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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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Foreword

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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.

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.

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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).

This edition of ISO 1740|IDF 6 cancels and replaces IDF 6B:1989. Only editorial changes have been made.

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 products[1)] 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, $c(C_{16}H_{37}NO)$ = 0,1 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 ($KHC_8H_4O_4$) 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, $\rho(C_{27}H_{30}O_5S)$ = 0,1 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

Dissolve known quantities of palmitic acid ($C_{16}H_{32}O_2$) 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 milkfat[2)] with aqueous potassium hydroxide solution [c(KOH) = 0,1 mol/l]. Then wash with water, centrifuge and filter though 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].

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).

7.3 Determination

7.3.1 Add to the test portion (7.2) 50 ml of the fat solvent (4.3) using a pipette or syringe (5.6) and dissolve the fat.

7.3.2 Titrate the dissolved fat with the tetra-*n*-butylammonium hydroxide standard solution (4.1) under a flow of nitrogen (5.9) until a yellow to faint greenish colour persists for at least 5 s.

Record the volume of solution (4.1) used to the nearest 0,01 ml.

IMPORTANT — To meet the requirements for precision, it is essential to exclude carbon dioxide from the titration vessel during the titration procedure; this can be achieved by carrying out the titration in an atmosphere of nitrogen.

Alternatively, the titration procedure may be carried out using automatic titration equipment and colorimetric determination of the endpoint of the titration (see [6] and [7]).

7.4 Blank test

Carry out a blank test simultaneously with the determination, using the same procedure and the same reagents, but omitting the test portion.

The value obtained in the blank test shall be less than 5 % of the lowest titration value determined on the test samples. When higher blank values are obtained, neutralize the fat solvent before use (see 4.3.2).

7.5 Check test

Carry out a check test at the start of each series of determinations, using the same procedure and the same reagents, but using the reference fat (4.4) in place of the test portion. The check test shall comprise at least two determinations for one reference fat.

Check whether the results meet the repeatability requirement (9.2). If so, take as the final result the arithmetic mean of the results obtained. In addition, the final result shall differ by less than 5 %, with a maximum of 0,05 mmol per 100 g of fat, from the value calculated in 4.4.1.

If the result does not fulfil these requirements, check separately the reagents, the equipment and the procedures.

8 Expression of results

Calculate the fat acidity of the test portion, w_a, in millimoles per 100 g of fat, using the following formula:

$$w_a = \frac{(V_1 - V_2)c}{m} \times 100$$

where

V_1 is the volume, in millilitres, of the tetra-*n*-butylammonium hydroxide standard solution (4.1) used in the titration of the dissolved test portion (7.3.2);

V_2 is the volume, in millilitres, of the tetra-*n*-butylammonium hydroxide standard solution (4.1) used in the titration of the blank (7.4);

c is the exact concentration, in moles per litre, of the tetra-*n*-butylammonium hydroxide standard solution (4.1);

m is the mass, in grams, of the test portion (7.2).

Calculate the fat acidity to two decimal places. If the repeatability has been checked and the requirements (see 9.2) are satisfied, take as the final result the arithmetic mean of the two results.

NOTE For the interest of those familiar with the other expressions used for the fat acidity (see Note 2 to Definition 2.1), the alternative methods of calculation are given below.

a) Calculate the acid value w_{ar}, in milligrams of potassium hydroxide per gram of fat, using the following formula:

$$w_{ar} = \frac{M_1 \, w_a}{100}$$

where

w_a is the fat acidity of the test portion, in millimoles per 100 g of fat;

M_1 is the relative molecular mass of potassium hydroxide (M_1 = 56,1).

b) Calculate the free fatty acids content w_{fa}, expressed as grams of oleic acid per 100 g of fat, using the following formula:

$$w_{fa} = \frac{M_2 \, w_a}{1\,000}$$

where

w_a is the fat acidity of the test portion, in millimoles per 100 g of fat:

M_2 is the relative molecular mass of oleic acid (M_2 = 282).

9 Precision

9.1 Interlaboratory test

The values of repeatability and reproducibility have been derived from the results of an interlaboratory test carried out and evaluated in accordance with ISO 5725:1986 [3] and published in [8].

The precision requirements are related to samples with a fat acidity in the range 0,20 mmol to 2,00 mmol per 100 g of fat. For higher fat acidity levels these requirements are not always attainable.

9.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 0,05 mmol per 100 g.

Reject both results if the difference exceeds 0,05 mmol per 100 g and carry out two new single determinations.

9.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 0,08 mmol per 100 g.