INTERNATIONAL STANDARD

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Animal and vegetable fats and oils — Determination of content of polar compounds

Corps gras d'origines animale et végétale — Détermination de la teneur en composés polaires

Reference number
ISO 8420:2002(E)

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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.

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

The main task of technical committees is to prepare International Standards. 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.

ISO 8420 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 8420:1990), which has been technically revised.

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

Animal and vegetable fats and oils — Determination of content of polar compounds

1 Scope

This International Standard describes a method for the determination of the content of polar compounds in animal and vegetable fats and oils, hereinafter referred to as fats.

Polar compounds are formed during the heating of fats and thus the method serves to assess the deterioration of frying fats with use.

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 661:1989, *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
polar compounds
constituents of fats which are determined by column chromatography under the conditions specified in this International Standard

NOTE 1 The polar compounds content is expressed as a mass fraction in percent.

NOTE 2 Polar compounds include polar substances which occur in unused fats, such as monoglycerides, diglycerides and free fatty acids, as well as polar transformation products formed during heating as occurs during the frying of food. Non-polar compounds are mostly unaltered triglycerides.

4 Principle

A test portion is separated by column chromatography into non-polar and polar compounds. The non-polar compounds are eluted then weighed. The polar compounds are determined by difference.

5 Reagents and materials

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

5.1 Silica gel, of particle size 0,063 mm to 0,200 mm (70 mesh to 230 mesh), such as Merck No. 7734[1], adjusted to a water content of 5 % (mass fraction) as follows.

Place approximately 180 g of silica gel in the porcelain dish. Dry in an oven at (160 ± 5) °C for at least 4 h with occasional stirring then cool in a desiccator to room temperature. Adjust the water content of the silica gel to 5 % (mass fraction) by placing 152 g of silica gel and 8 g of water in a 500 ml flask. Stopper the flask and shake on a shaking machine for 60 min.

Store the silica gel in a tightly closed container. Remaining parts of the conditioned silica gel have to be used within 24 h, as they cannot be reactivated and reconditioned.

5.2 Elution solvent, prepared by mixing 87 volumes of chromatographic quality light petroleum (boiling range 40 °C to 60 °C) and 13 volumes of stabilized diethyl ether (see warning in 9.4.6).

5.3 Sand, acid washed and calcined.

5.4 Cotton wool, surgical quality, non-absorbent.

5.5 Nitrogen, 99,0 % to 99,8 % purity.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Round- or flat-bottom glass flasks, with a ground-glass neck, of 250 ml capacity.

6.2 Chromatographic column, made of glass, of 21 mm internal diameter and 450 mm in length, equipped with a stopcock (preferably made of polytetrafluoroethylene) and having an internal ground-glass joint at the top.

6.3 Dropping funnel, of 250 ml capacity, with a ground-glass joint to fit the top of the column (6.2).

6.4 Glass rod, about 600 mm in length.

6.5 Rotary evaporator, or other apparatus for removing solvent under vacuum.

6.6 Shaking machine.

7 Sampling

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

Sampling is not part of this method specified in this International Standard. A recommended sampling method is given in ISO 5555.

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[1] Merck No. 7734 is the tradename of a product supplied by Merck. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

Semisolid and solid samples are heated to a temperature somewhat above their melting points and carefully mixed. Overheating should be avoided. Visible contaminations are removed by filtration after mixing. If water is present a hydrophobic filter shall be used.

9 Procedure

9.1 Preparation of the column

Using the glass rod (6.4), place a wad of the cotton wool (5.4) in the lower part of the column (6.2) and press it down. Pour about 30 ml of the elution solvent (5.2) into the column and remove air by pressing the cotton wool down with the rod.

In a beaker, prepare a slurry of 25 g of the silica gel (5.1) in about 80 ml of the elution solvent and pour this slurry into the column using a funnel. Complete the transfer of the silica gel into the column by rinsing the beaker with the elution solvent.

Open the stopcock and run off the elution solvent until the level of the elution solvent is about 100 mm above the silica gel. Level the silica gel by tapping the column.

Add about 4 g of the sand (5.3) through the funnel. Run off the supernatant elution solvent to within 10 mm of the sand layer.

Discard the elution solvent used in the preparation of the column.

9.2 Assessment of column efficiency

If desired, assess the column efficiency in accordance with annex A.

9.3 Test portion

Weigh (2,5 ± 0,1) g of the test sample (clause 8), to the nearest 0,001 g, into a 50 ml volumetric flask.

9.4 Determination

9.4.1 Dissolve the test portion (9.3) in about 20 ml of the elution solvent (5.2) by slight warming. Allow to cool to room temperature and dilute to 50 ml with elution solvent.

9.4.2 Using a pipette, transfer 20 ml of the test solution (9.4.1) to the prepared column (9.1). Avoid disturbing the surface of the sand.

9.4.3 Weigh, to the nearest 0,001 g, a 250 ml flask, previously dried at (103 ± 2) °C and cooled in the desiccator, and place it under the outlet of the column.

9.4.4 Open the stopcock and run off the solvent down to the level of the top of the sand layer, collecting the eluate (which contains non-polar compounds) in the 250 ml flask.

9.4.5 Continue the elution of non-polar compounds by adding 150 ml of the elution solvent (5.2) via a dropping funnel (6.3). Adjust the flowrate so that the 150 ml pass through the column in 60 min to 70 min.

After completion of the elution, wash any material adhering to the outlet of the column into the flask with the elution solvent, using a pipette or dropper.

If the polar compounds are required, for example for checking the efficiency of the column, they may be eluted using 150 ml of diethyl ether following the procedure described in 9.4.5 and 9.4.6.

Discard the silica gel after completing the elution(s).

9.4.6 Remove the solvent from the flask under low vacuum with the aid of a rotary evaporator (6.5) and a water bath controlled at a temperature no higher than 60 °C. Avoid losses due to foaming. Blow off residues of the solvent with nitrogen.

WARNING — Explosive peroxides may develop in diethyl ether. It is important therefore to use stabilized diethyl ether and to carry out the evaporation at as low a temperature as possible, carefully collecting the evaporated ether.

If a rotary evaporator is not available, evaporate the elution solvent in a stream of nitrogen.

9.4.7 Dry the flask in an oven at (103 ± 2) °C for 30 min. Cool down in a desiccator and weigh to the nearest 1 mg.

Heat again for 30 min under the same conditions, allow to cool and weigh.

The difference between the two weighings shall not exceed 1 mg. If it does, repeat the operations of heating, cooling and weighing until the difference between two successive weighings does not exceed 1 mg. Note the final mass of the flask.

If there is a significant increase in mass (over 1 mg), oxidation of a drying oil may be taking place. In this case, take the lowest weighing for the calculation.

9.5 Number of determinations

Carry out two determinations on test portions (9.3) taken from the same test sample (clause 8).

10 Expression of results

10.1 Calculation

The content of polar compounds, w, as a mass fraction in percent, is given by the formula

$$w = 100 - \frac{(m_1 - m_2) \times 100}{m}$$

where

m_1 is the mass, in grams, of the flask plus the mass of non-polar compounds (9.4.7);

m_2 is the mass, in grams, of the empty flask (9.4.3);

m is the mass, in grams, of the test portion in 20 ml of the sample solution (9.4.1); in this special case 2/5 of 2,5 g.

Take as the result the arithmetic mean of the two determinations provided that the requirements for repeatability (see 11.2) are satisfied.

Express the result to one decimal place.

11 Precision

11.1 Results of interlaboratory test

Details of an interlaboratory test are summarized in annex B. The values derived from this interlaboratory test may not be applicable to concentration ranges other than those given.

11.2 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, will in not more than 5 % of cases be greater than the repeatability limit r given in annex B.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the reproducibility limit R given in annex B.

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

Annex A
(informative)

Assessment of column efficiency

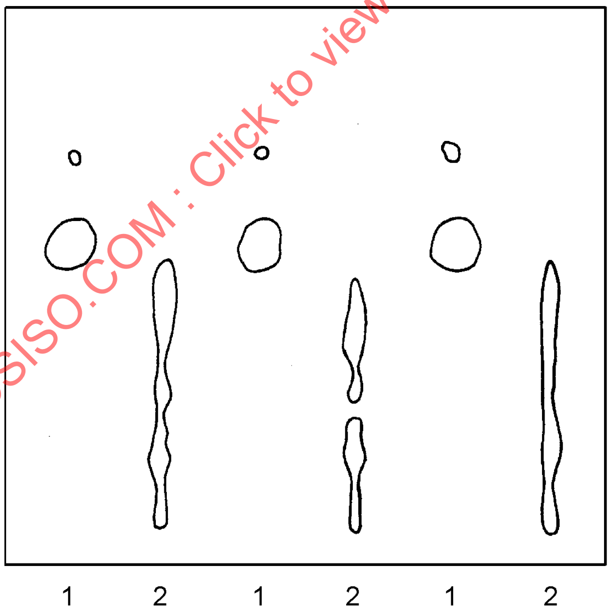
The column efficiency may be checked by using thin-layer chromatography as follows.

Prepare 10 % (mass fraction) solutions of the polar and non-polar compounds (separated as in 9.4) in chloroform, and apply 2 µl spots to a plate coated with a layer of silica gel 0,25 mm thick and without a fluorescence indicator.

Line a developing tank with filter paper to achieve saturation. Place the plate in the developing tank and carry out the development with a developing solvent consisting of a mixture of light petroleum (boiling range, 40 °C to 60 °C), diethyl ether and 100 % acetic acid (70 + 30 + 2 by volume). Allow the solvent front to ascend to a height of about 170 mm; this usually takes about 35 min. Remove the plate and allow the solvent to evaporate.

Spray the plate with a 100 g/l solution of 12-molybdophosphoric acid in ethanol. Allow the ethanol to evaporate and then heat the plate in an oven at a temperature of between 120 °C and 130 °C.

The efficiency of the separation can also be assessed by comparing the sum of the polar and non-polar compounds determined with the amount of test portion contained in 20 ml of the test solution (9.4.1). For samples containing large amounts of polar material, recovery of the test portion may be incomplete since small amounts of highly polar material (generally not more than 1 % to 2 %) are not eluted under the conditions specified in this International Standard.



Key

1 Non-polar compounds

2 Polar compounds

Figure A.1 — Chromatogram obtained after separation of frying fat into polar and non-polar compounds

Annex B
(informative)

Results of an interlaboratory test

An international collaborative test involving 16 laboratories in 6 countries was carried out on five samples of used frying oils.

The test was organized by DIN in 2000 and the results obtained were subjected to statistical analysis in accordance with ISO 5725-2 to give the precision data shown in Table B.1.

Table B.1 — Summary of statistical results

	Sample				
	A	B	C	D	E
Number of participating laboratories	15	16	15	15	16
Number of laboratories retained after eliminating outliers	13	13	11	14	10
Number of individual test results of all laboratories on each sample	26	26	22	28	20
Mean value, %	16,01	22,28	11,74	32,06	20,80
Repeatability standard deviation (s_r)	0,27	0,29	0,34	0,33	0,14
Repeatability coefficient of variation, %	1,7	1,3	2,9	1,0	0,7
Repeatability limit (r)	0,74	0,81	0,95	0,92	0,38
Reproducibility standard deviation (s_R)	1,04	1,29	0,56	1,66	0,61
Reproducibility coefficient of variation, %	6,5	5,8	4,8	5,2	3,0
Reproducibility limit (R)	2,5	2,3	1,7	2,2	1,2