INTERNATIONAL STANDARD

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Animal and vegetable fats and oils — Determination of residual technical hexane content

Corps gras d'origines animale et végétale — Dosage de l'hexane technique résiduel

Reference number
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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.

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 9832 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 9832:1992), of which it constitutes a minor revision to incorporate Amendment 1:1998.

Annex A of this International Standard is for information only.

INTERNATIONAL STANDARD **ISO 9832:2002(E)**

Animal and vegetable fats and oils — Determination of residual technical hexane content

1 Scope

This International Standard specifies a method for the determination of the residual technical hexane content of animal and vegetable fats and oils (referred to as fats hereinafter).

The method is suitable for the determination of hexane contents between 10 mg and 1 500 mg per kilogram of fat.

The method is not applicable to marine oils

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
residual technical hexane content
content of volatile hydrocarbons such as those remaining in fats following processing involving the use of hydrocarbon solvents, when determined by the method specified in this International Standard

NOTE The content is expressed as milligrams of hexane per kilogram of sample.

4 Principle

Desorption of volatile hydrocarbons by heating at 80 °C in a closed vessel after addition of an internal standard. Determination of the particular volatile hydrocarbons content of the headspace by gas chromatography using packed or capillary columns.

5 Reagents

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

5.1 Technical hexane, with a composition similar to that of hexane used in industrial processing or, if this is not available, ***n*-hexane**.

It is recommended that technical hexane be used for the calibration. This reagent usually has an *n*-hexane content of 50 % (by mass) and consists mainly of C_6 isomers but may include C_5 and C_7 hydrocarbons.

5.2 Internal standard, *n*-heptane.

If this is not available, cyclohexane may be used, provided that the solvent (5.1) used for the extraction or calibration has a negligible content of cyclohexane and/or *n*-heptane or components with similar retention times.

5.3 Carrier gas, e.g. hydrogen, nitrogen or helium, etc., thoroughly dried and with an oxygen content of less than 10 mg/kg.

5.4 Auxiliary gases, hydrogen (99,9 % pure, free from organic impurities) and air (free from organic impurities).

5.5 Calibration fat, freshly refined and deodorized vegetable fat, the technical hexane content of which is negligible.

This material should be free from peroxides or other components likely to decompose with the formation of volatile material which could be confused with hydrocarbons during the test.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Septum vials, of 20 ml capacity.

6.2 Septa, inert to fats and solvents, made of a material such as butyl rubber or red rubber free from hydrocarbon solvent residues and of a suitable quality that they will not swell under the conditions of use, **aluminium caps** suitable for use with the vials (6.1) and **crimping pliers**.

6.3 Tongs, suitable for holding the vials (6.1).

6.4 Syringes, of 10 µl capacity, used only for the analysis of residual technical hexane. They shall not be cleaned with hydrocarbon solvent.

6.5 Syringes, of 1 µl capacity, used only for the analysis of residual technical hexane. They shall not be cleaned with hydrocarbon solvent.

6.6 Syringes, of 1 000 µl capacity, gas-tight, used only for the analysis of residual technical hexane. They shall not be cleaned with hydrocarbon solvent.

6.7 Gas chromatograph, with a flame ionization detector and an integrator and/or recorder, equipped with either

a) a packed glass column, 2 m to 4 m long and of internal diameter 3,2 mm approximately, packed with an acid-washed and silanized diatomaceous earth support of particle size 150 µm to 180 µm (Chromosorb P NAW 60-80 mesh1) is suitable), and coated with 10 % squalane or any other phase permitting the chromatographic separation required, or

b) a glass capillary column, approximately 30 m long and of 0,3 mm internal diameter, coated with methylpolysiloxane of film thickness 0,2 µm.

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1) Chromosorb P NAW 60-80 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

The injector and detector temperature shall be set at 100 °C and the oven temperature at 50 °C.

If a capillary column [see b)] is used, the apparatus shall have a 1/100 split injection system.

NOTE For analyses in series, a headspace gas chromatograph with automatic sample injection and tempering bath has been shown to be satisfactory. In this case, manual injection is not necessary.

6.8 Heating bath, equipped with clamps for holding septum vials, regulated thermostatically at 80 °C ± 2 °C.

NOTE For continuous operation, glycerol is recommended as the heating medium.

6.9 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 transport and storage.

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

It is essential that the sample be protected from gain or loss of solvent residues.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 661, taking care to prevent gain or loss of solvent.

9 Procedure

9.1 Calibration

9.1.1 Weigh, to the nearest 0,01 g, 5 g of calibration fat (5.5) into each of seven vials (6.1). Close each vial with a septum and a cap (6.2).

To six of the seven vials (6.1) add, using a syringe (6.4 or 6.5), the quantity of solvent (5.1) specified in Table 1 to obtain the concentrations indicated. Do not add solvent to the seventh vial.

Shake vigorously, in the shaking machine (6.9) for 1 h at room temperature, the six vials to which solvent was added.

9.1.2 At the end of this time add, by means of a syringe (6.4), 5 µl ± 0,1 µl of internal standard (5.2) to each of the seven vials through the septum.

For hexane contents between 10 mg/kg and 20 mg/kg, it is preferable to add 2 µl of internal standard (5.2).

Mix the contents vigorously by hand for about 1 min by moving the vial with a circular motion in a horizontal plane in such a way that the fat does not touch the septum. If this happens, reject the vial and start again with a further portion of calibration fat.

CAUTION — If there is fat on the septum it will contaminate the needle when the headspace gas is sampled and the contaminant may be transferred to the column; it is particularly important that such contamination be avoided when using capillary columns.

At intervals of about 15 min (i.e. the retention time of the internal standard), place one vial at a time up to its neck in the heating bath (6.8) set at 80 °C to allow equilibrium between the fat and the gaseous phase to be reached.

Table 1 — Hexane content in the calibration samples

Volume of solvent (5.1) added µl	**Hexane content** (mg/kg) **when using** **technical hexane**	 ***n*-hexane**
0,5	67	66
1	134	132
2	268	264
4	536	528
7	938	924
10	1 340	1 320

9.1.3 From each vial which has been in the heating bath for 60 min ± 1 min, take (without removing it from the bath) 1 000 µl of the gaseous phase by means of a syringe (6.6) warmed to 60 °C. Immediately inject into the gas chromatograph the gaseous phase so removed.

9.1.4 From the chromatogram corresponding to the vial to which no solvent was added, calculate the hexane content A_c, expressed as a percentage of the total peak areas.

9.1.5 From each of the chromatograms corresponding to each of the vials to which solvent was added, calculate the calibration factor F using the formula

$$F = \frac{w_\mathrm{h} \cdot A_\mathrm{is}}{\left(A_\mathrm{t} - A_\mathrm{c} - A_\mathrm{is}\right) w_\mathrm{is}}$$

where

A_c is the hexane content calculated in 9.1.4;

A_is is the content of the internal standard in the calibration fat (5.5) with added solvent, expressed as a percentage of the total peak areas;

A_t is the total hydrocarbons content, including the internal standard, in the calibration fat (5.5) with added solvent, expressed as a percentage of the total peak areas;

w_h is the content of the solvent (5.1) in the calibration fat (5.5) with added solvent, expressed in milligrams per kilogram;

w_is is the content of the internal standard in the calibration fat (5.5) with added solvent, expressed in milligrams per kilogram, i.e. 680 for *n*-heptane or 780 for cyclohexane.

NOTE If only 2 µl of internal standard was added in 9.1.2, w_is is equal to 272 for *n*-heptane or 312 for cyclohexane.

Express the results to the third decimal place.

The calibration factors of the six calibration samples should be approximately equal. Calculate the arithmetic mean value $\overline{F}$, which should be about 0,45 for heptane. The factor $\overline{F}$ so evaluated can be used for determining hexane contents of less than 60 mg/kg. If the value of F found for the vial containing 0,5 µl of solvent (5.1) is significantly below the mean value $\overline{F}$, this deviation is probably due to the difficulty of introducing exactly 0,5 µl and this determination shall be eliminated or repeated.

The mean calibration factor for cyclohexane is normally about 0,57, whilst it is about 0,45 for *n*-heptane.

9.2 Determination

9.2.1 Weigh, to the nearest 0,01 g, a test portion of 5 g of the test sample (clause 8) into a vial (6.1) as quickly as possible. Close immediately with a septum and a cap (6.2).

9.2.2 Inject 5 µl of the internal standard (5.2) through the septum by means of a syringe (6.4). Mix the contents vigorously by hand for about 1 min by moving the vial with a circular motion in a horizontal plane in such a way that the fat does not touch the septum. If this happens, reject the vial and start again with a further test portion. (See CAUTION in 9.1.2.) Place the vial up to its neck in the heating bath (6.8) set at 80 °C for 60 min ± 1 min.

9.2.3 Then take 1 000 µl from the gaseous phase by means of a syringe (6.6) warmed to 60 °C, without removing the vial from the heating bath. Immediately inject into the gas chromatograph the gaseous phase so removed.

9.2.4 Determine the residual technical hexane content of the sample from the chromatogram (see the example given in Figure 1), measuring those peaks identified as being from hexane and not from decomposition products.

9.3 Number of determinations

Carry out the determination on two test portions from the same test sample in rapid succession.

10 Expression of results

The residual technical hexane content of the sample, w, in milligrams per kilogram, is given by the formula

$$w = \frac{\left(A'_{\mathrm{t}} - A'_{\mathrm{is}}\right) \cdot \overline{F} \cdot w'_{\mathrm{is}}}{A'_{\mathrm{is}}}$$

where

A'_{is} is the content of the internal standard in the sample, expressed as a percentage of the total peak areas;

A'_{t} is the total hydrocarbons content, including the internal standard, of the sample, expressed as a percentage of the total peak areas;

$\overline{F}$ is the mean calibration factor determined in 9.1.5;

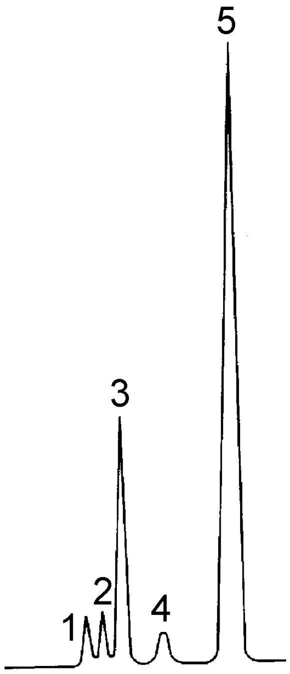
w'_{is} is the content of the internal standard in the sample, expressed in milligrams per kilogram, i.e. 680 for *n*-heptane or 780 for cyclohexane.

Take as the final result the arithmetic mean of the two determinations (9.3) provided that the repeatability requirement (11.2) is met. If the repeatability requirement is not met, disregard the results and carry out two new determinations on test portions taken from the same test sample.

11 Precision

11.1 Results of interlaboratory test

Details of an interlaboratory test on the precision of themethod are given in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.



Peak identification

1 2-Methylpentane
2 3-Methylpentane
3 *n*-Hexane
4 Methylcyclopentane
5 Cyclohexane (internal standard) (with other retention times: *n*-heptane)

Figure 1 — Example of a gas chromatogram of hexane hydrocarbons

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 A.

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 A.

NOTE It should be noted that the reproducibility limit values R cited in annex A apply in the particular case when the results of single determinations obtained by two laboratories are being compared. When following the method described and it is desired to compare the final results (which have been derived from the means of duplicate determinations) obtained by two laboratories, the values for R should be converted to the 95 % probability critical difference values, C_{95}, applicable to the means of two determinations using the following formula:

$$C_{95} = \sqrt{\left(R^2 - r^2\right)/2}$$

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 results;
- the test results obtained, or, if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Results of interlaboratory test

An international collaborative test involving 21 laboratories in 8 countries was carried out using the method given in this International Standard. The collaborative test was organized by the Federation of Oils, Seeds and Fats Associations (FOSFA International) in 1994. The results obtained were subjected to statistical analysis in accordance with ISO 5725 [2)] to give the precision date as shown in Table A.1.

The collaborative test was carried out on spiked samples of soyabean and sunflowerseed oils, as follows:

- samples A to D: sunflowerseed oil;
- samples E to H: soyabean oil.

Table A.2 shows the expected means of the samples provided to analysts.

Table A.2 also shows the actual means obtained and the repeatability limits found by the collaborative test.

Table A.1 — Summary of statistical results

	Sample							
	A	B	C	D	E	F	G	H
No. of laboratories originally instructed	21	21	21	21	21	21	21	21
No. of laboratories returning results	18	18	18	18	17	18	18	18
No. of laboratories with acceptable results	11	14	14	14	12	15	15	15
Overall mean (mg/kg)	1,55	392,6	1 059,1	757,5	1,65	374,5	1 043,4	808,8
Repeatability standard deviation, s_r (mg/kg)	0,09	10,06	20,43	24,9	0,39	8,31	25,8	18,9
Repeatability limit, r (mg/kg)	0,26	28,2	57,2	69,8	1,09	23,3	72,2	52,9
Reproducibility standard deviation, s_R (mg/kg)	0,58	22,1	73,3	55,9	0,65	26,9	55,9	48,2
Reproducibility limit, R (mg/kg)	1,62	61,8	205,2	156,4	1,83	75,4	156,6	135,1
Ratio R/r	6,1	2,2	3,6	2,2	1,7	3,2	2,2	2,6
Range of accepted results	1 to 2,8	342,4 to 428,2	881 to 1 214,1	588 to 873,7	0 to 3,1	326,2 to 447	896 to 1 153,6	693 to 888,0

[2)] ISO 5725:1986 (now withdrawn) was used to obtain the precision data.

Table A.2 — Comparison of expected and actual means and repeatability limits

Type of oil	Sample	Expected mean from homogeneity test	Overall mean from collaborative test	Interpolated repeatability limit	Repeatability limit from collaborative test
Sunflowerseed oil	A	1,30	1,55	< 1,8	0,26
	B	384,8	392,6	74,8	28,2
	C	1 100	1 059,1	> 202	57,2
	D	787	757,5	152	69,8
Soyabean oil	E	1,17	1,65	< 1,8	1,09
	F	361,4	374,5	70,4	23,3
	G	1 028	1 043,4	198	72,2
	H	779,6	808,8	151	52,9

Bibliography

[1] ISO 5555:1991, *Animal and vegetable fats and oils — Sampling*

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

[3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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