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Milk fat — Determination of the fatty acid composition by gas-liquid chromatography

Matières grasses du lait — Détermination de la composition des acides gras par chromatographie en phases gazeuse et liquide

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Foreword

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ISO 15885 | IDF 184 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.

Foreword

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 IDF 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 — Determination of the fatty acid composition by gas-liquid chromatography

1 Scope

This International Standard specifies a method for the determination of the fatty acid composition of milk fat and fat obtained from dairy products.

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.

ISO14156 | IDF 172, *Milk and milk products — Extraction methods for lipids and liposoluble compounds*

ISO 15884 | IDF 182, *Milk fat — Preparation of fatty acid methyl esters*

3 Terms and definitions

3.1
fatty acid composition of milk fat
mass fraction of individual fatty acids determined by the procedure specified in this International Standard

NOTE The fatty acid composition of milk fat (as grams of individual free acids) per 100 g of total fatty acids (free acids) is expressed as a mass fraction in percent.

4 Principle

Fatty acid methyl esters (FAME) of milk fat are prepared by transesterification. They are separated and determined by capillary gas-liquid chromatography. Individual FAME are quantified by reference to a milk fat of known composition.

5 Reagents

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

5.1 Reference fatty acid methyl esters (FAME) for identification purposes, of high purity (> 90 %), consisting of at least the saturated, even-numbered, straight-chain FAME with C4 to C22 atoms in addition to oleic, linoleic and linolenic acid methyl esters.

NOTE A set of reference fatty acid methyl esters can be obtained either as a commercially available mixture or as a laboratory-prepared mixture from single substances.

5.2 Reference milk fat, for quantification purposes.

Use a milk fat with known fatty acid composition (e.g. Reference material CRM 164[1)]).

5.3 Fat solvent: *n*-alkane (e.g. *n*-pentane, *n*-hexane or *n*-heptane), free of substances that appear in the region of interest in the chromatogram.

5.4 Carrier gas: hydrogen, helium or nitrogen, of purity of at least 99,999 %, with an oxygen content of below 2×10^{-6}.

5.5 Other gases, free from organic impurities (C_nH_m content of below 1×10^{-6}).

Use nitrogen and hydrogen, of purity of at least 99,995 %, and synthetic air.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Gas-liquid chromatograph, comprising the following.

6.1.1 Injector

Maintain injectors of the vaporizing type (split injector or programmed temperature injector, PTV) at a temperature of at least 220 °C (in the case of a PTV, the final injector temperature shall be at least 220 °C). In the case of a cold on-column injector, maintain the injector at a temperature a few degrees below the boiling point of the solvent.

6.1.2 Column oven, capable of running temperature programmes from near ambient temperature up to 260 °C.

6.1.3 Columns, narrow- or large-bore glass or silica capillary column, of suitable length and stationary phase thickness to attain the performance characteristics defined in Clause 7.

NOTE 1 Commercial stationary phases containing nitroterephthalic-acid-modified or unmodified poly(ethylene glycol) or other polar phases have been found to be suitable.

NOTE 2 The stationary phases may be substituted by other polar phases provided that a similar resolution of fatty acid methyl esters is obtained.

6.1.4 Flame ionization detector, capable of operating up to 20 °C above the final temperature of the column oven (6.1.2).

6.1.5 Carrier gas pneumatics, oxygen-diffusion-proof type, capable of maintaining the column head pressure to give a linear carrier gas velocity, with suitable flow controls to provide the requisite flow rates.

If using a vaporizing split injector, control the split vent flow to give a split ratio of 1:50 to 1:100.

NOTE Examples of flow rates are: nitrogen, 15 cm/s to 25 cm/s; helium, 25 cm/s to 35 cm/s; hydrogen, 35 cm/s to 55 cm/s at the initial oven temperature.

6.2 Injection syringe, of plunger-in-barrel type, manual with a maximum capacity of 10 µl, or auto-injector as recommended for the equipment.

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1) Reference material is available from Bureau of Reference, Commission of the European Communities, Brussels, Belgium. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO or IDF of this product.

Usually 10 µl syringes are graduated to 0,2 µl. If graduations of 0,1 µl are required, it is recommended to use 5 µl syringes.

6.3 Data system, capable of producing information required in Clauses 7, 10 and 11.

7 Performance specification

Prepare a test mixture consisting of methyl butyrate, methyl stearate and methyl oleate at a concentration of 0,1 mg/ml of each in the fat solvent (5.3). Separate this mixture by gas chromatography using the same operating conditions as applied to the milk fat samples. Any test substance peak should be more than three-quarters full-scale of the data system (6.3). The largest possible separation of methyl butyrate from the solvent peak and a resolution of 1,5 (baseline resolution) between methyl stearate and methyl oleate should be obtained.

Calculate the resolution, R, by using the following equation:

$$R = 2 \times d/(W_1 + W_2)$$

where

d is the numerical value of the distance between the respective peak maxima for methyl stearate and oleate, in millimetres;

W_1 is the numerical value of the width of the peak for methyl stearate, measured between the points of intersection of the tangents at the inflexion points of the curve with the baseline, in millimetres;

W_2 is the numerical value of the width of the peak for methyl oleate, measured between the points of intersection of the tangents at the inflexion points of the curve with the baseline, in millimetres.

8 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.

9 Preparation of test sample and test portions

For test samples of milk fat, see ISO 15884|IDF 182. For those of milk and milk products, see ISO 14156|IDF 172.

10 Procedure

10.1 Operating conditions

Select operating conditions [i.e. column length and inner diameter, stationary phase film thickness, initial oven temperature, temperature programme rate(s), final oven temperature, carrier gas flow rate] to fulfil the specifications of Clause 7.

10.2 Sample injection

In general, follow the manufacturer's instructions for the injector user.

In case of a vaporizing split injector, draw up 0,5 µl to 1,5 µl of the test portion (Clause 9) with a microsyringe (6.2). Withdraw the sample into the barrel of the syringe. Insert the needle into the heated injector and, after a dwell time of 3 s to 5 s, depress the plunger rapidly. Immediately thereafter, remove the syringe needle from the injector.

If injection is carried out by the cold on-column technique, dilute the test portion (Clause 9) sufficiently with a fat solvent (5.3) (e.g. a dilution ratio of 1:10). Inject the thus-prepared test portion at an oven temperature at or some degrees below the boiling point of the solvent used.

10.3 Qualitative analysis

Analyse the reference fatty acid methyl esters (5.1) under the same working conditions as used for the test portion. Record the retention times of the reference substances.

NOTE 1 Esters elute in order of increasing number of C atoms and in order of increasing number of double bonds for a given number (n) of C atoms (e.g. methyl palmitate elutes before methyl stearate). FAME with 18 C atoms elute in the order: methyl stearate, methyl oleate, methyl linoleate, methyl linolenate. Branched-chain FAME elute before the straight-chain esters with the same number of C atoms in the order: iso-branched [($n - 2$) methyl esters] before anteiso-branched [($n - 3$) methyl esters].

NOTE 2 The chromatogram shown in Figure 1 is an aid for the tentative identification of milk fat FAME.

Identify the peaks of the test sample by comparison with the retention data obtained with the reference mixture and by comparison with the chromatogram shown in Figure 1.

10.4 Quantitative analysis

10.4.1 General

Transmethylate the reference milk fat (5.2) as described in Clause 9. Analyse the obtained FAME mixture under the same working condition as used for the test portion. Terminate the recording of the chromatogram after complete elution of docosanoic acid (C22:0).

To estimate the percentage of a component represented by a peak in the chromatogram, use the method of normalization, which assumes that all components of the sample are represented in the chromatogram. In that case, the sum of the area under the relevant peaks represents 100 % of the sample constituents (100 % elution).

Determine the areas of peaks attributable to fatty acid methyl esters (i.e. all peaks in the chromatogram except the solvent peak, peaks due to addition of stabilizing agents and those which also appear in blank runs).

10.4.2 Calculation of the peak area

Calculate the percentage, P_{Ai}, of the total peak area represented by the peak of component i, by using the following equation:

$$P_{Ai} = \frac{A_i}{\sum A_i} \times 100\ \%$$

where

A_i is the numerical value of the peak area corresponding to component i;

$\sum A_i$ is the numerical value of the sum of all peak areas corresponding to the component fatty acid methyl esters.

Operating conditions

Column dimensions:	inner diameter 0,32 mm; length 30 m; film thickness 0,25 µm	Injector type	manual
		Injection type	split
Stationary phase	J&W DB wax	Injection volume	1 µl
Carrier gas	hydrogen	Split ratio	*ca*. 1:25 determined at 40 °C
Column head pressure	0,4 bar	Injector temperature	280 °C
Sample concentration	2 mg/ml dissolved in *n*-hexane	Detector temperature	250 °C

Figure 1 — Separation of methyl esters of milk fat fatty acids by GLC

10.4.3 Determination and calculation of the correction factors

10.4.3.1 Determination

To determine the correction factors, f_i, which are used to convert the peak area percentages into mass fractions in percent of components, the known mass fraction, ω_i, of the component i in the reference milk fat (5.2) is divided by the area-percentage of the corresponding peak in the chromatogram of the reference milk fat.

10.4.3.2 Calculation

Calculate the correction factor, f_i, by using the following equation:

$$f_i = \frac{\omega_i}{A_i} = \frac{\omega_i \cdot \sum A_i}{\sum \omega_i \cdot A_i}$$

where

ω_i is the mass fraction of component i in the reference milk fat (5.2), expressed as free fatty acid;

$\sum\omega_i$ is the sum of the mass fractions of the various components in the reference milk fat (5.2), expressed as free fatty acid.

10.4.3.3 Standardization of correction factor

Standardize the values of the individual correction factor with respect to the correction factor of methyl palmitate to obtain the relative correction factor of component i, f_i', by using the following equation:

$$f_i' = \frac{f_i}{f_\mathrm{p}}$$

where f_p is the value of the correction factor for methyl palmitate (f_i' for methyl palmitate = 1).

10.4.4 Determination of the test portion

Analyse the transmethylated test portion (Clause 9) and determine its peak areas as described in 10.4.2.

11 Calculation and expression of results

11.1 Calculation

Calculate the mass fraction of each individual component i in the test sample, $\omega_{i,\mathrm{s}}$, by using the following equation:

$$\omega_{i,\mathrm{s}} = \frac{f_i' \cdot P_{Ai,\mathrm{s}}}{\sum(f_i' \cdot P_{Ai,\mathrm{s}})} \times 100\ \%$$

where

$\omega_{i,\mathrm{s}}$ is the mass fraction, in percent, of the individual component i in the test sample, determined as grams of free fatty acid per 100 g of total fatty acids;

$P_{Ai,\mathrm{s}}$ is the percentage of the peak area representing component i in the test sample.

Include unidentified sample components (“unknowns”) in the final report, using a correction value, f', of 1 for the calculations.

11.2 Expression of results

Express the results to two decimal places.

12 Precision

12.1 Interlaboratory test

The values for repeatability and reproducibility limits cover the preparation of the methyl esters in accordance with ISO 15884 | IDF 182, together with the procedure of this International Standard. The values were derived from the results of an interlaboratory test carried out in accordance with ISO 5725-1 and ISO 5725-2.

12.2 Repeatability

The relative 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:

— for fatty acid components present in excess of 5 g per 100 g of total fatty acids: 5 %, with an absolute maximum of 1 g per 100 g;

— for fatty acid components present in amounts of 1 g to 5 g per 100 g of total fatty acids: 12 %, with an absolute maximum of 0,5 g per 100 g.

12.3 Reproducibility

The relative 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 not in more than 5 % of cases be greater than:

— for fatty acid components present in excess of 5 g per 100 g of total fatty acids: 15 %, with an absolute maximum of 4 g per 100 g;

— for fatty acids components present in amounts of 1 g to 5 g per 100 g of total fatty acids: 20 %, with an absolute maximum of 1 g per 100 g.

13 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);

e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Bibliography

[1] ISO 707, *Milk and milk products — Guidance on sampling*

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

[3] 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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