

SLOVENSKI STANDARD

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Nadomešča:

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Mleko, mlečni izdelki, formule za dojenčke in prehranska dopolnila za odrasle - Določanje sestave maščobnih kislin - Metoda kapilarne plinske kromatografije (ISO 16958:2026)

Milk, milk products, infant formula and adult nutritionals - Determination of fatty acids composition - Capillary gas chromatographic method (ISO 16958:2026)

Milch, Milcherzeugnisse, Säuglingsnahrung und Nahrungsergänzungsmittel für Erwachsene - Bestimmung der Fettsäurezusammensetzung - Verfahren mit Kapillargaschromatographie (ISO 16958:2026)

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 (ISO 16958:2026)

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67.100.10	Mleko in predelani mlečni proizvodi	Milk and processed milk products

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EUROPEAN STANDARD
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EN ISO 16958

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**Milk, milk products, infant formula and adult nutritionals -
Determination of fatty acids composition - Capillary gas
chromatographic method (ISO 16958:2026)**

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 (ISO 16958:2026)

Milch, Milcherzeugnisse, Säuglingsnahrung und Nahrungsergänzungsmittel für Erwachsene - Bestimmung der Fettsäurezusammensetzung - Verfahren mit Kapillargaschromatographie (ISO 16958:2026)

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European foreword

This document (EN ISO 16958:2026) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis" the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by July 2026, and conflicting national standards shall be withdrawn at the latest by July 2026.

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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
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ISO 16958:2026(en)
IDF 231:2026(en)**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).

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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.

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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.

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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).

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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 $\geq 1,5$ % (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/>

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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, $[\text{CH}_3(\text{CH}_2)_4\text{CH}_3]$, chromatography grade or *n*-heptane, $[\text{CH}_3(\text{CH}_2)_5\text{CH}_3]$, chromatography grade.

5.2 Methanol, $[\text{CH}_3\text{OH}]$, chromatography grade.

5.3 Water, HPLC grade or equivalent purity quality.

5.4 Sodium methoxide solution, $[\text{CH}_3\text{ONa}]$, 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, $[\text{HOC}(\text{COOH})(\text{CH}_2\text{COONa})_2 \cdot 1,5 \text{H}_2\text{O}]$.

5.7 Sodium chloride, $[\text{NaCl}]$.

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5.8 Neutralization solution, (di-sodium hydrogen citrate sesquihydrate 10 % m/v, sodium chloride 15 % m/v in water).

Weigh 50,0 g of di-sodium hydrogen citrate sesquihydrate and 75,0 g of sodium chloride in a 500 ml volumetric flask. Dissolve in 450 ml of water using a magnetic stirrer. Remove the magnetic stirrer, then make up to the mark with water.

Stored in the dark at 4 °C, this solution is stable for one month. Presence of salt crystals can appear in the solution during storage, but disappear after shaking.

Allow the solution to come to room temperature before use. This solution volume is sufficient to analyse approximately 40 samples or more. In cases of a smaller number of analyses (or single analysis), the mass and volume of solution can be adapted accordingly.

5.9 Tert-butyl methyl ether (MTBE), chromatography grade.

5.10 Methyl undecanoate (C11:0 FAME), of purity ≥ 99 % mass fraction.

5.11 Tritridecanoin (C13:0 TAG), of purity ≥ 99 % mass fraction.

5.12 C11:0 FAME/C13:0 TAG standard solution.

Into a 250 ml volumetric flask, weigh to the nearest 0,1 mg about 500 mg of tritridecanoin and 500 mg of methyl undecanoate. Dissolve and make up to the mark with MTBE.

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 or more. In cases of a smaller number of analyses, standard mass and volume of solvent can be adapted accordingly.

5.13 Octadecenoic acid methyl esters, *cis* and *trans* isomers mixture of C18:1 with *trans*-4 to *trans*-16 octadecenoic (all isomers) and principal *cis* isomers (concentration 2,5 mg/ml in methylene chloride).

NOTE This standard is commercially available from Sigma-Aldrich, brand of Merck (Cat. 40495-U), or Cayman Chemical (cat. 29363).¹⁾

5.14 Linoleic acid methyl esters, *cis* and *trans* isomers mixture of C18:2 with *trans*-9,*trans*-12-octadecadienoic acid (approximately 50 %), *cis*-9,*trans*-12-octadecadienoic acid (approximately 20 %), *trans*-9,*cis*-12-octadecadienoic acid (approximately 20 %) and *cis*-9,*cis*-12-octadecadienoic acid (approximately 10 %). Concentration 10 mg/ml in methylene chloride.

NOTE This standard is commercially available from Sigma-Aldrich, brand of Merck (Cat. L8404)¹⁾.

5.15 Linolenic acid methyl esters, *cis* and *trans* isomers mixture of C18:3 with:

- *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 3 % (mass fraction));
- *cis*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 7 % (mass fraction));
- *cis*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 7 % (mass fraction));
- *cis*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 15 % (mass fraction));
- *trans*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 7 % (mass fraction));

1) Sigma-Aldrich, brand of Merck, and Cayman Chemical are examples of suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by either ISO or IDF of the products named. Equivalent products may be used if they can be shown to lead to the same results.