BS EN ISO 12966-3:2016

BSI Standards Publication

Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters

Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH) (ISO 12966-3:2016)

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National foreword

This British Standard is the UK implementation of EN ISO 12966-3:2016. It supersedes BS EN ISO 12966-3:2009 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/307, Oilseeds, animal and vegetable fats and oils and their by-products.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Tierische und pflanzliche Fette und Öle - Gaschromatographie von Fettsäuremethylestern - Teil 3: Herstellung von Methylestern mittels Trimethylsulfoniumhydroxid (TMSH) (ISO 12966-3:2016)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

European foreword

This document (EN ISO 12966-3:2016) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 307 “Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis” the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2016, and conflicting national standards shall be withdrawn at the latest by December 2016.

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Endorsement notice

The text of ISO 12966-3:2016 has been approved by CEN as EN ISO 12966-3:2016 without any modification.

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Foreword

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 12966-3:2009), of which it constitutes a minor revision. The scope has been revised to state that the document is not applicable to milk and milk fat products.

ISO 12966 consists of the following part, under the general title *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters*:

— *Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters*

— *Part 2: Preparation of methyl esters of fatty acids*

— *Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)*

— *Part 4: Determination by capillary gas chromatography*

INTERNATIONAL STANDARD ISO 12966-3:2016(E)

Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters —

Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH)

1 Scope

This part of ISO 12966 specifies a rapid base-catalysed transesterification method for fats and oils with trimethylsulfonium hydroxide (TMSH) to prepare fatty acid methyl esters. The method is exclusively applicable to the preparation of methyl esters of fats and oils for gas liquid chromatographic (GLC) analysis. It is applicable to all fats and oils, but excluding those coming from milk and milk products. Isomerization of unsaturated fatty acids only occurs to a minor extent and isomerized fatty acids are only present at the determination limit. As isomerization takes place, the procedure is not recommended for conjugated linoleic acid (CLA).

Only about 70 % to 80 % of the free fatty acids are esterified. In the case of conjugated cyclopropyl and cyclopropenyl fatty acids, side reactions may occur, but these do not interfere with the determination of the fatty acids.

NOTE This part of ISO 12966 is based upon German Standard Method C-VI 11e (98) (see Reference [8]).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

3 Principle

The sample is dissolved in *tert*-butyl methyl ether (TBME) and mixed with a methanolic solution of trimethylsulfonium hydroxide. Glycerides are base-catalysed transesterified and fatty acid methyl esters are formed (see References [4] to [8]). Free fatty acids are converted to salts which are pyrolysed to methyl esters and dimethylsulfide in the injector. Excess reagent is also pyrolysed into methanol and dimethylsulfide. To obtain a complete pyrolytic reaction, a hot injector (split injection) of at least 250 °C is necessary.

For the determination of short-chain fatty acids (C_4 to C_8), valeric acid methyl ester is used as an internal standard. Lipids containing hydroxy groups are partially converted to the corresponding *O*-methyl ether derivatives which may interfere with fatty acid methyl esters in the GLC separation (Reference [9]). In the early part of the chromatogram (region of C_4), peaks may occur, which are from the reagent. These peaks are not taken into account.

4 Reagents

WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational, and personal safety measures shall be followed.

During the analysis, unless otherwise stated, use only reagents and solvents of recognized analytical grade.

4.1 ***tert*-Butyl methyl ether (TBME)**.

4.2 **Trimethylsulfonium hydroxide (TMSH)**, methanolic solution, amount of substance concentration $c(Me_3SOH)$ = 0,2 mol/l.

The content of the solution can be determined by acidimetry: dilute 5,0 ml of the solution with 10 ml of methanol, add two drops of phenolphthalein and titrate against 0,1 mol/l HCl. The concentration should be at least 0,15 mol/l.

NOTE The solution remains stable for at least two months when stored at 4 °C in small quantities in closed tubes.

5 Apparatus

Usual laboratory equipment, and in particular:

5.1 **Test tubes**, of capacity 2 ml (autosampler vials).

5.2 **Graduated pipettes**, of capacities 250 µl, 500 µl, and 1 000 µl, ISO 835[1] class A.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 12966. A recommended sampling method is given in ISO 5555[3].

7 Preparation of the test sample

The test sample shall be liquid, dry and clear. Proceed in accordance with ISO 661, but heat the sample to just above the melting point.

Solid samples are carefully melted at a temperature of maximum 10 °C above their melting point and mixed. Avoid overheating.

Samples containing water are dissolved in petroleum ether and dried for 30 min by addition of anhydrous sodium sulfate. The drying agent is removed by filtration through a fluted filter paper and the residue is carefully washed with petroleum ether. The solvent is subsequently removed with the aid of a rotary evaporator.

8 Procedure

8.1 Weigh, into a test tube (5.1), (10 ± 2) mg of the test sample.

8.2 Pipette (5.2) 500 µl of TBME (4.1) into the test tube and dissolve the sample, warming gently if necessary.

NOTE For the determination of short-chain fatty acids (C_4 to C_8), valeric acid methyl ester is used as an internal standard. The internal standard solution is used to dissolve the sample.

8.3 Pipette (5.2) 250 µl of TMSH solution (4.2) into the test tube and shake vigorously for about 30 s. After this, the solution is ready for injection into the gas chromatograph. As the methyl esters are formed during injection, an injector temperature of at least 250 °C is required.

If necessary, use a mixture of TBME and methanol (9 volumes + 1 volume) to dilute the solution.

IMPORTANT — Free fatty acids react with TMSH to form the corresponding salts, which are pyrolysed to methyl esters and dimethylsulfide in the injector. Therefore, an injector temperature of 250 °C is necessary. To prevent blocking, the capillary of the split vent shall have an appropriate internal diameter (>1 mm). It should be cleaned by regular heating or flushing with solvent. Moreover, the split vent valve shall be protected with an activated charcoal trap.

9 Test report

The test report shall include the following information:

a) result of the determination;

b) the method used, together with a reference to this part of ISO 12966, i.e. ISO 12966-3;

c) all information necessary for the complete identification of the sample;

d) all operating details not specified in this part of ISO 12966, or regarded as optional, together with details of any incident that may have influenced the result(s).

Bibliography

[1] ISO 835, *Laboratory glassware — Graduated pipettes*

[2] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

[3] ISO 5555, *Animal and vegetable fats and oils — Sampling*

[4] Butte W.J. Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulfonium hydroxide for transesterification. *J. Chromatogr. A*. 1983, **261** pp. 142–145

[5] Schulte E., & Weber K. Rapid preparation of fatty acid methyl esters from fats with trimethylsulfonium hydroxide or sodium methylate. *Fat Sci. Technol*. 1989, **91** pp. 181–183

[6] Arens M., Schulte E., Weber K. Fettsäuremethylester, Umesterung mit trimethylsulfoniumhydroxid [Fatty acid methyl esters, transesterification with trimethylsulfonium hydroxide]. *Fat Sci. Technol*. 1994, **96** pp. 67–68

[7] El-Hamdy A.H., & Christie W.W. Preparation of methyl esters of fatty acids with trimethylsulphonium hydroxide — An appraisal. *J. Chromatogr. A*. 1993, **630** pp. 438–441

[8] Deutsche Gesellschaft für Fettwissenschaft. *Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen/German standard methods for the analysis of fats and other lipids*. Wissenschaftliche Verlagsgesellschaft, Stuttgart, 2008

[9] Vosmann K., Schulte E., Klein E, Weber N. Reactions of lipids containing hydroxy groups with trimethylsulfonium hydroxide: Formation of *O*-methyl derivatives. *Lipids*. 1996, **31** pp. 349–352

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