International Standard

ISO 16958

IDF 231

Milk, milk products, infant formula and adult nutritionals — Determination of fatty acids composition — Capillary gas chromatographic method

Lait, produits laitiers, formules infantiles et produits nutritionnels pour adultes — Détermination de la composition en acides gras — Méthode de chromatographie en phase gazeuse sur colonne capillaire

Second edition
2026-01

Reference numbers
ISO 16958:2026(en)
IDF 231:2026(en)

© ISO and IDF 2026



COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2026

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11

Email: copyright@iso.org
Website: www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B
B-1030 Brussels
Phone: +32 2 325 67 40
Fax: +32 2 325 67 41
Email: info@fil-idf.org
Website: www.fil-idf.org

Published in Switzerland

Contents

Page

Foreword ... **iv**

1 Scope ... **1**

2 Normative references ... **1**

3 Terms and definitions ... **1**

4 Principle ... **2**

5 Reagents ... **2**

6 Apparatus ... **6**

7 Sampling ... **9**

8 Preparation of test sample ... **9**
8.1 Liquid and powder milk and infant formula with a fat content ≥ 1,5 % (mass fraction) ... 9
8.2 Liquid and powder milk and infant formula with a fat content < 1,5 % (mass fraction) ... 9
8.3 Cheese ... 9

9 Procedure ... **9**
9.1 Test portion ... 9
9.2 Quantitative determination ... 10
9.2.1 Determination of response factors ... 10
9.2.2 Determination of the test portion ... 10
9.2.3 Fatty acid identification ... 10

10 Calculation and expression of results ... **12**
10.1 Calculation ... 12
10.1.1 Calculation of response factor ... 12
10.1.2 Fatty acids on the product ... 13
10.1.3 Fatty acids on the total fat ... 13
10.1.4 Sum of class or group of fatty acids in 100 g product ... 13
10.1.5 Sum of class or group of fatty acids in 100 g fat ... 14
10.1.6 Performance of the transesterification ... 14
10.2 Expression of results ... 14

11 Precision ... **15**
11.1 Interlaboratory test ... 15
11.2 Repeatability ... 15
11.3 Reproducibility ... 15
11.4 Limit of detection (LOD) ... 15
11.5 Limit of quantitation (LOQ) ... 15

12 Test report ... **15**

Annex A (normative) **Groups or classes of fatty acids and individual fatty acids** ... **16**

Annex B (normative) **Gas–liquid chromatographic analysis** ... **19**

Annex C (informative) **Results of an interlaboratory trial** ... **29**

Bibliography ... **45**

© ISO and IDF 2026 – All rights reserved

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document 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, and in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products - Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). It is being published jointly by ISO and IDF and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2012.13: *Determination of labeled fatty acids content in milk products and infant formula*.

This second edition cancels and replaces the first edition (ISO 16958 | IDF 231:2015), of which it constitutes a minor revision.

The changes are as follows:

— references to other standards have been updated;

— information on standard solutions and chromatographic columns has been updated;

— the Bibliography has been expanded.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

© ISO and IDF 2026 – All rights reserved

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/ food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

IDF draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). IDF takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, IDF had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. IDF shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

This document was prepared by the IDF *Standing Committee on Analytical Methods for Composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with AOAC INTERNATIONAL. It is being published jointly by ISO and IDF and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2012.13: *Determination of labeled fatty acids content in milk products and infant formula*

All work was carried out by the ISO-IDF Project Group C11 of the Standing Committee on *Analytical Methods for Composition* under the aegis of its project leader, Mr Pierre-Alain Golay (CH).

© ISO and IDF 2026 – All rights reserved

Milk, milk products, infant formula and adult nutritionals — Determination of fatty acids composition — Capillary gas chromatographic method

1 Scope

This document specifies a method for the quantification of individual and/or all fatty acids content in the profile of milk, milk products, infant formula and adult nutritional formula, containing milk fat and/or vegetable oils, supplemented or not supplemented with oils rich in long chain polyunsaturated fatty acids (LC-PUFA). This also includes groups of fatty acids often labelled [i.e. *trans* fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3, omega-6 and omega-9 fatty acids] and/or individual fatty acids [i.e. linoleic acid (LA), α-linolenic acid (ALA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)].

The determination is performed by direct transesterification in food matrices, without prior fat extraction, and consequently it is applicable to liquid samples or reconstituted powder samples with water having total fat ≥ 1,5 % (mass fraction).

The fat extracted from products containing less than 1,5 % (mass fraction) fat can be analysed with the same method after a preliminary fat extraction using methods referenced in Clause 2. Dairy products, such as soft or hard cheeses with acidity level ≤ 1 mmol/100 g of fat, can be analysed after a preliminary fat extraction using methods referenced in Clause 2.

For products supplemented or enriched with PUFA with fish oil or algae origins, the evaporation of solvents is performed at the lowest possible temperature (e.g. max. 40 °C) to recover these sensitive fatty acids.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 648, *Laboratory glassware — Single-volume pipettes*

ISO 1740 | IDF 6, *Milkfat products and butter — Determination of fat acidity (Reference method)*

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

ISO 23319 | IDF 250, *Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

© ISO and IDF 2026 – All rights reserved

3.1
fatty acids content
mass fraction of individual or groups of substances

Note 1 to entry: The fatty acids content is determined by the procedure specified in this document. See Table A.1.

Note 2 to entry: The fatty acids content is expressed as a mass fraction in grams (or in milligrams) of the fatty acids per 100 g of product. Fatty acid results can be converted into other results expression formats (see 10.2).

4 Principle

Addition of the internal standard solution to the sample, preparation of fatty acid methyl esters (FAMEs) by direct transesterification with methanolic sodium methoxide for liquid samples; dissolution (i.e. reconstitution) in water for powder sample and direct transesterification with methanolic sodium methoxide. The same transesterification procedure is applied to fat extracted from various foods (e.g. low fat products, cheeses).

Separation of FAMEs is done by using capillary gas-liquid chromatography. Identification of FAMEs is done by comparison with the retention time of pure standards and quantification as fatty acids by reference to an internal standard (C11:0 FAME) and instrument response factors. Verification of the transesterification performance using a second internal standard (C13:0 TAG).

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 ***n*-Hexane,** [$CH_3(CH_2)_4CH_3$], chromatography grade or *n*-heptane, [$CH_3(CH_2)_5CH_3$], chromatography grade.

5.2 **Methanol,** [CH_3OH], chromatography grade.

5.3 **Water**, HPLC grade or equivalent purity quality.

5.4 **Sodium methoxide solution**, [CH_3ONa], dissolved in methanol 30 % m/v, or 25 % m/v, depending on local availability.

5.5 **Transesterification solution**, (sodium methoxide solution 5 % m/v in methanol).

Into a 250 ml volumetric flask, pipette 42 ml (or 50 ml) of sodium methoxide solution 30 % m/v (or 25 % m/v) and mix gently with 200 ml of methanol using a magnetic stirrer. Remove the magnetic stirrer (i.e. using a rigid rod with magnetic extremity), then cool to room temperature and make up to the mark with methanol.

Stored in the dark at 4 °C, this solution is stable for one week. Allow the solution to come to room temperature before use. This solution volume is sufficient to analyse approximately 40 samples. In cases of a smaller number of analyses, the reagent volume can be adapted accordingly.

Perform the transesterification reaction at ambient temperature (between 20 °C and 25 °C).

NOTE Value indicated in brackets () corresponds to sodium methoxide solution with 25 % m/v concentration.

5.6 **di-Sodium hydrogen citrate sesquihydrate**, [$HOC(COOH)(CH_2COONa)_2 \cdot 1{,}5\ H_2O$].

5.7 **Sodium chloride**, [NaCl].

© ISO and IDF 2026 – All rights reserved