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Animal and vegetable fats and oils — Determination of polymerized triglycerides content by high-performance size-exclusion chromatography (HPSEC)

Corps gras d'origines animale et végétale — Détermination de la teneur en triglycérides polymérisés par chromatographie liquide d'exclusion à haute performance (CLHP d'exclusion)

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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 16931 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

Annex A of this International Standard is for information only.

Animal and vegetable fats and oils — Determination of polymerized triglycerides content by high-performance size-exclusion chromatography (HPSEC)

1 Scope

This International Standard specifies a method using HPSEC to determine the contents of polymerized triglycerides in oils and fats which contain at least 3 % (from peak areas) of these polymers.

This method is applicable to frying fats, and fats and oils that have been thermally treated. It can also be applied to the determination of polymers in fats for animal feedstuffs.

NOTE 1 Polymerized triglycerides (strictly speaking dimeric and oligomeric triglycerides) are formed during the heating of fats and oils, thus the method serves to assess the thermal deterioration of frying fats with use.

NOTE 2 In the case of analysis of fats from animal feeding stuffs, the extraction method used can have an influence on the result (see ISO 6492).

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
polymerized triglycerides
constituents of heated fats and oils that are determined by HPSEC under the conditions specified in this International Standard

NOTE The content is expressed as a percentage of all peaks from eluted acylglycerides (TAGs, PTAGs, DAGs and MAGs).

4 Principle

The sample is dissolved in tetrahydrofuran then the polymerized triglycerides are separated by gel permeation chromatography according to molecular size. Detection of the compounds is realized by means of a refractive index detector.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Tetrahydrofuran, possibly stabilized with BHT (0,1%), degassed.

It is important that the tetrahydrofuran used to dissolve the sample has the same water content as the eluent, otherwise a negative peak can appear.

5.2 Toluene.

5.3 Sodium sulfate, anhydrous.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Solvent reservoir, of about 250 ml of capacity, with a polytetrafluoroethylene mobile-phase line filter (pore size 1 µm).

6.2 HPLC pump, pulseless, with a volume flow rate of 0,5 ml/min to 1,5 ml/min.

6.3 Injection valve, with a 10-µl loop and a suitable syringe with a volume of 50 µl to 100 µl, or **autosampler** with a 10 µl loop.

6.4 Stainless-steel column, 300 mm in length, and with 7,5 mm to 7,8 mm internal diameter, packed with a high-performance spherical gel made of styrene-divinylbenzene co-polymer; diameter of the particles: 5 µm; pore size 10 nm or the equivalent in terms of exclusion power and resolution (see 10.1).

A temperature control device for the column is recommended, to maintain the temperature of the column at between 30 °C and 35 °C.

If necessary, the column should be stored in toluene (5.2).

6.5 Detector, temperature-controlled refractive index detector with a sensitivity at full scale of at least 1×10^{-4} of the refractive index.

The ideal temperature for the detector is just above the ambient temperature (30 °C to 35 °C).

6.6 Recorder and/or **integrator**, or **computerized chromatography data system** (CDS), to allow display and accurate quantification of the peak areas.

7 Sampling

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

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

8 Preparation of test sample

The test sample shall be prepared in accordance with ISO 661.

9 Procedure

NOTE If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.1 and 9.2.

9.1 Starting up the HPLC equipment

It is advisable to follow carefully the manufacturer's recommendations. Switch on the system and pump tetrahydrofuran at a volume flow rate of between 0,5 ml/min and 1 ml/min to purge the whole system up to the injection valve. Connect the column to the injection valve and wash it with about 30 ml of tetrahydrofuran. Connect the column to the sample cell of the detector. Fill the reference cell with tetrahydrofuran. Adjust the mobile phase flow to between 0,5 ml/min and 1,0 ml/min. Wait until a convenient stabilization of the system (no appreciable deviation of the baseline) is obtained.

If the composition of the column is as indicated, an acceptable stabilization of the system should be obtained in about 15 min. With other column packings, the stabilization of the system may be more difficult. For example, changing the mobile phase should be done stepwise from toluene to tetrahydrofuran, with different mixtures, each containing 25 % more tetrahydrofuran. Acceptable stabilization is normally obtained in about 12 h.

9.2 Preparation of test portion and analysis

Weigh about 50 mg of fat and add 1 ml to 3 ml of tetrahydrofuran. Homogenize. Add 50 mg of anhydrous sodium sulfate and leave for 2 min to 5 min. Filter through a 1 µm size filter. Take with the syringe, 50 µl to 100 µl of that solution. Fill the injection loop, inject and switch on the integrator.

If using an autosampler, fill the sample vials of the autosampler, start the autosampler and switch on the integrator.

With a mobile phase volume flow rate of 1 ml/min, the analysis time is about 10 min.

The efficiency of the column, determined as the number of theoretical plates (n) for the monomeric triglycerides, should be at least 6 000.

10 Expression of results

10.1 Qualitative analysis

The chromatographic pattern of the determination shows a main peak representative of monomeric triglycerides (molecular mass about 900) and one or several smaller peaks with shorter retention times representative of polymerized triglycerides (dimers and upper oligomers).

Under suitable conditions, triglycerides (TAGs) and polymerized triglycerides (PTAGs) can be separated with good resolution [see Figure 1a) and b)] even at low levels of polymerized triglycerides.

However, in some cases (which seem to be connected to complex degradation phenomena), the peak pattern preceding the triglyceride peaks can be less clear with consequent difficulties for the calculations [Figure 1 c)].

10.2 Quantitative analysis

Calculation is carried out by the internal normalization method, assuming that all components of the sample which are eluted have the same response coefficient.

The polymerized triglycerides content is calculated from the formula:

$$P_{\mathrm{PTAG}} = \frac{\sum A_{\mathrm{PTAG}}}{\sum A_{\mathrm{tot}}} \times 100\ \%$$

where

P_{PTAG} is the percentage of polymerized triglycerides, based on peak areas;

A_{PTAG} is the sum of areas of the polymerized triglycerides peaks;

A_{tot} is the sum of the areas of all peaks representative of acylglycerides (TAGs, PTAGs, DAGs and MAGs).

For calculating A_{PTAG}, two cases are possible, as follows.

a) Good resolution between peaks ($R \geqslant 1$)

The general methods of integration (manual and electronic) may be used to calculate individual and total areas.

b) Poor resolution between peaks ($R < 1$)

It is assumed that all components eluted before ($d_r - 0{,}5\ w$) (see below) are polymerized triglycerides.

The resolution is calculated from:

$$R = \frac{D}{w}$$

where

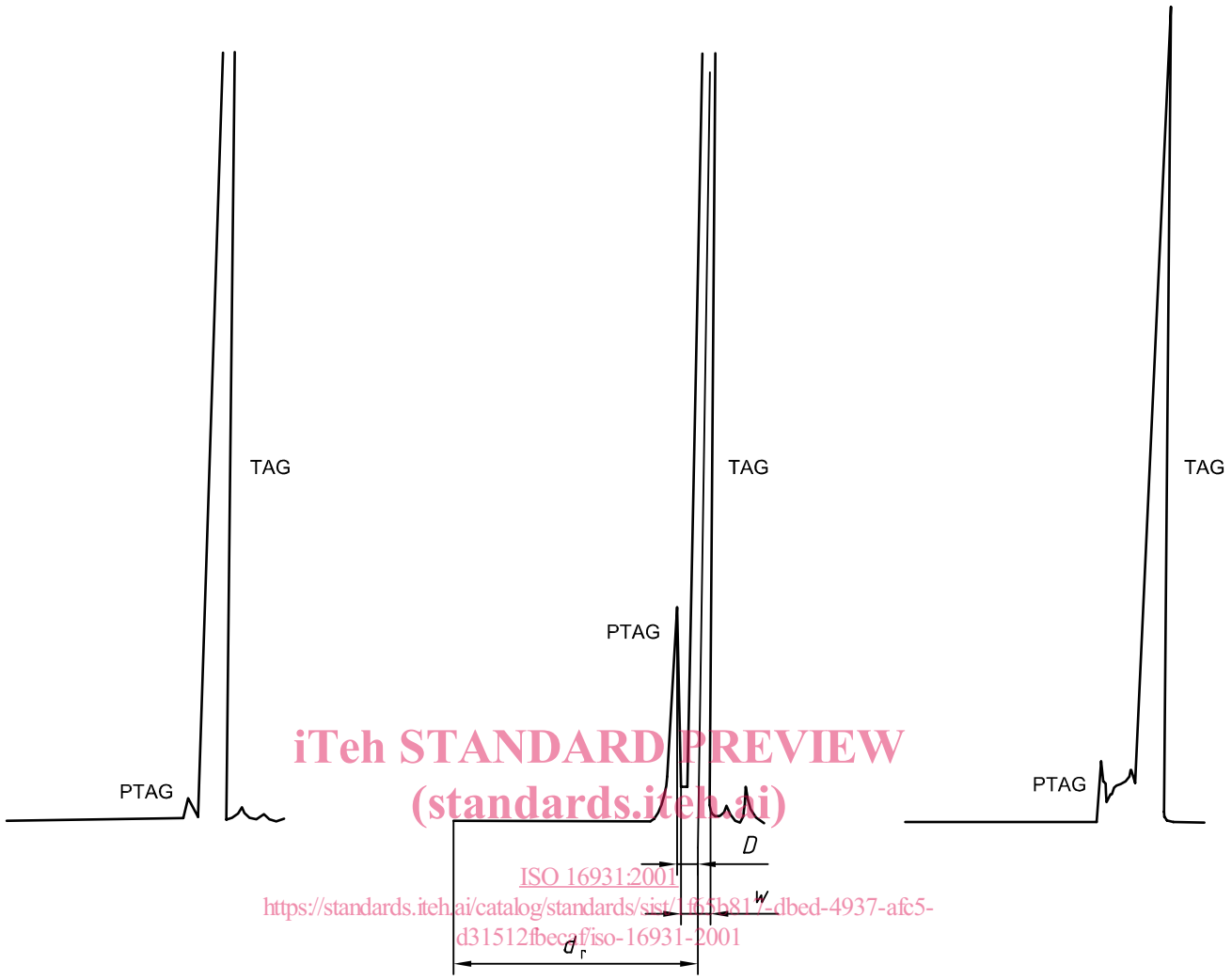
D is the distance, in millimetres, between the peak maxima of the non-polymerized triglycerides peak (TAGs) and the adjacent polymerized triglycerides peak (PTAGs);

w is the width, in millimetres, of the triglycerides peak at the baseline, measured between the points of intersection between the tangents and baseline;

d_r is the retention distance, in millimetres, from the beginning of the chromatogram to the peak maximum for triglycerides.

Using electronic integration, the integrator shall be carefully adjusted (backward horizontal integration) to integrate all the surfaces included between the curve and the baseline. If a manual technique is used, it is necessary to determine the triglycerides peak area by triangulation.

Express the results to one decimal place.



a) PTAG content 1 % **b) PTAG content 10 %** **c) PTAG content 10 %**

Figure 1 — HPSEC of triglycerides (TAGs) and polymerized triglycerides (PTAGs)

11 Precision

11.1 Interlaboratory tests

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.

11.2 Repeatability

When the values of 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, lie within the range of the mean values given in Table A.1, the absolute difference between the two test results obtained will in not more than 5 % of cases be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in Table A.1.