INTERNATIONAL STANDARD

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

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

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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 3596 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 3596 cancels and replaces ISO 3596-1:1988 and its Amendment 1:1997, 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 diethyl ether extraction

1 Scope

This International Standard specifies a 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.

A method given in ISO 18609 may be used when climatic conditions, or regulations, do not permit the use of diethyl ether.

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 Term and definition

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 a specified solvent, are not volatile under the specified operating conditions

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 diethyl ether. 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 **Diethyl ether**, freshly distilled, free from peroxides and residue.

5.2 Acetone.

5.3 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.

5.4 Potassium hydroxide, aqueous solution, c(KOH) ≈ 0,5 mol/l.

5.5 Phenolphthalein, 10 g/l solution in 95 % (by volume) 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 is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555 [1].

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 (5.3) 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.

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 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.

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 ethereal extract

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

CAUTION — 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 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 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).

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

9.6.1 Dry the residue in the oven (6.5) at 103 °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.

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

9.6.2 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 9.6.2), 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 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 An international collaborative test involving 51 laboratories in 16 countries was carried out on

Sample A: refined, bleached, deodorized soyabean oil, and

Sample B: dried, crude water-degummed soyabean oil,

using the diethyl ether method.

The test was organized by the Federation of Oils, Seeds and Fats Associations Ltd. (FOSFA International) in June 1995 and the results obtained were subjected to statistical analysis in accordance with ISO 5725[1)] to give the precision data shown in Table A.1.

Table A.1

	Soyabean oil	
	A	**B**
No. of participating laboratories after eliminating outliers	49	50
Mean value, % (by mass)	0,58	0,69
Repeatability standard deviation, s_r, % Repeatability limit r (2,8s_r), % Coefficient of variation of repeatability, %	0,025 0,07 4,3	0,027 0,08 3,9
Reproducibility standard deviation, s_R, % Reproducibility limit R (2,8s_R), % Coefficient of variation of reproducibility, %	0,22 0,62 37,9	0,24 0,67 34,7

A.2 Another international collaborative test involving 43 laboratories in 17 countries took place in July 1989 on crude Japanese fish oil.

The test was organized by the Federation of Oils, Seeds and Fats Associations Ltd. (FOSFA International) and the results obtained were subjected to statistical analysis in accordance with ISO 5725[1)] to give the precision data shown in Table A.2.

1) ISO 5725:1986 (now withdrawn), was used to obtain the precision data.

Table A.2

	Fish oil
No. of participating laboratories after eliminating outliers	37
Mean value, % (by mass)	0,81
Repeatability standard deviation, s_r, % Repeatability limit r (2,8s_r), % Coefficient of variation of repeatability, %	0,02 0,06 2,46
Reproducibility standard deviation, s_r, % Reproducibility limit R (2,8s_R), % Coefficient of variation of reproducibility, %	0,29 0,81 35,8

A.3 A third international collaborative test involving 10 laboratories was organized by IUPAC between 1976 and 1997. The results obtained were subjected to statistical analysis in accordance with ISO 5725[1)] to give the precision data shown in Table A.3.

Table A.3

	Refined soyabean oil	Refined tallow	Crude rapeseed oil
No. of participating laboratories after eliminating outliers	10	10	10
Means value, % (by mass)	0,630	0,253	1,432
Repeatability standard deviation, s_r, % Repeatability limit r (2,8s_r), % Coefficient of variation of repeatability, %	0,032 0,089 5,0	0,024 0,067 9,3	0,068 0,19 24,7
Reproducibility standard deviation, s_R, % Reproducibility limit R (2,8s_R), % Coefficient of variation of reproducibility, %	0,140 0,397 22,3	0,154 0,435 60,9	0,137 0,389 9,6