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Milk fat — Preparation of fatty acid methyl esters

Matières grasses du lait — Préparation des esters méthyliques d'acides gras

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Foreword

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ISO 15884 | IDF 182 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

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IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Fat*, of the Standing Committee on *Main Components in Milk*, under the aegis of its project leader, Dr F. Ulberth (AT).

Milk fat — Preparation of fatty acid methyl esters

1 Scope

This International Standard specifies a method for the preparation of fatty acid methyl esters from milk fat and fat obtained from dairy products.

The method is not suitable for the analysis of partially lipolysed milk fat (fat acidity > 1 mmol of free fatty acids per 100 g of fat). In such a case, the alternative method described in Annex A can be used.

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 2446, *Milk — Determination of fat content (Routine method)*

ISO 14156|IDF 172, *Milk and milk products — Extraction methods for lipids and liposoluble compounds*

ISO 15885|IDF 184, *Milk fat — Determination of the fatty acid composition by gas-liquid chromatography*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
fatty acid methyl esters of milk fat
methyl esters of fatty acids prepared by the procedure specified in this International Standard

4 Principle

Methyl esters of milk fat fatty acids are prepared by base-catalysed methanolysis of the glycerides in an essentially non-alcoholic solution. After a certain reaction time, the mixture is neutralized by the addition of crystalline sodium hydrogen sulfate to avoid saponification of preformed esters.

5 Reagents

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

5.1 **Solvent**: *n*-pentane, *n*-hexane or *n*-heptane.

5.2 **Methanol**, containing not more than a mass fraction of 0,5 % water.

5.3 Sodium hydrogen sulfate monohydrate ($NaHSO_4 \cdot H_2O$).

5.4 Transesterification reagent, potassium hydroxide (KOH) or sodium methoxide ($NaOCH_3$), methanolic solution of approximately 2 mol/l.

Dissolve 11,2 g of KOH in 100 ml of methanol and mix well.

Alternatively, dissolve 10,8 g of $NaOCH_3$ in 100 ml of methanol and mix well.

The sodium methoxide methanolic solution may also be prepared by dissolving 4,6 g of metallic sodium in methanol or by diluting a commercially available solution of approximately 5,4 mol/l (e.g. Fluka 71748[1)]). Special precautions, however, shall be taken when handling metallic sodium.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Balance, capable of weighing to the nearest 1 mg.

6.2 Test tube, of diameter 16 mm, of length 100 mm, fitted with PTFE-lined screw cap.

6.3 Graduated pipettes, of capacity 1 ml and 10 ml.

6.4 Vortex mixer.

6.5 Centrifuge, capable of operating at 350 *g* ± 50 *g*.

It shall also be provided with a speed indicator indicating the number of revolutions per minute, with a maximum tolerance of ± 50 r/min. For acceleration details, see ISO 2446.

7 Sampling

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

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

8 Preparation of test sample

Follow the extraction method specified in ISO 14156 | IDF 172.

The extracted fat may be stored in a freezer for no longer than 1 month.

9 Procedure

Weigh, to the nearest 5 mg, 100 mg of the prepared test sample (Clause 8) in a test tube (6.2). Dissolve the test portion sample in 5 ml of solvent (5.1) and mix. Add 0,2 ml of the transesterification reagent (5.4) and cap the tube.

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1) Fluka is the tradename of a reagent commercially available. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

Mix the contents of the tube vigorously with the vortex mixer (6.4) for 1 min. After an additional reaction time of 5 min, add 0,5 g of solid sodium hydrogen sulfate (5.3) and mix again. Place the test tubes with the test portion in the centrifuge (6.5) and centrifuge for 3 min at room temperature.

After centrifuging, take an aliquot from the obtained clear supernatant of the test portion for the gas-liquid chromatographic analysis. Proceed as specified in ISO 15885 | IDF 184.

If necessary, decant the ester solution and store it at normal refrigeration temperatures or, preferably, in a deep-freezer for several days. Precautions shall be taken to avoid losses due to the volatility of methyl esters of milk fat fatty acids.

NOTE *N*-Acyl lipids (e.g. sphingolipids) are not properly transesterified by base-catalysed methanolysis. These substances occur primarily in lipids of products derived from whey.

10 Test report

The test report shall specify:

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

b) the sampling method used, if known;

c) the test method used, together with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

Annex A
(normative)

Alternative procedure using acid-catalysed transesterification of glycerides

A.1 Introduction

If a partially hydrolysed milk fat is to be converted to fatty acid methyl esters, use acid-catalysed transesterification of glyceride-bound acids with concomitant esterification of free fatty acids as specified in this annex.

A.2 Reagents

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

A.2.1 **Sulfuric acid**, ρ_{20} (H_2SO_4) = 1,84 g/ml.

A.2.2 **Methanol**, containing not more than a mass fraction of 0,5 % water.

A.2.3 **Esterification reagent**.

While cooling, add slowly 1 ml of sulfuric acid (A.2.1) to 15 ml of methanol (A.2.2).

A.3 Apparatus

Usual laboratory equipment and, in particular, the following.

A.3.1 **Balance**, capable of weighing to the nearest 10 mg.

A.3.2 **Graduated pipettes**, of capacities 1 ml and 5 ml.

A.3.3 **Glass ampoules**, of capacity 5 ml.

Instead of glass ampoules, another suitable derivatization vial of similar dimensions may be used, provided that a leaktight closure prevents losses of volatile esters.

A.3.4 **Oven**, capable of operating at between 100 °C and 110 °C, or **water bath**, capable of boiling.

A.4 Preparation of test sample

Prepare the test sample as specified in Clause 8.

A.5 Procedure

Weigh, to the nearest 10 mg, 2 g of the prepared sample into a glass ampoule (A.3.3). Add 0,4 ml of esterification reagent (A.2.3). Flame seal the glass ampoule. Heat the glass ampoule and its contents for 3 h in the oven (A.3.4) set at between 100 °C and 110 °C, with intermittent mixing of the ampoule contents.

Cool the glass ampoule and its contents to room temperature and allow for complete phase separation. Open the glass ampoule and take an aliquot of the upper phase for further analysis. Dilution of the formed esters with an appropriate solvent may be necessary.