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Milk and milk products — Determination of the titratable acidity of milk fat

Lait et produits laitiers — Détermination de l'acidité titrable de la matière grasse laitière

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Contents

Page

Foreword

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ISO/TS 22113|IDF/RM 204 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.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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ISO/TS 22113|IDF/RM 204 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by Joint ISO-IDF Project Group (C01) on *Determination of titratable acidity of fat (BDI method)* of the *Standing Committee on Analytical Methods for Composition (SCAMC)* under the aegis of its project leader, P. Trossat (FR).

Milk and milk products — Determination of the titratable acidity of milk fat

1 Scope

This Technical Specification specifies a routine method for determining the titratable acidity of milk fat.

The method is applicable to milk fat obtained from:

a) raw milk;

b) heat-treated milk;

c) milk reconstituted from milk powder;

d) cream with any fat content, provided the product is diluted so as to obtain a mass fraction of between 4 % and 6 % fat.

The method is not applicable to fermented milk or milk that has undergone bacterial or enzymatic damage.

NOTE 1 The titration procedure can also be applied to fat separated from several other dairy products.

NOTE 2 This Technical Specification is designed for batches of test samples of between five and several hundred test portions per day.

2 Principle

An amount of sample is thoroughly mixed with a solution containing sodium tetraphosphate and a surface-active agent. The mixture is heated in a boiling water bath to obtain separation of fat. A known quantity of extracted fat is dissolved in an organic solvent and titrated with alcoholic alkali.

3 Reagents

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

3.1 Phosphoric acid solution, $c(H_3PO_4) \approx 1$ mol/l.

3.2 BDI [1] **reagent**. Dissolve 70 g of sodium tetraphosphate in about 700 ml distilled water without additional warming and mix.

1) The acronym “BDI” stands for Bureaux of Dairy Industries; this organization first developed this method.

Add 30 g of octylphenylpoly(ethyleneglycol) [2)] and mix again. Adjust the pH to 6,6 with phosphoric acid solution (3.1), if needed. Dilute to 1 l with water and mix. If necessary, readjust the pH with phosphoric acid solution (3.1).

If stored in a refrigerator and in the dark, the BDI reagent is stable for 1 month.

NOTE Sodium tetraphosphate is a polyphosphate containing sodium tetraphosphate $(NaPO_3)_4$ as the main component besides some other polyphosphates.

3.3 Thymol blue solution, $c(C_{27}H_{30}O_5S) = 0{,}1$ g/l in propan-2-ol.

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

3.4 Fat solvent solution. Mix one volume of thymol blue solution (3.3) with four volumes light petroleum with a boiling range between 60 °C and 80 °C.

The fat solvent solution can be stored in the dark for up to 1 month.

3.5 Potassium hydrogen phthalate solution, $c(KHC_8H_4O_4) = 0{,}01$ mol/l.

Dissolve 1,021 1 g of potassium hydrogen phthalate in a 500 ml one-mark volumetric flask (4.11). Dilute to the 500 ml mark with water and mix.

3.6 Tetra-*n*-butylammonium hydroxide solution, $c(C_{16}H_{37}NO) = 0{,}01$ mol/l in a propan-2-ol and methanol mixture.

Dilute one volume of tetra-*n*-butylammonium hydroxide, $c[(C_4H_9)_4NOH] = 0{,}1$ mol/l in a propan-2-ol and methanol mixture, with nine volumes of propan-2-ol to obtain a final concentration of $c(C_{16}H_{37}NO) = 0{,}01$ mol/l.

The concentration of the tetra-*n*-butylammonium hydroxide solution may change on storage and when being transferred to the burette. For these reasons, determine the actual concentration of the solution to four decimal places before use by titration against a standard solution of potassium hydrogen phthalate (3.5) using the thymol blue solution (3.3) as indicator.

If the burette is fitted with a facility to exclude the entry of carbon dioxide, the concentration is stable for 1 month.

3.7 Pilot fat and reference fat.

3.7.1 Pilot fat. Melt some anhydrous milk fat (e.g. 1 000 g) having a fat acidity level of between 0,5 mmol/100 g and 1,0 mmol/100 g of fat. Divide the melted anhydrous milk fat sample into subsamples (e.g. of 5 g each).

If stored in a freezer at −20 °C or below, the pilot fat subsamples can be kept for at least 2 years.

The pilot fat samples can be used for checking the reproducibility of the results obtained by the titration procedure (7.2), either during a single work session or between work sessions over a long period of time (several months to years).

3.7.2 Reference fat. Reference fat samples consist of milk fat of low fat acidity (basic fat) spiked with increasing levels of palmitic acid (C_{16}) within the range 0,5 mmol/100 g to 1,5 mmol/100 g per 100 g fat.

2) Triton X-100 is an example of a suitable product available commercially. This information is given for the convenience of users of this document, and does not constitute an endorsement by ISO and IDF of this product.

The accuracy of the titration procedure can be checked by using the regression Equation (1):

$$b(C_{16}) = \alpha + \beta\Delta b \quad (1)$$

where

$b(C_{16})$ is the amount of palmitic acid, expressed in mmol per 100 g fat, added to the basic fat;

Δb is the BDI value of the spiked samples decreased by the BDI value of the basic fat (blank).

The preparation and the guidelines for use of these reference fat samples are described in Annex C.

4 Apparatus

Usual laboratory equipment and, in particular, the following.

4.1 Delivery pipettes or **syringes**, capacities 10 ml, 25 ml, and 50 ml.

4.2 Fat separation tubes, consisting of a bulk vat surmounted by a narrow stem for collecting the small quantity of fat extracted from the reagent mixture. The diameter of the stem shall be large enough to allow the calibrated syringe (4.5) to take a fat sample. Models of fat separation tubes are given in Annex A. Butyrometers according to ISO 3432|IDF 221[3] can also be used.

NOTE The fat separation is enhanced by centrifugation, especially in tubes with narrow stems.

4.3 Water bath, capable of maintaining a temperature of 45 °C ± 1°C.

4.4 Boiling water bath, capable of maintaining a temperature of ≥ 95 °C.

4.5 Calibrated syringe, adjustable and capable of delivering a known quantity of milk fat of about 0,25 g at 45 °C, being accurate to 2 mg of milk fat.

NOTE From experience, transfer of a quantity of fat can be done accurately and conveniently by using a positive displacement pipette.

4.6 Titration vessel, capacity of between 10 ml and 100 ml depending on the volumes of test samples to be titrated in one titration run, provided with a stirring device.

4.7 Microburette, graduated in divisions of at least 0,002 ml.

4.8 Nitrogen supply, free of carbon dioxide.

4.9 Gas washbottle, containing light petroleum with a boiling range of 60 °C to 80 °C, connected to the nitrogen supply (4.8) and the titration vessel (4.6).

4.10 Colorimeter, with dip-probe, suitable for measuring at a wavelength of between 600 nm and 620 nm, connectable to the titration vessel (4.6).

4.11 One-mark volumetric flasks, capacities 100 ml to 500 ml, ISO 1042,[2] class A.

NOTE 1 The titration vessel (4.6), the microburette (4.7) for delivering the non-aqueous titrant tetra-*n*-butylammonium hydroxide (3.6), the nitrogen supply (4.8) through a gas washbottle (4.9) and the dip-probe connected to the colorimeter (4.10) are assembled in a typical device (see Annex B) for consecutive titration of several samples in one and the same volume of fat solvent.

NOTE 2 A simpler device for manual titration and visual determination of the endpoint of titration can be set up without a colorimeter with dip-probe.

5 Sampling

Sampling is not part of the method specified in this Technical Specification. A recommended sampling method is given in ISO 707|IDF 50.[1]

It is important the laboratory receive a truly representative sample which has not been damaged or changed during transport or storage.

6 Preparation of test samples

6.1 Storage and preservation

The milk or cream test samples shall have been stored and transported at 0 °C to 4 °C (milk powder can be stored at ambient temperature) and be analysed within 36 h.

For prolonged storage or storage in a refrigerator at ~5 °C, it is recommended that test samples be preserved by means of hydrogen peroxide at a final concentration of 0,2 g/l H_2O_2. In this case, the test samples can be stored for 4 days.

6.2 Pretreatment of test sample

6.2.1 Milk sample

Mix gently by inverting the test sample several times, without increasing its temperature.

6.2.2 Cream sample

Dilute cream sample using the corresponding skim milk or water to obtain a mass fraction of between 4 % and 6 % fat.

Using water to dilute cream results in an underestimation of the free fatty acid (FFA) level compared to the parent milk. In these cases, use a correction programme to obtain accurate results (see Reference [8]).

6.2.3 Milk powder sample

Dissolve around 13 g of milk powder in a 100 ml one-mark volumetric flask (4.11). Add 60 ml of water and mix using a mixer at room temperature for 70 min. Dilute to the 100 ml mark with water and mix.

7 Procedure

7.1 Separation of fat

Mix 3,5 parts (±3 %) of test sample (milk, cream diluted or reconstituted milk powder) (6.2) to 1 part (±1,5 %) of BDI reagent (3.2) in the tube for fat separation using the following amounts:

a) when using a MONED tube (4.2), mix 31 ml ± 1 ml of test sample (6.2) and 8,9 ml ± 0,1 ml of BDI reagent (3.2);

b) when using a Van Gulik butyrometer (4.2), mix 16,0 ml ± 0,5 ml of test sample (6.2) and 4,5 ml ± 0,1 ml of BDI reagent (3.2);

c) when using other tubes, mix volume fractions of the test sample (6.2) and the BDI reagent in ratio 3,5 + 1 using volumes such that a fat column exists in the stem of the extraction tube (4.2).

Immediately after filling, close the fat separation tube and mix its content.

For a test sample taken from raw milk, mix gently by inverting the tube several times. For test samples taken from heat-treated milk or reconstituted milk powder, shake more intensively in order to achieve good separation of the fat.

As soon as possible, but within 5 min, place the tube in the boiling water bath (4.4) maintained at a temperature of ≥95 °C for 15 min. Take care that the temperature of the water bath stays above 95 °C and its water level above the upper level of the tube content.

For tubes with narrow stems and milk samples other than raw milk, it can prove necessary to centrifuge the tubes to achieve better fat separation.

In the case of a bad fat separation, place the tubes in a refrigerator to solidify the fat. After reheating in the boiling water bath, fat separation is enhanced. In any case, the fat shall be limpid and free from any particles.

Once the fat extraction is achieved, put the tube in the water bath (4.3) at 45 °C. Ensure that its water level remains above the upper level of the tube content.

7.2 Titration

Perform the titration in the titration vessel (4.6) under a carbon dioxide-free atmosphere. Connect the titration vessel to the nitrogen supply (4.8) coming from the gas washbottle (4.9). Regularly fill the gas washbottle to compensate for the evaporation of the petroleum ether.

Transfer a suitable volume of fat solvent (3.4) and 0,25 g of pilot fat (3.7.1) into the titration vessel (4.6) being free from carbon dioxide by nitrogen flushing (4.8).

Control the wavelength setting of the colorimeter. Adjust the colorimeter scale at 0 % (dark) and at 100 % (fat solvent with fat sample) transmission.

Adjust the endpoint of the titration at 70 % on the transmission scale. Neutralize the fat solvent with the tetra-*n*-butylammonium hydroxide solution (3.6).

Using the calibrated syringe (4.5), add a known quantity of about 0,25 g of the pilot fat and titrate. Always repeat the procedure five times to fulfil the requirements mentioned for repeatability (9.2).

If the results obtained for the pilot fat are out of the range of the repeatability limits, check the titration device (Annex B) and the titration procedure.

Using the calibrated syringe (4.5), transfer a test portion of about 0,25 g of the prepared fat sample (7.1) to the titration vessel and titrate.

Replace the fat solvent with fresh solvent when three titrations have been carried out per 2 ml fat solvent (e.g. 60 titrations in a volume of 40 ml fat solvent).

When titrating a small number of test samples only, the endpoint titration can be estimated by visual observation of the change in colour (yellow to faint greenish). At least two titrations can be carried out in 5 ml fat solvent.

8 Calculation and expression of results

8.1 Calculation

Calculate the fat acidity of the test sample, b_{H^+}, expressed in millimoles per 100 g of fat, by using Equation (2):

$$b_{H^+} = \frac{Vc}{m} \times 100 \quad (2)$$