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Second edition
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Wheat flour and durum wheat semolina — Determination of impurities of animal origin

Farines de blé tendre et semoules de blé dur — Détermination des impuretés d'origine animale

Reference number
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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

This second edition cancels and replaces the first edition (ISO 11050:1993), which has been technically revised. The main changes compared with the previous edition are as follows:

— the Scope has been widened;

— the protocol has been improved to make it easier;

— the figures have been updated.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Wheat flour and durum wheat semolina — Determination of impurities of animal origin

1 Scope

This document specifies a method for determining the content of impurities of animal origin in wheat flours, with or without additives and having an ash yield not exceeding a mass fraction of 0,75 %, and in durum wheat semolinas.

This method permits the separation and quantification of contamination of animal origin, such as insects at all stages of their development and their fragments, rodent hairs and their fragments, and mites.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
impurity of animal origin
matter of animal origin [larvae, nymphs or adults of *insects* (3.10) and their fragments, rodent hairs and their fragments, *mites* (3.3)] separated from the product under the conditions specified in this document

3.2
abdomen
rear part of the body of an *insect* (3.10), excluding the head and thorax, commonly with eight or more segments when complete

3.3
mite
very small arthropod belonging to the class Arachnida, often living in large numbers

3.4
appendice
distinctly differentiated prolongation of the body of an arthropod

EXAMPLE Legs, wings, antennae, *urogomphi* (3.16).

3.5
cephalic capsule
head capsule
sclerous part of an exuvia that once contained the head of a larva

3.6
caterpillar
larvae of *Lepidoptera* spp.

Note 1 to entry: Butterfly or moth is the adult stage and chrysalis is the pupal stage.

3.7
scale
bristle (3.13) that has evolved into a flat structure resembling a fish scale and that covers the parts of the body of certain *insects* (3.10), in particular the wings of *Lepidoptera* spp.

3.8
wing case
elytron
hardened front wing of *Coleoptera* spp.

Note 1 to entry: It is used as a fixed wing in flight and as protective cover for the membranous hind wing.

3.9
false leg
proleg
fleshy extension of the lower part of the *abdomen* (3.2) of some larvae, sometimes with a crown of fine hooks (crochets) of chitin

Note 1 to entry: These help with attachment to the substrate, and in movement. Lepidopterous larvae have at least two pairs of false legs, towards the rear of the body.

3.10
insect
class of animals within the phylum Arthropoda, some of which are recognized pests of stored foodstuffs

3.11
mandible
toughened (sclerotized) mouthpart of *insects* (3.10)

Note 1 to entry: It is used for the dilaceration or grinding of food.

3.12
pericarp
external envelope of seeds that forms the bran after the grain has been crushed and the flour separated

3.13
bristle
fine but stiff hair of any length present on the cuticle of *insects* (3.10)

Note 1 to entry: Sensory hairs are called “setae”.

3.14
stage
state of development of an *insect* (3.10) or *mite* (3.3)

EXAMPLE Egg, larva, nymph, pupa, adult.

3.15
juvenile stage
pre-adult *stage* (3.14) of *insects* (3.10)

EXAMPLE Egg, larva, nymph, pupa.

Note 1 to entry: This term is most often applied to the active stages of larva and nymph.

3.16
urogomphi
pointed extensions of the cuticle of the final abdominal segment of some insect larvae

Note 1 to entry: They are common, and sometimes diagnostic, features of many *Coleoptera* spp.

Note 2 to entry: The abdominal extensions of a cockroach are called "cerci".

4 Principle

Hydrolysis of a test portion with a solution of hydrochloric acid at boiling point. Concentration of the insoluble particles (impurities other than those of animal origin may be present) at a water/oil interface. Separation by filtration on a filter paper, microscopic examination and counting under reflected light of the impurities of animal origin.

5 Reagents

Only use reagents of a recognized analytical grade and demineralized water or water of equivalent purity.

5.1 Ethanol, a volume fraction of 95 %.

5.2 Ethanol solution, a volume fraction of 50 %.

5.3 Ethanol/glycerol, 1 + 1 mixture by volume.

5.4 Hydrochloric acid solution, concentrated at 35 % to 37 %.

5.5 Paraffin oil (known as "Vaseline oil"), fluid, having a viscosity not exceeding 60 mPa·s at 20 °C.

5.6 Liquid detergent, non-foaming.

EXAMPLE Extran, Biodeck 4, Decon 90.[1)]

5.7 Liquid detergent, a volume fraction of 1 % aqueous solution of the detergent (5.6) in a washing bottle.

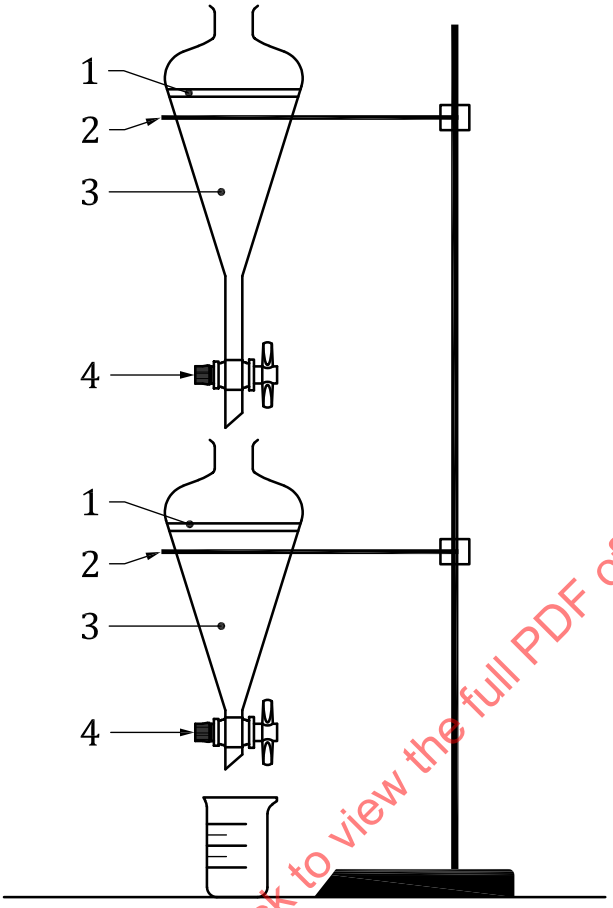
5.8 Dearomatized white spirit (possible use).

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

1) These are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

6.1 Separating funnels, conical, of 1 000 ml capacity, fitted with a non-lubricated polytetrafluoroethylene (PTFE) tap. See the recommended set-up shown in Figure 1.



Key

1 light hydrocarbon phase
2 support ring
3 "heavy" aqueous phase
4 PTFE tap

Figure 1 — Separation apparatus

6.2 Tall-form beaker, of at least 800 ml capacity, fitted with a watch glass made of Pyrex®[2] or PFTE and of appropriate dimensions to serve as a lid.

6.3 Crystallizing dish or **pan,** of at least 5 l capacity and of a height slightly less than that of the beaker (6.2), suitable for use as a cooling bath.

6.4 Graduated cylinders, of 25 ml, 50 ml and 500 ml capacity.

6.5 Washing bottles, with a flexible tube.

2) Pyrex® is the trademark of a product registered by Corning Inc. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.6 Filter paper[3], ash free, with rapid filtration characteristics, of 90 mm diameter, corresponding to that of the filtration unit (6.7), on which fine parallel lines are drawn, spaced 5 mm apart, using a lead pencil.

6.7 Filtration unit, of the Büchner funnel type, removable, suitable for accommodating the filter paper (6.6), and fitted with a conical adapter bung for connection to the filtration flask (6.15).

6.8 Analytical balance, for precision weighing to within 0,1 g.

6.9 Optical microscope or stereoscopic microscope, known as a "binocular magnifying glass", capable of producing magnifications close to ×25 and ×50, of very high optical quality, used in conjunction with:

a) **eyepieces**, producing a magnification of ×15 or ×20, thus enabling a total maximum magnification of the object being observed of ×75 or ×80 (depending on the model); and, possibly,

b) a **micrometer eyepiece**, to measure the dimensions of any impurities with better precision.

6.10 Petri dish, made of plastic or glass, with a diameter of 90 mm or 100 mm.

6.11 Fine needle, made of steel, mounted in needle-holding chuck.

6.12 Plastic stirring rod or **glass agitator,** fitted with a rubber or plastic protective end.

6.13 Magnetic stirrer/heater, thermostatically controlled, enabling water to be brought to boiling point.

6.14 Spring clips or special flexible clips, for holding the filter paper (6.6).

6.15 Filtration flask, of 1 l capacity, capable of being connected preferably to the vacuum pump (6.17) or else to a water suction pump (6.17).

6.16 Dropper.

6.17 Vacuum pump, enabling a residual pressure of below 1 000 Pa (10 mbar) to be achieved or, if this is not available, a **water suction pump.**

NOTE The duration of filtration is increased considerably if a water suction pump is used.

6.18 Temperature-adjustable oven, capable of being maintained at 37 °C to 40 °C, which may be used to dry the filters.

7 Sampling

For the use of this test method, it is essential that all equipment used for sampling is thoroughly cleaned between each sampling operation.

It is strongly recommended that users of this document ascertain, where possible, that these requirements are met during the sampling procedure.

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 24333.

3) Whatman® Ashless, Grade 41 Filtration Paper is an example of a suitable filter paper available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

A laboratory sample of at least 600 g is required. It shall be kept in a cold chamber at a temperature of 4 °C if the analysis is not performed immediately.

8 Procedure

IMPORTANT — All handling operations shall take place in clean premises, away from air currents or preferably beneath a non-ventilated canopy. Before use, all apparatus shall be washed in demineralized water. After rinsing and draining until completely dry, all containers shall be turned over to prevent any contamination.

8.1 Test portion

Mix the sample thoroughly using a long-handled spatula, then, taking parts from several places, weigh 50 g ± 0,1 g of the product into the beaker (6.2).

8.2 Hydrolysis

8.2.1 Take 300 ml of demineralized water with the cylinder (6.4). Dilute the test portion using the stirring rod (6.12), adding the water in small amounts to avoid the formation of lumps. Rinse the edges of the beaker and then the stirring rod with demineralized water. Then place the stirring rod in a container to protect it from dust, for example, in a cylinder.

8.2.2 Place the beaker on the magnetic stirrer (6.13). Introduce the magnetic bar, previously rinsed in demineralized water, and then regulate the stirrer to a low speed of rotation. Add to the solution 20 ml of concentrated hydrochloric acid (5.4), measured in a graduated cylinder (6.4). Switch on the heating element and slowly bring the contents of the beaker (6.2) to boiling point (to avoid carbonization due to formation of a starch paste). When a smooth paste has been achieved, add 30 ml of paraffin oil (5.5) measured in a graduated cylinder (6.4). Allow to boil for 30 min with gentle stirring.

8.2.3 Place the beaker, again covered with its watch glass (6.2), in the crystallizing dish or pan (6.3) filled with cold water until a near-ambient temperature is obtained.

8.2.4 See Annex C for a diagram of the procedure.

8.3 Separation of impurities

8.3.1 Set up the two separating funnels (6.1) in such a way that the upper funnel can drain directly into the lower funnel (see Figure 1).

8.3.2 Pour 30 ml of paraffin oil (5.5) into the lower separating funnel.

8.3.3 Remove the magnetic bar from the beaker and rinse it using the ethanol solution (5.2), collecting the rinsings in the beaker. Transfer the contents of the beaker into the upper separating funnel. Rinse the stirring rod and the walls of the beaker using the washing bottle (6.5) with the ethanol solution (5.2), carefully scraping the walls of the beaker with the stirring rod, and transfer the rinsings to the upper separating funnel. If necessary, the cleaning operation should be completed with the ethanol solution (5.2) using the same procedure as described above.

8.3.4 Remove the upper separating funnel from its support and swirl the contents for 2 min using a circular motion so as to cause the liquid to flow in a thin layer around the walls. Replace the separating funnel on its support, rinse the walls of the funnel with the ethanol solution (5.2) and leave it to stand for at least 1 h.

8.3.5 Drain off the major part of the aqueous phase into the lower separating funnel, allowing a few millilitres to remain in the upper funnel (i.e. a layer about 3 cm thick).

8.3.6 Remove the lower separating funnel from its support and swirl the contents in the same way as described in 8.3.4.

8.3.7 Add directly to the upper separating funnel about 300 ml of the ethanol solution (5.2), allowing the solution to run down the wall.

8.3.8 Remove the upper separating funnel from its support and swirl the contents in the same way as described in 8.3.4.

8.3.9 Leave the two separating funnels to stand for at least 1 h.

8.3.10 Discard the major part of the aqueous phase in the lower separating funnel allowing a few millilitres to remain (which represents a layer of about 3 cm).

8.3.11 Repeat steps 8.3.5 to 8.3.8.

8.3.12 Leave the two separating funnels to stand for at least 30 min and drain off the aqueous phase.

8.3.13 Add to each separating funnel about 50 ml of dearomatized white spirit (5.8) or about 20 ml of non-foaming liquid detergent solution (5.7).

8.3.14 The contents of the two funnels will be ready for filtration at the same time. If necessary, repeat steps 8.3.10, 8.3.5 and 8.3.6. Leave the lower separator funnel to stand for at least 30 min.

8.4 Filtration

8.4.1 Place the filter paper prepared as per 6.6 in the filtration unit (6.7) mounted on the filtration flask (6.15) connected to the vacuum pump (6.17). Moisten the filter and switch on the vacuum pump.

8.4.2 Transfer the contents of the two separating funnels directly into the filtration unit and rinse them carefully using the non-foaming detergent solution (5.7).

NOTE To facilitate reading, filtration can be performed on a number of filters.

8.4.3 Switch off the pump. Remove the filter with the clip (6.14) and place it in the bottom of a Petri dish containing a few drops of the ethanol/glycerol mixture (5.3).

NOTE It is possible to dry the filters using the oven (6.18).

8.5 Microscopic examination

IMPORTANT — The operator shall be capable of recognizing the debris of insects and/or mites dispersed on the filter as distinct from fragments of bran in the flour.

Using the microscope (6.9), identify the following impurities on each ruled band of the filter:

a) rodent hair and fragments of rodent hair;

b) whole insects (larva, nymph or adult);

c) insect fragments (including butterfly scales);

d) whole mites.

Count the number of impurities of size greater than 30 µm in each category.

Fragment identification may be facilitated by use of a mounted needle (6.11) to probe the various organic substances present on the filter.

Optionally also note the presence and nature of any impurities that are not of human or animal origin, and impurities of human or animal origin that are not specified in categories a) to d).

Annex A provides information on the characteristics of fragments found on the filters.

Annex D provides a chronology of operations and timetable for information.

8.6 Number of determinations

Carry out two determinations on test portions taken from the same laboratory sample.

9 Expression of results

If the repeatability requirement is satisfied (see Clause 10), express the results separately for each test as the number of impurities found in each category.

If the repeatability requirement is not satisfied, carry out two new determinations after homogenization of the laboratory sample.

10 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material tested in the same laboratory by the same operator using the same apparatus and within a short interval of time, shall not be greater than 10 fragments if the average number of fragments is lower than 50. In other cases, the repeatability limit is 20 % of the average number of fragments.

11 Test report

The test report shall include all information necessary for the complete identification of the sample.

It shall specify the following:

— the sample;

— the International Standard used (including its year of publication);

— the method used (if the standard includes several);

— the result(s), including a reference to the clause that explains how the results were calculated;

— any deviations from the procedure;

— any unusual features observed;

— the date of the test.

An example is given in Annex B.

Annex A
(informative)

Characteristics of fragments found on the filters

The distinction between vegetable and animal fragments is based on their general appearance and structural characteristics.

The complete juvenile stages of insects are easily recognized. Fragments of insects and some heavily sclerotized mites range in colour from light brown to grey/brown, and have a surface of shiny appearance or of a pattern of small knobs, depressions, pits or regular striations. Food storage mites are usually translucent white. Fragments of vegetable origin have a matt appearance and are generally a light reddish brown in colour.

Those insect fragments found will originate more often than not from the appendages (legs and antennae) or from particularly robust parts of the body such as the mandibles. In the case of fragments from other parts of the body (e.g. head, wing cases, abdomen), these can be distinguished by their translucent appearance and, in the case of the largest, by their construction, which is in the form of juxtaposed plates. Unlike the fragments of the pericarp of seeds, which exhibit cellular walls (visible at the magnifications indicated in the procedure) with thick cellulose membranes, no cellular structure can be distinguished in the cuticle of insects upon examination under a microscope. The surface of insect fragments generally exhibits fine hollow spotting in an irregular pattern, strewn with small circular depressions, at the centre of which it is occasionally possible to distinguish the base of hairs or bristles (see Figure A.1).

Rodent hairs have an internal structure in the form of transverse black streaks of irregular shape occurring over the entire length of the hair. These streaks can be more or less distinct, depending on the state of digestion of the hair. However, human hairs and the hairs of domestic animals exhibit a continuous structure without streaks.

Whole insect cuticles have the form of very thin fragments, which can be quite large in size. They are quite easy to identify since they still carry hairs (bristles) or exhibit the characteristic forms of the organ they enclosed (cephalic capsule for the head, form of the limbs, circular hooks on the false legs of caterpillars, etc.).

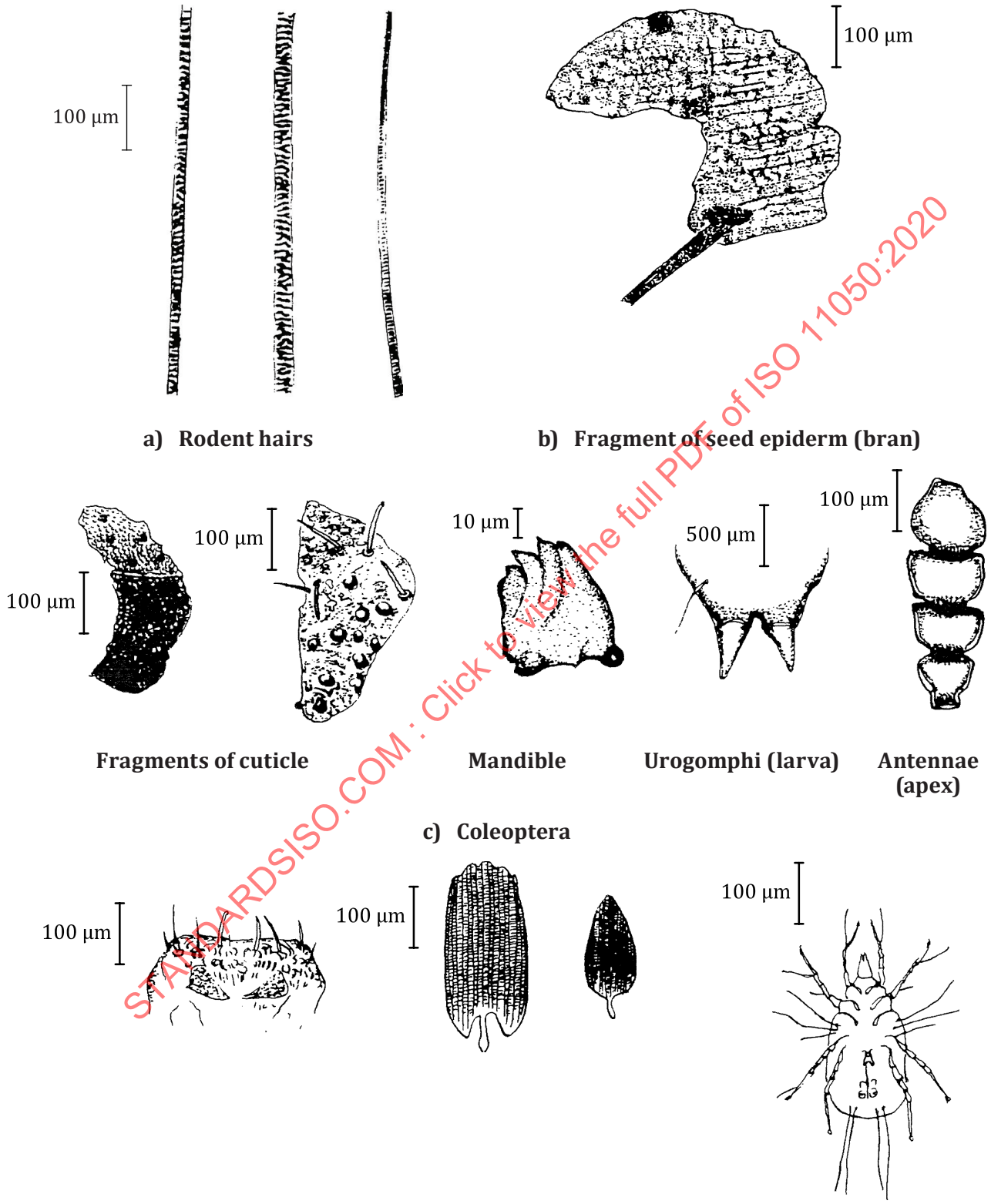


Figure A.1 — Different types of fragment found on the filters

Annex B
(informative)

Test report — Determination of animal impurities in accordance with this document

B.1 Identification of the sample

— Type of product:

— Sample reference:

— Date of reception of the sample:

— Date of analysis:

B.2 Results of quantifications

Test portion no.	1	2
Rodent hair and fragments of rodent hair		
Whole insects (larva, nymph or adult)		
Insect fragments (including butterfly scales)		
Whole mites		

B.3 Observations

— International Standard used (including year of publication):

— Method used (if the standard includes several):

— Reference to the clause that explains how the results were calculated:

— Any deviations from the procedure:

— Any unusual features observed:

Annex C
(informative)

Diagram of the procedure