INTERNATIONAL STANDARD

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Animal and vegetable fats and oils — Determination of unsaponifiable matter — Method using hexane extraction

Corps gras d'origines animale et végétale — Détermination de la teneur en matières insaponifiables — Méthode par extraction à l'hexane

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 18609 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This first edition of ISO 18609 cancels and replaces ISO 3596-2:1988 and its Amendment 1:1999, of which it constitutes a minor revision.

Annex A of this International Standard is for information only.

Animal and vegetable fats and oils — Determination of unsaponifiable matter — Method using hexane extraction

1 Scope

This International Standard specifies a 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.

CAUTION — In comparison with the method given in ISO 3596, however, the present method gives results which are systematically low.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

3 Terms and definitions

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

3.1
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

The fat or oil is saponified by boiling under reflux with an ethanolic potassium hydroxide solution. The unsaponifiable matter is extracted from the soap solution by hexane or, failing this, light petroleum. The solvent is evaporated and the residue is weighed after drying.

5 Reagents

Use only reagents of recognized analytical grade, and distilled or deionized water or water of 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 % (by volume) solution.

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

5.4 **Potassium hydroxide,** ethanolic solution, c(KOH) ≈ 1 mol/l.

Dissolve 60 g of potassium hydroxide in 50 ml of water and dilute to 1 000 ml with 95 % (by volume) 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 is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555 [2].

It is important the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of 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 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.

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 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 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 in the oven (6.5), then 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

9.6.1 Dry the residue for 15 min in the oven (6.5) set at 103 °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.

9.6.2 If a correction for free fatty acids is considered necessary, after weighing the residue dissolve it in 4 ml of the hexane (5.1) 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

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

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 and equals 0,28 Vc

where

V is the volume, in millilitres, of the standard volumetric ethanolic potassium hydroxide solution used for the titration (9.6.2);

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 Precision

Details of interlaboratory tests on the precision of the method are summarized in annex A. The values derived from these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory tests

A.1 Participation

Fourteen laboratories from six different countries (France, Germany, Hungary, Malaysia, Netherlands, United Kingdom) took part in a collaborative study organized by the Institut des corps gras Centre technique industriel.

A.2 Samples

Three samples were provided:

— Sample A: crude sunflower oil;

— Sample B: crude palm oil;

— Sample C: crude tallow.

A.3 Results

Tables A.1, A.2 and A.3 give the results obtained by the laboratories for the three samples A, B and C. Table A.4 gives the statistical results, sample by sample.

Two laboratories were eliminated for sample A on basis of the Cochran (Laboratory 6) and Dixon (Laboratory 9) tests, one for sample C on basis of the Dixon test (Laboratory 11) and none for sample B.

The mean values for the unsaponifiable matter content for the three samples were between 0,15 % and 0,58 % (by mass).

Repeatability limits are about 0,06 % (by mass) and repeatability coefficient of variation values are between 3,6 % and 10,5 %.

Reproducibility limits are about 0,18 % (by mass) and reproducibility coefficient of variation values are between 9 % and 36 %.