INTERNATIONAL STANDARD

ISO 8420

First edition
1990-11-01

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

Corps gras d'origines animale et végétale — Dosage des composés polaires

Reference number
ISO 8420:1990(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 voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 8420 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

Annex A of this International Standard is for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

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

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.

NOTE 1 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 references

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

ISO 661:1989, *Animal and vegetable fats and oils — Preparation of test sample.*

ISO 5555:—[1], *Animal and vegetable fats and oils — Sampling.*

3 Definition

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

polar compounds: Constituents of fats which are determined by column chromatography under the conditions specified in this International Standard.

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

Separation of a test portion by column chromatography into non-polar and polar compounds. Elution of the non-polar compounds and weighing of them. Determination of the polar compounds by difference.

5 Reagents and materials

All reagents shall be of recognized analytical grade and the water used shall be distilled 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[2], adjusted to a water content of 5 % (*m*/*m*) as follows.

Place a shallow layer of the silica gel in a porcelain dish, dry in an oven at 155 °C to 160 °C for at least 4 h with occasional stirring and cool in a desiccator to room temperature. Adjust the water content of the silica gel to 5 % (*m*/*m*) 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 20 min. Determine the water content by drying at 155 °C to 160 °C and, if necessary, adjust to 5 % (*m*/*m*) ± 0,2 % (*m*/*m*).

1) To be published. (Revision of ISO 5555:1983)

2) Merck No. 7734 is the trade-name 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 the product named. Equivalent products may be used if they can be shown to lead to the same results.

Store the silica gel in a tightly closed container. If not used within 24 h, check the moisture content and adjust if necessary.

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 to 9.5.4).

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 flasks**, with a ground 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.

7 Sampling

Sampling shall have been 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 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 remove air by pressing the cotton wool down with the rod. Pour about 30 ml of the elution solvent (5.2) into the column.

In a 100 ml 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 Blank test

Pass 150 ml of the elution solvent through the column. Collect the solvent in a 250 ml flask (6.1), weighed to the nearest 1 mg, evaporate the solvent as described in 9.5.4 and weigh again to the nearest 1 mg. Calculate the mass, in grams, of the blank by subtraction.

9.3 Assessment of column efficiency

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

9.4 Test portion

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

9.5 Determination

9.5.1 Dissolve the test portion (9.4) 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.

Dry a 250 ml flask (6.1) under conditions similar to those used when it contains the eluted compounds (see 9.5.4). Weigh the flask and place it under the column.

9.5.2 Using a pipette, transfer 20 ml of the test solution (9.5.1) into the prepared column (9.1). Avoid disturbing the surface of the sand.

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.

9.5.3 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 flow-rate 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.

NOTE 2 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 9.5.3 and 9.5.4 .

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

9.5.4 Remove the solvent from the flask, preferably under 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.

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.

NOTE 3 If a rotary evaporator is not available evaporate the elution solvent in a stream of nitrogen.

Shortly before the end of the evaporation, introduce nitrogen (5.5) into the system to complete the evaporation.

9.5.5 Weigh the flask containing the eluted compounds to the nearest 1 mg.

9.6 Number of determinations

Carry out two determinations on test portions (9.4) taken from the same test sample (clause 8).

10 Expression of results

The content of polar compounds, as a percentage by mass, is given by the formula

$$\frac{m_1 - (5/2)m_2 - m_3}{m_1} \times 100$$

where

m_1 is the mass, in grams, of the test portion (9.4);

m_2 is the mass, in grams, of the non-polar fraction;

m_3 is the mass, in grams, of the blank (9.2).

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 inter-laboratory test

An inter-laboratory test, carried out at the international level in 1979 by the International Union of Pure and Applied Chemistry (IUPAC), in which 10 laboratories participated, each of which carried out two determinations on each sample, gave the statistical results (evaluated in accordance with ISO 5725[3]) shown in table 1.

11.2 Repeatability

The difference between the values of two determinations, carried out in rapid succession by the same operator using the same apparatus on the same test sample, shall not exceed 1 % (m/m) (absolute value).

11.3 Reproducibility

The difference between the values of the final result obtained by two laboratories using this method for the analysis of the same laboratory sample are not expected to exceed 2 % (m/m) (absolute value).

12 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 International Standard, 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.

3) ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

Table 1

Sample	Soyabean oil/palm oil mixture	Soyabean oil/palm oil mixture	Unmixed palm oil	Hydrogenated soyabean oil
Number of laboratories retained after eliminating outliers	9	9	8	8
Mean [% (*m*/*m*)]	7,3	8,0	11,5	25,9
Standard deviation of repeatability, s_r [% (*m*/*m*)]	0,33	0,36	0,23	0,52
Coefficient of variation of repeatability (%)	4,5	4,5	2,0	2,0
Repeatability 2,8 s_r [% (*m*/*m*)]	0,9	1,0	0,7	0,8
Standard deviation of reproducibility, s_R [% (*m*/*m*)]	0,39	0,37	0,48	1,47
Coefficient of variation of reproducibility (%)	5,3	4,6	4,2	3,0
Reproducibility, 2,8 s_R [% (*m*/*m*)]	1,1	1,0	1,4	2,2

Annex A
(informative)

Assessment of column efficiency

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

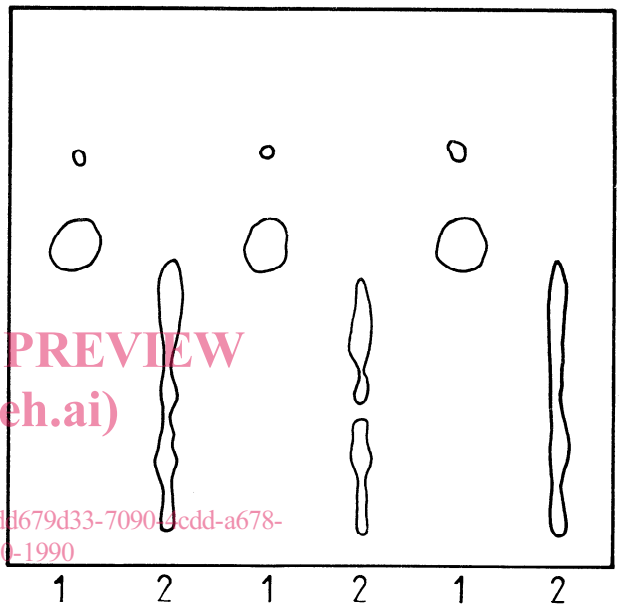
Prepare 10 % (*m*/*m*) solutions of the polar and non-polar compounds (separated as in 9.5) in chloroform, and apply 2 µl spots onto a plate coated with a layer of silica gel 0,25 mm thick and without a fluorescence indicator.

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 120 °C to 130 °C.

Figure A.1 shows an example of a chromatogram obtained after separation of frying fat into polar and non-polar compounds.

NOTE 4 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 (9.5.1). 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.



1 Non-polar compounds
2 Polar compounds

Figure A.1 — Example of a chromatogram

UDC 665.2/.3:543.544.42

Descriptors: agricultural products, food products, animal fats, vegetable fats, animal oils, vegetable oils, chemical analysis, determination of content, polar compounds.

Price based on 5 pages