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Milk fat 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)

Reference number
ISO 1740:1991(E)

Foreword

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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.

International Standard ISO 1740 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, in collaboration with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC) and will also be published by these organizations.

This second edition cancels and replaces the first edition (ISO 1740:1980), the scope of which has been technically revised.

Annex A of this International Standard is for information only.

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Milk fat 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 milk fat products (as defined in FAO/WHO Standard A-2[1]) and in butter.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 707:1985, *Milk and milk products — Methods of sampling*.

FAO/WHO Standard A-2, elaborated under the Code of Principles concerning milk and milk products, 8th edition, 1984, Rome, Food and Agriculture Organization of the United Nations/World Health Organization.

3 Definition

For the purposes of this International Standard, the following definition applies.

fat acidity of a milk fat product or butter: The 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.

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

NOTE 1 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).

4 Principle

In the particular case of butter, preliminary separation of the fat from the melted butter by centrifuging.

In an oven, filtration of the melted milk fat product or fat from butter through a filter paper.

Dissolution of the filtrate in a mixture of propan-2-ol and light petroleum, and titration with standard volumetric tetra-*n*-butyl ammonium hydroxide solution using thymol blue as indicator.

5 Reagents

All reagents shall be of recognized analytical grade.

5.1 Tetra-*n*-butyl ammonium hydroxide, $c(C_{16}H_{37}NO) = 0{,}1$ mol/l, volumetric solution in propan-2-ol/methanol mixture, 3 + 1 (V/V).

NOTE 2 The concentration of the standard volumetric tetra-*n*-butyl ammonium hydroxide 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.

1) FAO/WHO Standard A-2, Section A for anhydrous milk fat, anhydrous butteroil and butteroil, and Section B for ghee.

However, if the burette is fitted with a facility to exclude the entry of carbon dioxide, the concentration of the standard volumetric tetra-*n*-butyl ammonium hydroxide 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 (8.5) using the reference fat (5.4).

5.2 Thymol blue, $\varrho(C_{27}H_{30}O_5S) = 0{,}1$ g/l, indicator solution 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.

5.3 Fat solvent

5.3.1 Mix one volume of thymol blue solution (5.2) 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.

5.3.2 If blank tests (8.4) give high results, neutralize the fat solvent with the standard volumetric tetra-*n*-butyl ammonium hydroxide solution (5.1) until a faint greenish colour is obtained.

5.4 Reference fat (for checking periodically the whole titration procedure)

5.4.1 Preparation of reference fat samples

Dissolve known quantities of palmitic acid ($C_{16}H_{32}O_2$) in washed milk fat (see 5.4.2). 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 as millimoles of palmitic acid added per mass of milk fat, and expressed as millimoles per 100 g of fat.

NOTE 3 This calculated value may serve as a reference value.

5.4.2 Washed milk fat

Wash a good quality milk fat[2] with aqueous potassium hydroxide solution [c(KOH) = 0,1 mol/l]. Then wash with water, centrifuge and filter through a filter paper.

5.4.3 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.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 Balance, having an accuracy of approximately 5 mg.

6.2 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 [1]).

6.3 Centrifuge tubes.

6.4 Glass funnels and **filter paper** (medium grade).

6.5 Delivery pipettes or **syringes**, of 5 ml to 10 ml capacity.

6.6 Delivery pipettes or **syringes**, of 50 ml ± 0,5 ml capacity.

6.7 Titration vessels, for example conical flasks of approximately 100 ml to 250 ml capacity.

6.8 Burette, graduated in divisions of 0,02 ml.

6.9 Nitrogen, free from carbon dioxide.

6.10 Oven, electrically heated, capable of being maintained at 50 °C ± 2 °C.

7 Sampling

Sampling shall have been carried out in accordance with ISO 707.

8 Procedure

NOTE 4 If it is required to check the repeatability, carry out the operations specified in 8.3 on two test portions taken from the same test sample, using the same apparatus within the shortest feasible time interval.

8.1 Preparation of the test sample

8.1.1 Butter

Set the oven (6.10) at 50 °C ± 2 °C.

Melt an appropriate quantity of the sample in a centrifuge tube (6.3) in the oven and separate the fat by centrifuging at a radial acceleration of at least

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

350*g* in the centrifuge (6.2) 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.

8.1.2 Milk fat products (anhydrous milk fat, anhydrous butteroil or anhydrous butterfat, butteroil or butterfat, ghee)

Set the oven (6.10) at 50 °C ± 2 °C.

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

8.2 Test portion

Weigh, to the nearest 0,01 g, 5 g to 10 g of the test sample (8.1) into a titration vessel (6.7), transferring the fat using a pipette or syringe (6.5).

8.3 Determination

8.3.1 Add to the test portion (8.2) 50 ml of the fat solvent (5.3) using a pipette or syringe (6.6) and dissolve the fat.

8.3.2 Titrate the dissolved fat with the standard volumetric tetra-*n*-butyl ammonium hydroxide solution (5.1) under a flow of nitrogen (6.9) until a yellow to faint greenish colour persists for at least 5 s.

Record the volume of solution (5.1) 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 end-point of titration (see [2] and [3]).

8.4 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 5.3.2).

8.5 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 (5.4) in place of the test sample. The check test shall comprise at least two determinations for one reference fat.

Check whether the results meet the repeatability requirement (10.1). 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 5.4.1.

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

9 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 standard volumetric tetra-*n*-butyl ammonium hydroxide solution (5.1) used in the titration of the dissolved test sample (8.3.2);

V_2 is the volume, in millilitres, of the standard volumetric tetra-*n*-butyl ammonium hydroxide solution (5.1) used in the titration of the blank (8.4);

c is the exact concentration, in moles per litre, of the standard volumetric tetra-*n*-butyl ammonium hydroxide solution (5.1);

m is the mass, in grams, of the test portion (8.2).

Calculate the fat acidity to two decimal places.

If the repeatability has been checked and the requirements (see 10.1) are satisfied, take as the final result the arithmetic mean of the two results.

NOTE 5 For the interest of those familiar with the other expressions used for the fat acidity (see note 1 to clause 3), the alternative methods of calculation are given below.

a) Calculate the acid value w_{ar}, in milligrams per gram, 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:

$$w_{fa} = \frac{m_2 w_a}{1000}$$

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 ($m_2 = 282$).

10 Precision

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.

NOTE 6 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 [4], and published in [5].

10.1 Repeatability

The absolute difference between two 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 short intervals of time, shall not exceed 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.

10.2 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 shall not exceed 0,08 mmol per 100 g.

11 Test report

The test report shall specify the method used, whether the repeatability has been checked, and the results obtained. It shall also mention all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all information necessary for the complete identification of the sample.