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МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

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

Part 2 :
Rapid method using hexane extraction

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

Partie 2 : Méthode rapide par extraction à l'hexane

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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 3596-2 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

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

Part 2 : Rapid method using hexane extraction

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1 Scope

This part of ISO 3596 specifies a rapid method using three hexane extractions for the determination of the unsaponifiable matter content of animal and vegetable fats and oils.

The method is applicable to all fats and oils but not to waxes. In comparison with the reference method given in ISO 3596-1, however, it gives results which are systematically low, particularly with certain fats and oils having a high unsaponifiable matter content, for example fats and oils of marine animals.

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 hexane, 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 hexane or, failing this, light petroleum. 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 ***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.

5.2 **Ethanol**, 10 % (V/V) solution.

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

5.4 **Potassium hydroxide**, ethanolic solution, $c(\mathrm{KOH}) \approx 1$ 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.

6 Apparatus

Usual laboratory equipment 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 250 ml capacity, with stopcocks and stoppers made of polytetrafluoroethylene.

6.4 Boiling water-bath.

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

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 (5.4) and some anti-bumping granules. Attach the reflux condenser (6.2) to the flask and boil the contents gently for 1 h. Stop heating. Add 50 ml of water through the top of the condenser and swirl.

9.3 Extraction of the unsaponifiable matter

After cooling, transfer the solution to a 250 ml separating funnel (6.3). Rinse the flask and the anti-bumping granules several times with the hexane (5.1), 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.

Extract the aqueous ethanolic soap solution twice more, each time in the same way with 50 ml of the hexane. Collect the three hexane extracts in one separating funnel.

9.4 Washing of the hexane extract

Wash the combined extracts three times with 25 ml portions of the ethanol solution (5.2), shaking vigorously and drawing off the aqueous ethanolic solution after each wash. Draw off each washing solution leaving 2 ml, then rotate the separating funnel around its axis. Wait some minutes to allow the remaining aqueous ethanolic layer to collect. Draw this off, closing the stopcock when the hexane solution reaches the bore of the stopcock.

Continue to wash with the ethanol solution until the washings no longer give a pink colour on the addition of a drop of the phenolphthalein solution (5.3).

9.5 Evaporation of the solvent

Transfer the hexane solution quantitatively, a little at a time if necessary, 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).

9.6 Drying the residue and determination

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

Alternatively, attach the flask to the apparatus for drying under vacuum (6.5) and dry on the boiling water-bath under the maximum vacuum of the water pump for about 15 min. Allow to cool to room temperature under the maximum vacuum of the water pump, carefully wipe the flask, 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 constant mass is not obtained after three periods of drying, the unsaponifiable matter is probably contaminated and the determination shall be repeated.

NOTE — If a correction for free fatty acids is considered necessary, after weighing the residue dissolve it in 4 ml of the diethyl ether and then add 20 ml of ethanol previously neutralized to a faint pink colour in the presence of the phenolphthalein (5.3) 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

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

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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), and equals 0,28 Vc

where

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

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