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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
ISO 11050:1993(E)

Foreword

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Annexes A, B, C and D of this International Standard are for information only.

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

1 Scope

This International Standard 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 0,63 % (*m*/*m*), and in durum wheat semolinas.

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

2 Definition

For the purposes of this International Standard, the following definition applies.

2.1 impurities of animal origin: Matter of animal origin (eggs, larvae, nymphs or adults of insects and their fragments, rodent hairs and their fragments, mites and their fragments) separated from the product under the conditions specified in this International Standard.

3 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/hydrocarbon interface. Separation by filtration on a filter paper or membrane, microscopic examination, and counting under reflected light, of the impurities of animal origin.

4 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

All the reagents used shall be filtered carefully before use or after their preparation. Such filtration may be performed using a filter cloth with a maximum mesh size of 10 µm to 30 µm and which is resistant to acids and solvents (of the nylon or polyethylene fibre type).

4.1 Ethanol or **methanol**, 95 % (*V*/*V*).

4.2 Ethanol or **methanol solution**, 50 % (*V*/*V*).

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

4.4 Hydrochloric acid solution, concentrated (ρ_{20} = 1,18 g/ml).

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

4.6 Liquid detergent, non-foaming.

4.7 Liquid detergent, 1 % (*V*/*V*) solution of the detergent (4.6) in a washing bottle.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Separating funnels, conical, of 1 000 ml capacity, fitted with a non-lubricated tap with a flexible tube and a Mohr clip (rubber-tube clip) (see the recommended set-up shown in figure 1).

5.2 Tall-form beaker, of 800 ml capacity, fitted with a watch glass made of pyrex and of appropriate dimensions to serve as a lid.

5.3 Crystallizing dish or **pan**, of at least 5 litre capacity, and of a height slightly less than that of the beaker (5.2), suitable for use as a cooling bath.

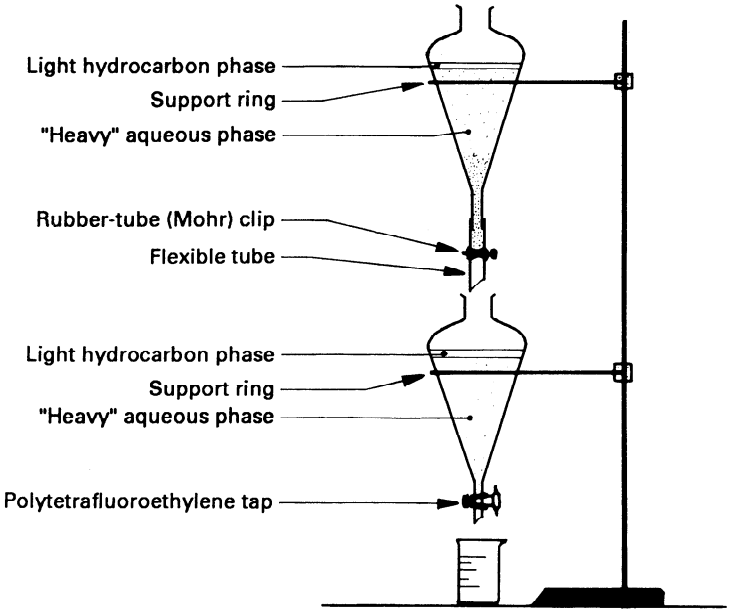

Figure 1 — Separation apparatus

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

5.5 Washing bottles, of 1 litre capacity, graduated in 50 ml increments, and fitted with a flexible tube.

5.6 Stretchable protective film, waxed or made of a plastic material.

5.7 Filter paper, ash-free, with rapid filtration characteristics[1], of diameter corresponding to that of the filtration unit (5.8) (i.e. 50 mm or 90 mm), or **filtration membrane**, of 47 mm to 50 mm diameter, made of cellulose nitrate and having a porosity of 5 µm or 8 µm, on which fine parallel lines are drawn, spaced 5 mm apart, using a ball-point pen or hard lead pencil.

5.8 Filtration unit, of the Büchner funnel type, suitable for accommodating the filter (5.7), and fitted with a conical adaptor bung for connection to the filtration flask (5.16).

5.9 Analytical balance, accurate to within 0,1 g.

5.10 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

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

5.11 Petri dish, sterile, made of plastic or glass, with a diameter of 90 mm.

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

5.13 Glass rod, fitted with a rubber or plastic protective end.

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

5.15 Spring clips, suitable for holding the filter papers or filtration membranes (5.7).

5.16 Filtration flask, of 1 litre capacity, capable of being connected preferably to the vacuum pump (5.18), or to a water suction pump (5.18).

5.17 Dropper

5.18 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**.

1) Whatman 41 is an example of a suitable filter paper available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

NOTE 1 The duration of filtration will need to be increased considerably if a water suction pump is used.

5.19 Oven, capable of being maintained at 37 °C to 40 °C.

6 Sampling

For the purposes of this test method, it is essential that all equipment used for sampling was thoroughly cleaned between each sampling operation by using, for example, filtered compressed air and **not** by using brushes or textile materials.

It is strongly recommended that users of this International Standard ascertain, where possible, that this requirement was met during the sampling procedure.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 2170[2].

A laboratory sample of at least 600 g is required.

7 Procedure

IMPORTANT — All handling operations shall take place in clean premises, away from air currents, or preferably beneath a non-ventilated canopy. All the apparatus shall be washed in filtered water, rinsed, drained until dry and then covered with a protective film (5.6) until use.

7.1 Test portion

With the laboratory sample still in its packaging, mix it thoroughly using a long-handled spatula. By taking samples from several places, weigh 50 g of the product into the beaker (5.2).

7.2 Hydrolysis

7.2.1 Add 100 ml of filtered water, a little at a time, to the test portion in the beaker, whilst stirring continuously with the glass rod (5.13) to avoid the formation of lumps. Rinse the sides of the beaker and the glass rod with 200 ml of filtered water. Then place the glass rod in a container to protect it from dust, for example in a cylinder fitted with a lid.

7.2.2 Place the beaker on the magnetic stirrer (5.14). Introduce the magnetic bar, previously rinsed in filtered water, and then regulate the stirrer to a low speed of rotation. Add to the solution, a little at a time, 20 ml of concentrated hydrochloric acid (5.4), measured in a graduated cylinder (5.4). Cover the beaker with a watch glass. Switch on the heating element of the magnetic stirrer and slowly bring the contents of the beaker 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 (4.5) measured in a graduated cylinder (5.4). Allow to boil for 30 min, with gentle stirring.

7.2.3 Cover the beaker with protective film (5.6) and allow the contents to cool to near-ambient temperature in the crystallizing dish or pan (5.3) in which cold water is circulating.

7.3 Separation of impurities

7.3.1 Set up the separating funnels (5.1) in such a way that the upper funnel drains directly into the lower funnel (see figure 1).

7.3.2 Pour 30 ml of paraffin oil (4.5) into the lower separating funnel.

7.3.3 Remove the magnetic bar from the beaker and rinse it using the alcohol solution (4.2), collecting the rinsings in the beaker. Transfer the contents of the beaker with the aid of the glass rod (7.2.1) into the upper separating funnel. Rinse the glass rod and the walls of the beaker using the washing bottle (5.5), with 30 ml to 50 ml of the alcohol solution (4.2), scraping carefully the walls of the beaker with the glass rod, and transfer the rinsings to the upper separating funnel. If necessary, the cleaning operation should be completed using about 10 ml of ethanol or methanol (4.1), using the same procedure as described above.

7.3.4 Make up the contents of the upper separating funnel with the alcohol solution (4.2) in such a way that the level of the liquid reaches the widest part of the funnel (100 ml to 250 ml of the alcohol solution will have to be added, depending on the quantities used during rinsing).

Remove the separating funnel from its support and, keeping it vertical, 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 and leave it to stand for at least 1 h.

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

7.3.6 Remove the lower separating funnel from its support and swirl the contents in the same way as described in 7.3.4 for the upper separating funnel. Replace the separating funnel on the stand and leave it to stand for 1 h.

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2) ISO 2170:1980, *Cereals and pulses — Sampling of milled products.*

7.3.7 Discard the major part of the aqueous phase, allowing a few millilitres (i.e. a layer about 3 cm thick) to remain in the lower funnel.

7.3.8 Add directly to the upper separating funnel 300 ml of the alcohol solution (4.2), allowing the solution to run down the wall. Swirl the contents for 2 min, in the same way as described in 7.3.4, and leave to stand for 1 h.

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

7.3.10 Add 300 ml of the alcohol solution (4.2) to each of the separating funnels, allowing the solution to run down the wall. Swirl the contents of each funnel for 2 min, in the same way as described in 7.3.4, and leave to stand for 30 min.

7.3.11 Discard the major part of the aqueous phase in each funnel, allowing a few millilitres to remain.

7.3.12 Repeat the operations described in 7.3.10 if necessary.

NOTE 2 The contents of the two funnels will be ready for filtration at approximately the same time.

7.4 Filtration

7.4.1 Place the filter (5.7) in the filtration unit (5.8). Attach the unit to the filtration flask (5.16) and connect the flask to the vacuum pump (5.18). Moisten the filter with a small quantity of paraffin oil (4.5) and switch on the vacuum pump.

7.4.2 Transfer the contents of the two separating funnels directly into the filtration unit.

7.4.3 Add, using the dropper (5.17), about four drops of the detergent (4.6) to the upper separating funnel, and then add 10 ml of filtered water. Fit a bung to the funnel and mix the contents vigorously by swirling them around the wall and inverting the funnel several times.

Replace the separating funnel on its support and allow the washing product to flow into the lower separating funnel. Fit a bung to the lower separating funnel and mix its contents as described above. Replace the separating funnel on its support, and allow the rinsing product to flow into the filtration apparatus.

7.4.4 Rinse the walls of each separating funnel, using the washing bottle (5.5), with 20 ml of the alcohol solution (5.2), rinsing first the upper separating funnel and then the lower separating funnel. Allow the solution to flow onto the filter in the filtration unit and rinse the reservoir of the filtration unit with the alcohol solution. Rinse the base of the cylindrical part with ethanol or methanol (4.1), and then, using the washing bottle, with a small quantity of the detergent solution (4.7) in order to cause any impurities which frequently remain behind at this point to pass on to the filter.

7.4.5 Remove the filter using the spring clip (5.15), and place it in the bottom of a Petri dish. Place the dish, partly covered with its lid or covered with an inverted funnel (in order to avoid accidental pollution) in the oven (5.19) set at 37 °C to 40 °C. Once the filter is dry, moisten it with a few drops of the ethanol/glycerol mixture (4.3), using the dropper (5.17).

7.5 Microscopic examination

(See annex A and annex D.)

IMPORTANT — The operator shall be capable of differentiating the debris of insects or mites from fragments of pericarp which are present in the flour, sometimes in large quantities.

Using the microscope (5.10) at a magnification of × 25 and then × 50, identify the following impurities on each ruled band of the filter:

a) rodent hair and fragments of hair;

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

c) insect fragments (including butterfly scales), insect eggs, whole mites and their fragments.

Count the number of impurities of size greater than 30 µm in each category and, if required, determine the minimum size of the impurities in each category by using the micrometer eyepiece. If required, the impurities grouped together in category c) may be quantified separately.

It may be necessary to use a magnification of × 75 or × 80 to study impurities which are difficult to identify.

It may be useful to use a mounted needle (5.12) to probe the various organic substances present on the filter or to move them into a clean area in the centre of the filter.

Note also the presence and nature of any impurities which are not of human or animal origin and of any impurities of human or animal origin but which are not specified in the categories a) to c) above. Give a detailed description of such impurities for specification in the test report (for example, coloured threads of a synthetic material, metallic debris, mineral particles, human hairs, cat fur, bird feathers or down, etc.).

7.6 Number of determinations

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

8 Expression of results

Provided that the repeatability requirement (clause 9) is satisfied, express the results separately for each filter 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.

If at least one rodent hair or fragment of rodent hair is found in one of the test portions, carry out four new determinations and report the results separately for each of the six determinations.

9 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, shall not be greater than 10 fragments.

10 Test report

(See the example given in annex B.)

The test report shall specify

— the method of sampling (if known) and whether the special requirements of clause 6 were met,

— the method used,

— the test result(s) obtained, and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results.

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

Annex A
(informative)

Definitions and characteristics of fragments found on the filters

A.1 Definitions

For the purposes of this annex, the following definitions apply.

A.1.1 abdomen: Rear part of the body of an insect, excluding head and thorax, commonly with eight or more segments when complete.

A.1.2 appendices: Distinctly differentiated prolongations of the body of an arthropod; e.g. legs, wings, antennae, urogomphi.

A.1.3 bristles: Fine but stiff hairs of any length present on the cuticle of insects.

NOTE 3 Special sensory hairs are called setae.

A.1.4 caterpillars: Larvae of *Lepidoptera* spp.

NOTE 4 Butterfly or moth is the adult stage and the chrysalis is the pupal stage.

A.1.5 cephalic capsule: Sclerous part of an exuvia which once contained the head of a larva. Also called head capsule.

A.1.6 ecdysis; exuviation: Casting of the juvenile insect cuticle, thereby allowing growth to take place.

NOTE 5 The old cuticle is called the exuvia.

A.1.7 exuviae: Cuticle cast during ecdysis.

A.1.8 false legs; pro-legs: Fleshy extensions of the lower part of the abdomen of some larvae, sometimes with a crown of fine hooks (crochets) of chitin. 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.

A.1.9 feelers: A non-scientific term for the sensory appendages situated on the head capsule of insects. They may be close to the eyes, and called antennae, or associated with the mouthparts and generally called palps.

A.1.10 insects: A class of animals within the phylum Arthropoda, some of which are recognized pests of stored foodstuffs.

A.1.11 juvenile stages: The pre-adult stages of insects; e.g. egg, larva, nymph and pupa.

NOTE 6 This term is most often applied to the active stages of larva and nymph.

A.1.12 labrum: The upper lip, covering the mouth opening, of some larval and adult insects.

A.1.13 mandibles: Toughened (sclerotized) mouthparts of insects, used for dilaceration or grinding of food.

A.1.14 mites: Very small arthropods belonging to the class *Arachnida*, sub-class *Acarina*, often living in large numbers.

A.1.15 pericarp: External envelope of seeds which forms the bran after the grain has been crushed and the flour separated.

A.1.16 scales: Bristles which have evolved into flat structures resembling fish scales and which cover parts of the body of certain insects, in particular the wings of *Lepidoptera* spp.

A.1.17 stage: The state of development of an insect or mite; e.g. egg, larva, nymph, pupa, adult.

A.1.18 urogomphi: Pointed extensions of the cuticle of the final adbominal segment of some insect larvae. They are common, and sometimes diagnostic, features of many *Coleoptera* spp.

NOTE 7 The abdominal extensions of a cockroach are called cerci.

A.1.19 wing case; elytron: Hardened front wing of *Coleoptera* spp., used as fixed wing in flight and as protective cover for the membranous hind wing.

A.2 Characteristics

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

The complete juvenile stages of insects and mites 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. The 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 or mite fragments which are 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, etc.), 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 the entire length of the hair. These streaks may be more or less distinct, depending on the state of digestion of the hair. However, human hairs and the fur of domestic animals exhibit a continuous structure without streaks.

Whole insect cuticles have the form of very thin fragments which may 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 which they enclosed (cephalic capsule of the head, form of the limbs, circular hooks on the false legs of caterpillars, etc.).