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Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides

Lait et produits laitiers — Détermination de la pureté des matières grasses laitières par analyse chromatographique en phase gazeuse des triglycérides

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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 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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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). It is being published jointly by ISO and IDF.

This second edition cancels and replaces the first edition (ISO 17678 | IDF 202:2010), which has been technically revised. The following changes have been made:

— the Scope has been restricted to exclude milk fat obtained from special feeding practices and from whey;

— the Scope has been extended to include milk fat obtained from cheese showing low lipolysis;

— the Normative references have been updated to reflect the modified scope;

— a method has been added for the fat extraction from cheese;

— 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.

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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.* It is being published jointly by ISO and IDF.

The work was carried out by the Joint ISO/IDF Action Team C23 of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader, Mr J. Molkentin (DE).

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Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides

1 Scope

This document specifies a reference method for the determination of milk fat purity using gas chromatographic analysis of triglycerides. The method utilizes the differences in triglyceride fingerprint of milk fat from the individual triglyceride fingerprints of other fats and oils to determine samples which are outside the range normally observed for milk fat. This is achieved by using the defined triglyceride formulae based on the normalized weighted sum of individual triglyceride peaks which are sensitive to the integrity of the milk[6][7]. The integrity of the milk fat can be determined by comparing the result of these formulae with those previously observed for a range of pure milk fat samples[12]. Both vegetable fats and animal fats such as beef tallow and lard can be detected.

The method is applicable to bulk milk, or products made thereof, irrespective of the variation in common feeding practices, breed or lactation conditions. In particular, the method is applicable to fat extracted from milk products purporting to contain pure milk fat with unchanged composition, such as butter, cream, milk and milk powder.

Because a false-positive result can occur, the method does not apply to milk fat related to these circumstances:

a) obtained from bovine milk other than cow's milk;

b) obtained from single cows;

c) obtained from cows whose diet contained a particularly high proportion of vegetable oils such as rapeseed, cotton or palm oil, etc.;

d) obtained from cows suffering from serious underfeeding (strong energy deficit);

e) obtained from colostrum;

f) subjected to technological treatment such as removal of cholesterol or fractionation;

g) obtained from skim milk, buttermilk or whey;

h) obtained from cheeses showing increased lipolysis;

i) extracted using the Gerber, Weibull–Berntrop or Schmid–Bondzynski–Ratzlaff methods, or that has been isolated using detergents (e.g. the Bureau of Dairy Industries method).

With the extraction methods specified in i), substantial quantities of partial glycerides or phospholipids can pass into the fat phase.

NOTE 1 In nature, butyric (*n*-butanoic) acid (C4) occurs exclusively in milk fat and enables quantitative estimations of low to moderate amounts of milk fat in vegetable and animal fats to be made. Due to the large variation of C4, for which the approximate content ranges from 3,1 % fat mass fraction to 3,8 % fat mass fraction, it is difficult to provide qualitative and quantitative information for foreign fat to pure milk fat ratios of up to 20 % mass fraction[11].

NOTE 2 In practice, quantitative results cannot be derived from the sterol content of vegetable fats, because they depend on production and processing conditions. Furthermore, the qualitative determination of foreign fat using sterols is ambiguous.

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NOTE 3 Due to special feeding practices such as those related to c) and d), false-positive results have sometimes been reported for milk from certain Asian regions[15]. Moreover, grass-only diets such as mountain and, in particular, highland pasture feeding sometimes cause false-positive results, which can be substantiated by a content of conjugated linoleic acid (C18:2 c9t11) of ≥ 1,3 % fatty acid mass fraction[16][17]. Nevertheless, results conforming to the criteria of milk fat purity specified in this document are accepted, even if samples were undoubtedly produced under conditions reported in this note, including those described in h).

NOTE 4 In cases where a positive result is suspected to be caused by circumstances related to c) or d), another analytical method, such as fatty acid or sterol analysis, can be applied to confirm the finding. Due to similar or increased limitations (e.g. as described in NOTE 1 and NOTE 2), a negative result obtained by another method is not appropriate to contrastingly confirm milk fat purity.

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 1211 | IDF 1, *Milk — Determination of fat content — Gravimetric method (Reference method)*

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

ISO 1736 | IDF 9, *Dried milk and dried milk products — Determination of fat content — Gravimetric method (Reference method)*

ISO 2450 | IDF 16, *Cream — Determination of fat content — Gravimetric method (Reference method)*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 7328 | IDF 116, *Milk-based edible ices and ice mixes — Determination of fat content — Gravimetric method (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.

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

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

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

3.1
milk fat purity
absence of vegetable and animal fats determined by the procedure specified in this document

Note 1 to entry: The purity is determined using *S*-values, which are calculated from the content of triglycerides. Triglyceride mass fractions are expressed as percentages.

4 Principle

Fat extracted from milk or milk products is analysed by gas chromatography (GC) using a packed or a short capillary column to determine triglycerides (TGs), separated by total carbon numbers. By inserting the mass fraction, expressed as a percentage, of fat molecules of different sizes (C24 to C54, using even C numbers only) into suitable TG formulae, *S*-values are calculated. If the *S*-values exceed the limits established with pure milk fat, the presence of foreign fat is detected.

NOTE 1 The suitability and equivalence of both packed and capillary columns have been demonstrated previously[8][9][10].

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