INTERNATIONAL STANDARD

ISO 12966-3

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

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

Corps gras d'origines animale et végétale — Chromatographie en phase gazeuse des esters méthyliques d'acides gras —

Partie 3: Préparation des esters méthyliques à l'aide d'hydroxyde de triméthylsulfonium (TMSH)

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 2.

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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 12966-3 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegatable fats and oils*.

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 2: Preparation of methyl esters of fatty acids*

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

The following part is in preparation:

— *Part 4: Determination of* cis-*,* trans-*, saturated, mono- and polyunsaturated fatty acids in vegetable or non-ruminant oils and fats*

The following part is planned:

— Part 1: Guidelines on gas chromatography

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 including milk fat and blends containing milk fat. 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). As CLA is not correctly analysed, this method is not applicable to the determination of the complete fatty acid composition of milk fat samples.

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 referenced documents are indispensable for the application of this document. 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.