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Animal and vegetable fats and oils — Determination of unsaponifiable matter —

Part 1 :
Method using diethyl ether extraction (Reference method)

Corps gras d'origines animale et végétale — Détermination de la teneur en matières insaponifiables —

Partie 1 : Méthode par extraction à l'oxyde diéthylique (méthode de référence)

Reference number
ISO 3596-1 : 1988 (E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 3596-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

ISO 3596 consists of the following parts, under the general title *Animal and vegetable fats and oils — Determination of unsaponifiable matter*:

Part 1 : Method using diethyl ether extraction (Reference method)

Part 2 : Rapid method using hexane extraction

Annex A of this part of ISO 3596 is for information only.

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Animal and vegetable fats and oils — Determination of unsaponifiable matter —

Part 1 :
Method using diethyl ether extraction (Reference method)

1 Scope

This part of ISO 3596 specifies the reference method using diethyl ether extraction for the determination of the unsaponifiable matter content of animal and vegetable fats and oils.

This method is not applicable to waxes and moreover gives approximate results with certain fats of high unsaponifiable matter content, for example with fats derived from marine animals.

Annex A describes a method using nine hexane extractions which gives similar results for many animal and vegetable fats and oils, but because of differences of solubility of some constituents of the unsaponifiable matter in the two solvents, the results may not be identical, the hexane method giving lower results. The hexane method may be used when climatic conditions, or regulations, do not permit the use of diethyl ether.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 3596. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 3596 are encouraged to investigate the possibility of applying the most recent editions of the standards listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 661 : 1980, *Animal and vegetable fats and oils — Preparation of test sample.*

ISO 5555 : 1983, *Animal and vegetable fats and oils — Sampling.*

3 Definition

For the purposes of this part of ISO 3596, the following definition applies.

unsaponifiable matter : All the substances present in the product which, after saponification of the latter by potassium hydroxide and extraction by a specified solvent, are not volatile under the specified operating conditions.

NOTE — The unsaponifiable matter includes lipids of natural origin such as sterols, higher hydrocarbons and alcohols, aliphatic and terpenic alcohols, as well as any foreign organic matter extracted by the solvent and not volatile at 103 °C (e.g. mineral oils) that may be present.

4 Principle

Saponification of the fat or oil by boiling under reflux with an ethanolic potassium hydroxide solution. Extraction of the unsaponifiable matter from the soap solution by diethyl ether. Evaporation of the solvent and weighing of the residue after drying.

5 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled water or water of at least equivalent purity.

5.1 Diethyl ether, freshly distilled, free from peroxides and residue.

5.2 Acetone.

5.3 Potassium hydroxide, ethanolic solution, $c(KOH) \approx 1$ mol/l.

Dissolve 60 g of potassium hydroxide in 50 ml of water and dilute to 1 000 ml with 95 % (*V*/*V*) ethanol. The solution should be colourless or straw-yellow.

5.4 Potassium hydroxide, aqueous solution, $c(KOH) \approx 0{,}5$ mol/l.

5.5 Phenolphthalein, 10 g/l solution in 95 % (*V*/*V*) ethanol.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Round-bottomed flasks, of 250 ml capacity, with ground neck.

6.2 Reflux condenser, with ground joint to fit the flasks (6.1).

6.3 Separating funnels, of 500 ml capacity, with stopcock and stopper made of polytetrafluoroethylene.

6.4 Boiling water-bath.

6.5 Oven, capable of being maintained at 103 °C ± 2 °C.

7 Sampling

Sampling shall be carried out in accordance with ISO 5555.

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

9 Procedure

9.1 Test portion

Weigh, to the nearest 0,01 g, about 5 g of the test sample (clause 8) into a 250 ml flask (6.1).

9.2 Saponification

Add 50 ml of the potassium hydroxide solution (5.3) and a few anti-bumping granules. Attach the reflux condenser (6.2) to the flask and boil the contents gently for 1 h. Stop heating. Add 100 ml of water through the top of the condenser and swirl.

NOTE — If the extraction of the unsaponifiable matter is carried out with a view to the determination of the composition of tocopherols, the addition of pyrogallol is necessary and the extraction should be completed quickly (say, within 30 min).

9.3 Extraction of the unsaponifiable matter

After cooling, transfer the solution to a 500 ml separating funnel (6.3). Rinse the flask and the anti-bumping granules several times with the diethyl ether (5.1), using 100 ml in all, and pour these rinsings into the separating funnel. Stopper and shake vigorously for 1 min, periodically releasing pressure by inverting the separating funnel and cautiously opening the stopcock.

Allow to stand until there is complete separation of the two phases. Then run off the lower layer as completely as possible into a second separating funnel.

NOTE — If an emulsion is formed, destroy it by adding small quantities of ethanol or concentrated potassium hydroxide or sodium chloride solution.

Extract the aqueous ethanolic soap solution twice more, each time in the same way with 100 ml of the diethyl ether. Collect the three ether extracts in one separating funnel containing 40 ml of water.

9.4 Washing of the ethereal extract

Gently rotate the separating funnel containing the combined extracts and the 40 ml of water.

NOTE — Violent shaking at this stage may result in emulsions.

Allow the layers to separate completely and draw off the lower aqueous layer. Wash the ethereal solution twice more with 40 ml portions of water, shaking vigorously each time and discarding the lower aqueous layer after separation. Draw off each washing solution leaving 2 ml, then rotate the separating funnel around its axis. Wait some minutes to allow the remaining aqueous layer to collect. Draw this off, closing the stopcock when the ethereal solution reaches the bore of the stopcock.

Wash the ethereal solution successively with 40 ml of the potassium hydroxide solution (5.4), 40 ml of water, and again with 40 ml of potassium hydroxide solution, then at least twice more with 40 ml of water.

Continue to wash with water until the washings no longer give a pink colour on the addition of a drop of the phenolphthalein solution (5.5).

9.5 Evaporation of the solvent

Transfer the ethereal solution quantitatively, a little at a time, through the top of the separating funnel into a 250 ml flask (6.1), previously dried at 103 °C ± 2 °C in the oven (6.5), cooled and weighed to the nearest 0,1 mg. Evaporate the solvent on a boiling water-bath (6.4).

Add 5 ml of acetone (5.2) and evaporate the volatile solvent completely in a gentle current of air, holding the flask obliquely while turning it in a boiling water-bath.

9.6 Drying the residue and determination

Dry the residue in the oven (6.5) at 103 °C ± 2 °C for 15 min, with the flask in an almost horizontal position. Allow to cool in a desiccator and weigh to the nearest 0,1 mg.

Repeat the drying for successive 15 min periods until the loss of mass between two successive weighings is less than 1,5 mg. If

a constant mass is not obtained after three periods of drying, the unsaponifiable matter is probably contaminated and the determination shall be repeated.

NOTES

1 If available, a vacuum rotary evaporator may be used, particularly if the unsaponifiable matter is to be examined further.

2 If a correction for free fatty acids is considered necessary, after weighing the residue dissolve it in 4 ml of the diethyl ether (5.1) and then add 20 ml of ethanol previously neutralized to a faint pink colour in the presence of the phenolphthalein (5.4) as indicator. Titrate with standard volumetric ethanolic potassium hydroxide solution, c(KOH) = 0,1 mol/l, to the same final colour. Calculate the mass of free fatty acids as oleic acid and correct the mass of the residue accordingly (see clause 10).

9.7 Number of determinations

Carry out two determinations on the same test sample.

9.8 Blank test

Carry out a blank test, using the same procedure and the same quantities of all the reagents, but omitting the test portion. If the residue exceeds 1,5 mg, investigate the technique and the reagents.

10 Expression of results

The unsaponifiable matter content, expressed as a percentage by mass of the sample, is equal to

$$\frac{100\,(m_1 - m_2 - m_3)}{m_0}$$

where

m_0 is the mass, in grams, of the test portion;

m_1 is the mass, in grams, of the residue;

m_2 is the mass, in grams, of the residue obtained with the blank;

m_3 is the mass, in grams, of free fatty acids, if any (see 9.6, note 2), and equals 0,28 Vc

where

V is the volume, in millilitres, of the standard volumetric ethanolic potassium hydroxide solution used for titration;

c is the exact concentration, in moles per litre, of the standard volumetric ethanolic potassium hydroxide solution.

Take as the result the arithmetic mean of the two determinations.

11 Test report

The test report shall specify the method used and the result obtained. It shall also mention all operating details not specified in this part of ISO 3596, or regarded as optional, together with details of any incidents which may have influenced the result.

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

Annex A
(informative)

Method using nine hexane extractions

A.1 Principle

Saponification of the fat or oil by boiling under reflux with an ethanolic potassium hydroxide solution. Extraction of the unsaponifiable matter from the soap solution by hexane or, failing this, light petroleum. Evaporation of the solvent and weighing of the residue after drying.

A.2 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled water or water of at least equivalent purity.

A.2.1 ***n*-Hexane** or, failing this, **light petroleum,** distilling between 40 °C and 60 °C, bromine number less than 1. Both solvents shall be free from residue.

A.2.2 Ethanol, 50 % (*V*/*V*), neutral (colourless) to phenolphthalein.

A.2.3 Potassium hydroxide, ethanolic solution, $c(KOH) \approx 4$ mol/l, recently prepared as follows.

Dissolve 60 g of potassium hydroxide in 95 % (*V*/*V*) ethanol and dilute to 250 ml. The solution should be colourless or straw-yellow.

A.2.4 Potassium hydrogencarbonate, 10 g/l aqueous solution.

A.2.5 Phenolphthalein, 10 g/l solution in 95 % (*V*/*V*) ethanol.

A.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

A.3.1 Round-bottomed flasks, of 250 ml capacity, with ground neck.

A.3.2 Reflux condenser, with ground joint to fit the flasks (A.3.1).

A.3.3 Separating funnels, of 250 ml and 1 000 ml capacity, with stopcocks and stoppers made of polytetrafluoroethylene.

A.3.4 Boiling water-bath.

A.3.5 Oven, capable of being maintained at 103 °C ± 2 °C, or **apparatus for drying under vacuum,** e.g. rotary evaporator or similar apparatus.

A.4 Sampling

Sampling shall be carried out in accordance with ISO 5555.

A.5 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

A.6 Procedure

A.6.1 Test portion

Weigh, to the nearest 0,01 g, about 5 g of the test sample (clause A.5) into a 250 ml flask (A.3.1).

A.6.2 Saponification

Add 25 ml of the potassium hydroxide solution (A.2.3) and some anti-bumping granules. Attach the reflux condenser (A.3.2) to the flask and boil the contents gently for 1 h. Stop heating. Add 50 ml of the potassium hydrogencarbonate solution (A.2.4) through the top of the condenser and swirl.

NOTE — If the extraction of the unsaponifiable matter is carried out with a view to the determination of the composition of tocopherols, the addition of pyrogallol is necessary and the extraction should be completed quickly (say, within 30 min).

A.6.3 Extraction of the unsaponifiable matter

After cooling, transfer the solution to a 250 ml separating funnel (A.3.3). Rinse the flask and the anti-bumping granules several times with the hexane (A.2.1), using 50 ml in all, and pour these rinsings into the separating funnel. Stopper and shake vigorously for 1 min, periodically releasing pressure by inverting the separating funnel and cautiously opening the stopcock.

Allow to stand until there is complete separation of the two phases. Then run off the lower layer as completely as possible into a second separating funnel.

NOTE — If an emulsion is formed, destroy it by adding small quantities of ethanol or concentrated potassium hydroxide or sodium chloride solution.