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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

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Contents

~~Forewords~~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 ~~documents~~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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~~The committee responsible for this~~This document ~~is~~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 ~~has undergone~~it constitutes a minor revision.

The ~~main~~ 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.

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~~This document was prepared by the IDF *Standing Committee on Analytical Methods for Composition* and ~~the~~ ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 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 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 % ~~m/m.~~(mass fraction).

The fat extracted from products containing less than 1,5 % ~~m/m~~(mass fraction) fat can be analysed with the same method after a preliminary fat extraction using methods referenced in Clause 2. Dairy products, ~~like~~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 ~~should be~~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 ~~whole~~the text in such a way that some or ~~in part, are normatively referenced in~~all of their content constitutes requirements of 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 23319 | IDF 250, *Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method*~~

ISO 1740 | IDF 6, ~~*Milk fat*~~ *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/

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

Note 2 to entry: The fatty ~~acid~~ 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.