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**Milkfat products and butter —
Determination of fat acidity (Reference
method)**

*Produits à matière grasse laitière et beurre — Détermination de l'acidité
de la matière grasse (Méthode de référence)*

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 1740|IDF 6 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. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition of ISO 1740|IDF 6 cancels and replaces ISO 1740:1991, of which it constitutes a minor revision. Only editorial changes have been made.

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Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

ISO 1740|IDF 6 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. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Group of Experts, *Free fatty acids* (E39), under the aegis of its project leader, Mr A. Jellema (NL).

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Milkfat products and butter — Determination of fat acidity (Reference method)

1 Scope

This International Standard specifies a method for the determination of the acidity of the fat contained in milkfat products¹⁾ and in butter.

2 Terms and definitions

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

2.1

fat acidity of a milkfat product or butter

amount of alkali required to neutralize the free fatty acids in the test portion, as determined using the method specified in this International Standard, divided by the mass of the test portion

NOTE 1 The fat acidity is expressed in millimoles per 100 g of fat.

NOTE 2 The following alternative methods of expression of fat acidity have been used in the past but they are no longer recommended:

- a) the number of milligrams of potassium hydroxide required to neutralize the free acids contained in 1 g of fat (equal to the acid value);
- b) the number of grams of oleic acid per 100 g of fat (equal to the percentage of free fatty acids).

3 Principle

In the particular case of butter, the fat is first separated from the melted butter by centrifuging.

In an oven, the melted milkfat product or fat from butter is filtered through a filter paper.

The filtrate is dissolved in a mixture of propan-2-ol and light petroleum, then titrated with tetra-*n*-butylammonium hydroxide standard solution using thymol blue as indicator.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

1) As defined in FAO/WHO Standard A-2, Section A for anhydrous milkfat and anhydrous butter oil, and Section B for ghee.

4.1 Tetra-*n*-butylammonium hydroxide standard solution, $c(\text{C}_{16}\text{H}_{37}\text{NO}) = 0,1 \text{ mol/l}$, in propan-2-ol/methanol mixture, 3 + 1 (volume fraction).

The concentration of the tetra-*n*-butylammonium hydroxide standard solution may change on storage and when being transferred to the burette. For these reasons, the actual concentration of the solution should be determined to four decimal places immediately before use by titration against a standard solution of potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$) using thymol blue as indicator.

However, if the burette is fitted with a facility to exclude the entry of carbon dioxide, the concentration of the tetra-*n*-butylammonium hydroxide standard solution is stable for longer periods. In this case the actual concentration of the solution need be checked only for each series of determinations by carrying out a check test (7.5) using the reference fat (4.4).

4.2 Thymol blue solution, $\rho(\text{C}_{27}\text{H}_{30}\text{O}_5\text{S}) = 0,1 \text{ g/l}$, in propan-2-ol.

Dissolve 0,1 g of sodium salt of thymol blue in 100 ml of propan-2-ol to prepare a stock solution. Before use, dilute one volume of this stock solution with nine volumes of propan-2-ol.

4.3 Fat solvent

4.3.1 Mix one volume of thymol blue solution (4.2) with four volumes of light petroleum (boiling range 60 °C to 80 °C). Store this mixture in the dark. The mixture may be stored for up to 1 month.

4.3.2 If blank tests (7.4) give high results, neutralize the fat solvent with the tetra-*n*-butylammonium hydroxide standard solution (4.1) until a faint greenish colour is obtained.

4.4 Reference fat (for checking periodically the whole titration procedure).

4.4.1 Preparation of reference fat samples [ISO 1740:2004](https://standards.iteh.ai/catalog/standards/sist/5e98f5b6-2fe4-4b54-8846-4d2350111111/iso-1740-2004)

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Dissolve known quantities of palmitic acid ($\text{C}_{16}\text{H}_{32}\text{O}_2$) in washed milkfat (see 4.4.2). Suitable concentrations of palmitic acid are 0,5 mmol to 2,0 mmol of palmitic acid per 100 g of fat.

Calculate the fat acidity of the reference fat samples in millimoles of palmitic acid present in 100 g of reference fat.

NOTE This calculated value may serve as a reference value.

4.4.2 Washed milkfat

Wash a good quality milkfat²⁾ with aqueous potassium hydroxide solution [$c(\text{KOH}) = 0,1 \text{ mol/l}$]. Then wash with water, centrifuge and filter through a filter paper.

4.4.3 Storage

Dispense the reference fat into bottles and seal them hermetically. If the fat is to be used within 4 weeks, the bottles may be stored in the dark at a temperature not exceeding 4 °C. If it is necessary to keep the fat for a longer period, freeze it immediately and store in the dark.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

2) For the specification of "good quality", see FAO/WHO Standard A-2, Section A.

- 5.1 Analytical balance**, capable of weighing to the nearest 0,01 g.
- 5.2 Centrifuge**, capable of producing a radial acceleration of at least 350 g, with a swing-out rotor, for example a so-called Gerber centrifuge (see ISO 2446^[2]).
- 5.3 Centrifuge tubes**.
- 5.4 Glass funnels and filter paper** (medium grade).
- 5.5 Delivery pipettes or syringes**, of capacity 5 ml to 10 ml.
- 5.6 Delivery pipettes or syringes**, of capacity 50 ml \pm 0,5 ml.
- 5.7 Titration vessels**, for example conical flasks of capacity approximately 100 ml to 250 ml.
- 5.8 Burette**, graduated in divisions of 0,02 ml.
- 5.9 Nitrogen**, free from carbon dioxide.
- 5.10 Oven**, electrically heated, capable of being maintained at 50 °C \pm 2 °C.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707^[1].

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7 Procedure

7.1 Preparation of test sample

7.1.1 Butter

Set the oven (5.10) at 50 °C.

Melt an appropriate quantity of the sample in a centrifuge tube (5.3) in the oven and separate the fat by centrifuging at a radial acceleration of at least 350 g in the centrifuge (5.2) for 5 min. Filter the warm separated butterfat through a folded dry filter paper in the oven. The filtered butterfat shall be clear and visibly free from water and non-fatty compounds.

7.1.2 Milkfat products (anhydrous milkfat, anhydrous butter oil or anhydrous butterfat, butter oil, butterfat or ghee).

Set the oven (5.10) at 50 °C.

Melt an appropriate quantity of the milkfat product in the oven and filter it through a folded dry filter paper in the oven.

7.2 Test portion

Weigh, to the nearest 0,01 g, 5 g to 10 g of the test sample (7.1) into a titration vessel (5.7), transferring the fat using a pipette or syringe (5.5).