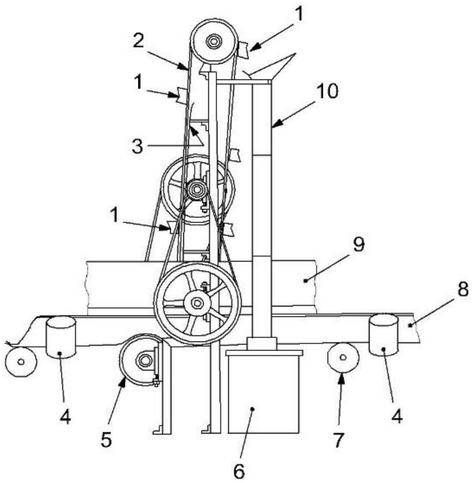
Key

1 groundnuts flow
2 vertical chute
3 rotating cup
4 flow
5 sample flow

Figure A.5 — Rotating cup sampling device

A.4 Bucket elevator sampling devices

This type of sampling device samples groundnuts from a moving belt or conveyor. Buckets travelling in a continuous loop take samples over the entire width of the groundnuts flow because the configuration of the lateral rollers concentrates the groundnuts on the belt. Once the buckets have pivoted around the upper roller, the samples are delivered into the hopper (see Figure A.6).



Key

1. sampling bucket(s)
2. sampling bucket belt
3. belt guide
4. balance weight
5. special roller
6. samples container
7. conveyor roller
8. carrier belt
9. safety panel
10. hopper

Figure A.6 — Bucket elevator sampling device

NOTE : as they are elevated, the buckets sample the groundnuts from a belt or conveyor and, once they have pivoted around the upper roller, deliver the samples into the hopper.

Annex B
(informative)

Examples of instruments used to sample static products and instruments used to divide samples

B.1 groundnuts sampling instruments

B.1.1 Instruments used to sample static bulk products in tote bags and rigid containers

B.1.1.1 Manual concentric tapered sampling probes

B.1.1.1.1 Open or closed shaft: with one or several apertures. See Figures B.1 and B.2.

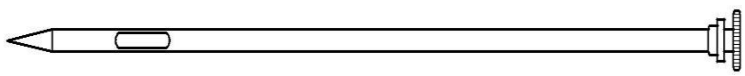


Figure B.1 — Open shaft with single aperture

Figure B.2 — Open shaft with several apertures or closed shaft with compartments and several apertures

B.1.1.1.2 Open shaft with sequentially staggered apertures: several apertures. See Figure B.3.

Figure B.3 — Open shaft with several sequentially staggered apertures

B.1.1.2 Gravity-type sampling probes with extension rods and T-shaped handles

B.1.1.2.1 Gravity-type sampling probe: concentric. See Figure B.4.

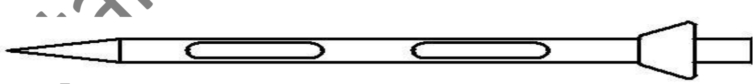


Figure B.4 — Concentric gravity-type tapered probe head

B.1.1.2.2 Gravity-type sampling probe: cup-type. See Figure B.5.

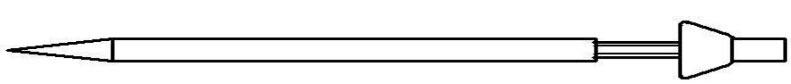


Figure B.5 — Cup-type (head represented in open position)

5 B.1.1.3 Mechanical sampling devices

B.1.1.3.1 There are three main categories of mechanical sampling devices (see B.1.1.3.2 to B.1.1.3.4).

B.1.1.3.2 Gravity-type sampling device. See Figure B.6.

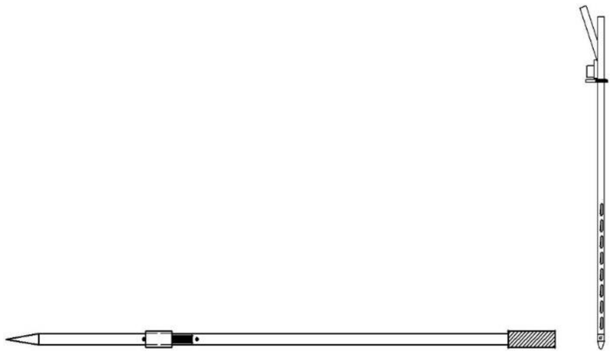
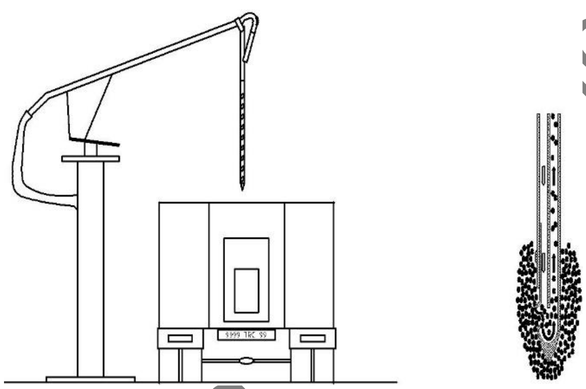


Figure B.6 — Gravity-type sampling device

B.1.1.3.3 Suction sampling device (sometimes called “vacuum sampling device”). See Figure B.7.



Example of sampling from a lorry

Figure B.7 — Suction (or “vacuum”) sampling device

B.1.1.3.4 Pneumatic sampling device (not represented)

B.1.2 Instruments used to take samples from sacks or bags including bulk sacks

B.1.2.1 Tapered sampling probes for sacks

Minimum diameter: 17 mm; aperture: 40 mm × 15 mm. See Figure B.8.

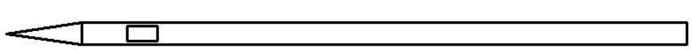


Figure B.8 — Tapered sampling probe for sacks

B.1.2.2 "Walking stick"-type sampling probe

Concentric tubes, minimum diameter: 20 mm:

6 Open shaft: with one or several apertures;

7 With compartments: with one or several apertures (see Figure B.9).

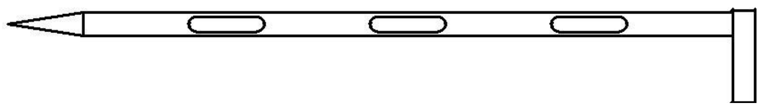


Figure B.9 — "Walking stick"-type, concentric sampling probe for sacks: open shaft with several compartments

B.1.2.3 Cone-shaped sampling device

See Figure B.10.

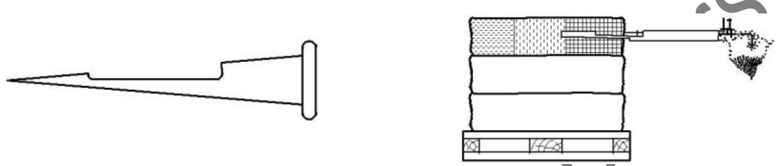


Figure B.10 — Cone-shaped sampling device

B.1.2.4 Gravity-type sampling probes with extension rods and T-shaped handles for open-topped sacks

See Figures B.4 and B.5.

B.1.2.5 Archimedes' screw sampling probe

Generally, a small, portable, electric sampling probe. See Figure B.11.

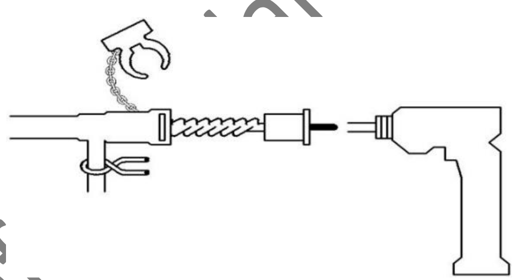


Figure B.11 — Archimedes' screw sampling probe (portable)

B.2 Instruments used to sample milled products, excluding products in granular form

B.2.1 Instruments used to sample static bulk products

B.2.1.1 Identical to those used to sample groundnuts (B.1.1), except for mechanical sampling devices. Only two types of mechanical sampling device are suitable for the sampling of milled products (see B.2.1.2 and B.2.1.3). In general, pneumatic sampling devices are not suitable for this usage.

B.2.1.2 Electromechanical Archimedes' screw sampling probe (Figure B.12).

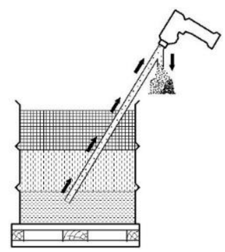


Figure B.12 — Electromechanical Archimedes' screw sampling probe

B.2.1.3 Gravity-type mechanical sampling device (Figure B.6).

B.2.2 Instruments used to take samples from sacks and bags

Identical to those used to sample cereals (B.1.2).

B.3 Instruments used to divide samples

Made of materials which are not liable to contaminate samples.

B.3.1 Quartering irons

See Figure B.13.

B.3.2 Multiple-slot dividers (with partitions and plates)

B.3.2.1 Small laboratory dividers for milled samples.

Minimum of 12 slots; chutes of: 12.7 mm.

B.3.2.2 Medium-sized dividers for samples of groundnuts in the form of grain.

Minimum of 18 slots; chutes of: 12.7 mm.

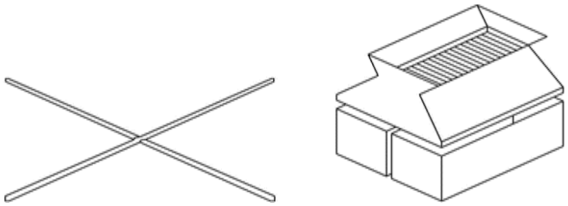


Figure B.13 — Quartering iron

B.3.3 Cone-shaped dividers (Boerner type)

See Figure B.14.

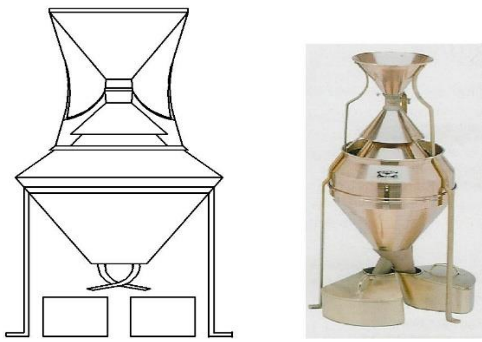
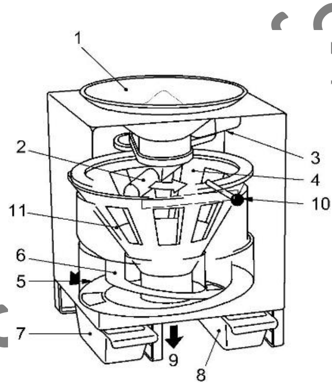


Figure B.14 — Cone-shaped divider (Boerner type)

B.3.4 Rotating mechanical divider

Allows multiple samples to be obtained simultaneously. See Figure B.16.



Key

1. loading hopper
2. rotating chute
3. driving motor
4. cone-shaped hopper with eight apertures
5. sub-sample collection
6. sub-sample collection spout
7. two sub-sample collection boxes
8. excess grain evacuation, grain to put back in the divider
9. adjustment of flaps to modify division factor
10. one of eight adjustable apertures

Figure B.15 — Rotating mechanical divider