SLOVENSKI STANDARD
SIST EN ISO 16958:2020

01-julij-2020

Mleko, mlečni proizvodi, hrana za dojenčke in prehranska dopolnila za odrasle - Določevanje sestave maščobnih kislin - Kapilarna plinska kromatografija (ISO 16958:2015)

Milk, milk products, infant formula and adult nutritionals - Determination of fatty acids composition - Capillary gas chromatographic method (ISO 16958:2015)

Milch, Milcherzeugnisse, Säuglingsnahrung und Nahrungsergänzungsmittel für Erwachsene - Bestimmung der Fettsäurenzusammensetzung - Verfahren mit Kapillargaschromatographie (ISO 16958:2015)

Lait, produits laitiers, formules infantiles et produits nutrionnels pour adultes - Détermination de la composition en acides gras - Méthode de chromatographie en phase gazeuse sur colonne capillaire (ISO 16958:2015)

Ta slovenski standard je istoveten z: **EN ISO 16958:2020**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.100.10	Mleko in predelani mlečni proizvodi	Milk and processed milk products

SIST EN ISO 16958:2020 **en**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 16958

June 2020

ICS 67.100.10

English Version

Milk, milk products, infant formula and adult nutritionals - Determination of fatty acids composition - Capillary gas chromatographic method (ISO 16958:2015)

Lait, produits laitiers, formules infantiles et produits nutrionnels pour adultes - Détermination de la composition en acides gras - Méthode de chromatographie en phase gazeuse sur colonne capillaire (ISO 16958:2015)

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Ref. No. EN ISO 16958:2020 E

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European foreword

The text of ISO 16958:2015 has been prepared by Technical Committee ISO/TC 34 "Food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 16958:2020 by Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis" the secretariat of which is held by NEN.

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Endorsement notice

The text of ISO 16958:2015 has been approved by CEN as EN ISO 16958:2020 without any modification.

INTERNATIONAL STANDARD

ISO 16958

IDF 231

First edition
2015-11-01

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

Reference numbers
ISO 16958:2015(E)
IDF 231:2015(E)

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Forewords

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 documents 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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The committee responsible for this document is 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. 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*.

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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ISO 16958 | IDF 231 was prepared by the IDF Standing Committee on *Analytical Methods for Composition* and the ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee 5 on *Milk and milk products* (ISO/TC 34/SC 5), 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).

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

1 Scope

This International Standard specifies a method for the quantification of individual and/or all fatty acids 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 % m/m.

The fat extracted from products containing less than 1,5 % m/m fat can be analysed with the same method after a preliminary fat extraction using methods referenced in Clause 2. Dairy products, like 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 should be performed at the lowest possible temperature (e.g. max. 40 °C) to recover these sensitive fatty acids.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 1735 | IDF 5, *Cheese and processed cheese products — Determination of fat content — Gravimetric method (Reference method)*

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

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

3 Terms and definitions

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

3.1
fatty acids content
mass fraction of individual or groups of substances determined by the procedure specified in this International Standard

Note 1 to entry: See Table A.1.

Note 2 to entry: The fatty acid content is expressed as a mass fraction in grams (or in milligrams) of the fatty acids per 100 g of product (see Table A.1). 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 using capillary gas-liquid chromatography. Identification of FAMEs 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.

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 300 ml volumetric flask, pipette 50 ml (or 60 ml) of sodium methoxide solution 30 % m/v (or 25 % m/v) and mix gently with 250 ml of methanol using a magnetic stirrer. Remove the magnetic stirrer, 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 case 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, [$HOC(COOH)(CH_2COONa)_2.1{,}5\ H_2O$].

5.7 Sodium chloride, [NaCl].

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 may 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 case 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 case 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 Supelco Inc, brand of Sigma-Aldrich (Cat. 40495-U)[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 Supelco Inc, brand of Sigma-Aldrich (Cat. 47791)[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 % m/m),

— *cis*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 7 % m/m),

— *cis*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 7 % m/m),

— *cis*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 15 % m/m),

— *trans*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 7 % m/m),

— *trans*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 15 % m/m),

— *trans*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (approximately 15 % m/m), and

— *trans*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (approximately 30 % m/m).

Concentration 10 mg/ml in methylene chloride.

NOTE This standard is commercially available from Supelco Inc, brand of Sigma Aldrich (Cat. 47792)[1)]. This standard contains all *trans* isomers of C18:3 (eight in total) but their abundance and ratio are different to those observed in refined/deodorized oils and fats.

5.16 Methyl octadecadienoate conjugated acids, mixture of C18:2 *cis*-9,*trans*-11 and *cis*-10,*trans*-12-octadecadienoate conjugated acids, of purity ≥ 99 % mass fraction.

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1) Supelco Inc., brand of Sigma Aldrich, is an example 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 product named. Equivalent products may be used if they can be shown to lead to the same results.