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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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Foreword

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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.

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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/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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a Standing Committee may decide to publish an other type of normative document which is called by IDF: *Reviewed method*. Such a method represents an agreement between the members of a Standing Committee and is accepted for publication if it is approved by at least 50 % of the committee members casting a vote. A *Reviewed method* is equal to an ISO/PAS or ISO/TS and will, therefore, also be published jointly under ISO conditions.

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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):

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

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):

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

where

- V is the volume, in millilitres, expressed to three decimal places, of the tetra-*n*-butylammonium hydroxide solution (3.6) used in the titration;
- c is the exact concentration, in moles per litre, expressed to four decimal places, of the tetra-*n*-butylammonium hydroxide solution (3.6);
- m is the mass, in grams, expressed to three decimal places, of fat transferred with the calibrated syringe into the titration vessel.

8.2 Expression of results

Express the test results to two decimal places.

9 Precision

9.1 Interlaboratory test

The values for the repeatability derived from this interlaboratory test were determined in accordance with ISO 5725-1[4] and ISO 5725-2.[5] However, only three laboratories participated in the test.

The values obtained, therefore, should only be considered as being indicative. Details of the interlaboratory test on the precision of the method are given in Annex D.

9.2 Repeatability

The absolute difference between two individual single test results, obtained with 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 indicatively in not more than 5 % of cases be greater than 0,072 mmol/100 g.

10 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, together with reference to this Technical Specification (ISO/TS 22113|IDF/RM 204:2012);

d) all operating details not specified in this Technical Specification, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained;

f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Models for the fat separation tubes

Dimensions in millimetres

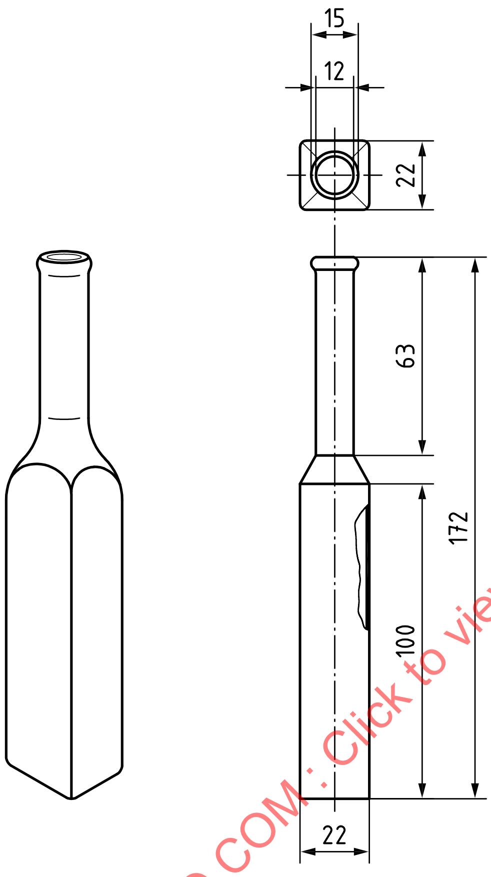
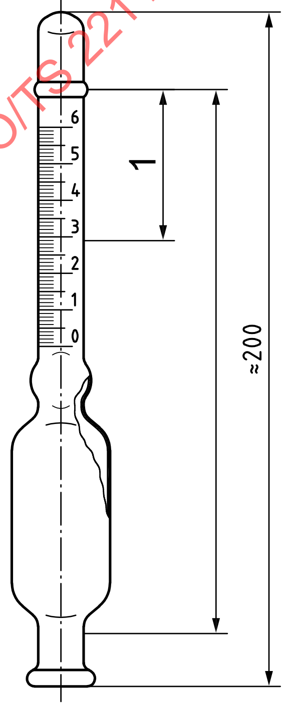


Figure A.1 — MONED tube

Dimensions in millimetres



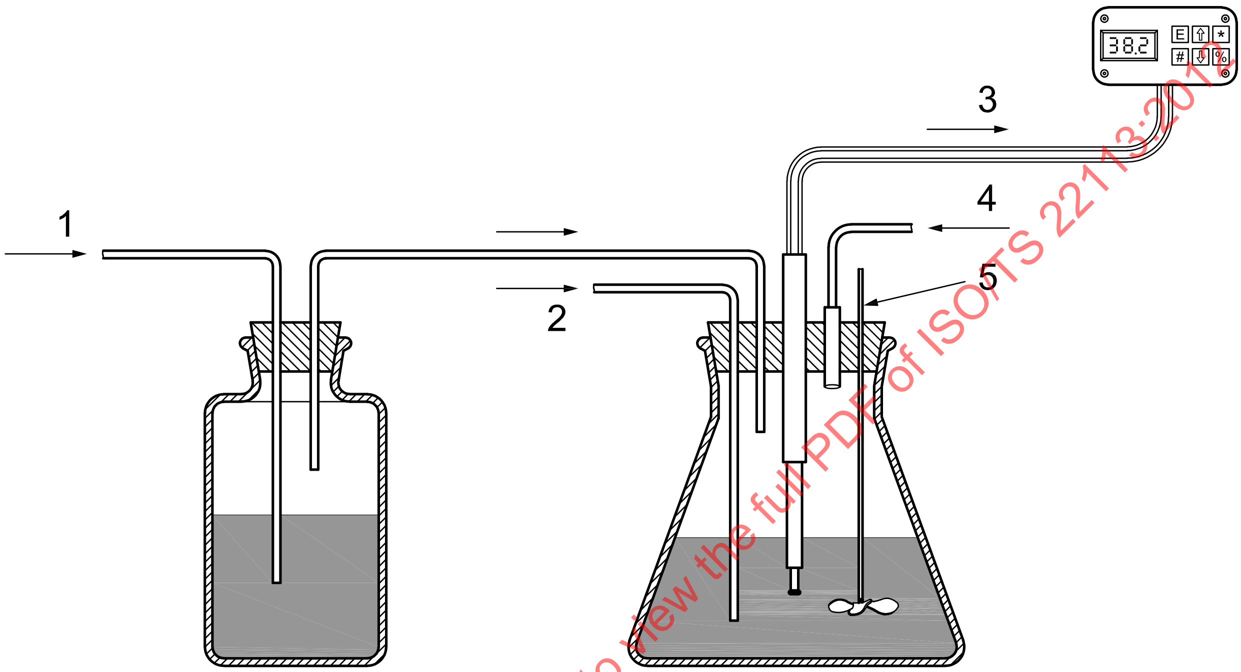
Key

1 extracted fat

Figure A.2 — General model of a fat extraction tube

Annex B
(informative)

Typical titration device for consecutive titration of several samples in one volume of fat solvent



Key

1 nitrogen supply through a gas washbottle containing petroleum ether
2 inlet for basic non-aqueous titrant tetra-*n*-butylammonium hydroxide
3 optical probe connected to a colorimeter
4 inlet for the introduction of the fat solvent and the fat samples
5 stirring device

Figure B.1 — Typical titration device for consecutive titration

Annex C
(informative)

Guidelines for the preparation and implementation of reference fat samples for the follow-up of the titration procedure

C.1 Preparation of the reference fat samples

C.1.1 Basic milk fat

Obtain an adequate amount of milk fat of low fat acidity. Melt it at 38 °C.

C.1.2 Preparation of reference fats spiked with palmitic acid (C_{16})

C.1.2.1 **Reference fat A**, spiked with 1,25 mmol of palmitic acid per 100 g fat.

Weigh to the nearest 0,1 mg, 0,962 g ± 0,001 g of palmitic acid. Dissolve the palmitic acid in about 300 g of basic milk fat (C.1.1) and mix.

Weigh, to the nearest 0,01 g, the total mass of the thus prepared reference fat A.

In general, palmitic acid is not 100 % pure. The purity, therefore, has to be taken into account when weighing the palmitic acid. For instance, by implementing palmitic acid containing "at least 98 %", the mass of palmitic to be weighed shall be about 0,962/0,98 = 0,982 g instead of 0,962 g.

Calculate the level, $b(\mathrm{C}_{16})_{1,25}$ of the palmitic acid in the reference fat A, in millimoles per 100 g, by using Equation (C.1):

$$b(\mathrm{C}_{16})_{1,25} = \frac{w m_1 \times 10^5}{m_2 M} \qquad \text{(C.1)}$$

where

w is the numerical value of the purity, as a mass fraction, of the palmitic acid (w normally is between 0,98 and 1,00);

m_1 is the mass, in grams, of palmitic acid for the preparation of reference fat A;

m_2 is the mass, in grams, of the total amount of prepared reference fat A;

M is the molecular mass, in grams, of palmitic acid (M = 256,43 g/mol).

C.1.2.2 **Reference fat B**, spiked with 1,00 mmol of palmitic acid per 100 g fat.

Weigh, to the nearest 0,01 g, 80 g ± 0,01 g of reference fat A (C.1.2.1). Dissolve the reference fat in 20 g ± 0,01 g of basic milk fat (C.1.1) and mix.

Weigh to the nearest 0,01 g, the mass of the reference fat B thus prepared.

Calculate the level, $b(C_{16})_{1,00}$, of the palmitic acid in the reference milk fat B, expressed in millimoles per 100 g, by using Equation (C.2):

$$b(C_{16})_{1,00} = b(C_{16})_{1,25} \frac{m_3}{m_4} \tag{C.2}$$

where

m_3 is the mass, in grams, of the reference fat A dissolved in the basic milk fat;

m_4 is the mass, in grams, of the total amount of prepared reference fat B.

C.1.2.3 Reference fat C, spiked with 0,75 mmol of palmitic acid per 100 g fat.

Weigh, to the nearest 0,01 g, 60 g ± 0,01 g of reference fat A (C.1.2.1). Dissolve the reference fat A in about 40 g ± 0,01 g of basic milk fat (C.1.1) and mix.

Weigh to the nearest 0,01 g, the mass of the reference fat C thus prepared.

Calculate the level, $b(C_{16})_{0,75}$, of the palmitic acid in the reference milk fat C, expressed in millimoles per 100 g, by using Equation (C.3):

$$b(C_{16})_{0,75} = b(C_{16})_{1,25} \frac{m_5}{m_6} \tag{C.3}$$

where

m_5 is the mass, in grams, of the reference fat A dissolved in the basic milk fat;

m_6 is the mass, in grams, of the total amount of prepared reference fat C.

C.1.2.4 Reference fat D, spiked with 0,50 mmol of palmitic acid per 100 g fat.

Weigh, to the nearest 0,01 g, 40 g ± 0,01 g of reference fat A. Dissolve reference fat A in 60 g ± 0,01 g of basic milk fat (C.1.1) and mix.

Weigh, to the nearest 0,01 g, the mass of the reference fat D thus prepared.

Calculate the level, $b(C_{16})_{0,50}$, of the palmitic acid in the reference milk fat D, expressed in millimoles per 100 g, by using Equation (C.4):

$$b(C_{16})_{0,50} = b(C_{16})_{1,25} \frac{m_7}{m_8} \tag{C.4}$$

where

m_7 is the mass, in grams, of the reference fat A dissolved in the basic milk fat;

m_8 is the mass, in grams, of the total amount of prepared reference fat D.

C.2 Implementation of the reference fat samples

C.2.1 Determination of the fat acidity of the reference fat

According to the procedure (7.2), determine the fat acidity of the basic milk fat (C.1.1) (blank), b_0, and the fat acidity values, $b_{1,25}$, $b_{1,00}$, $b_{0,75}$, and $b_{0,50}$, of the four reference fat samples.

C.2.2 Calculations and assessment of the results

Table C.1 — Calculations

Sample identity	Level of palmitic acid in the reference fat samples $b(C_{16})_{i,\text{ref}}$	BDI value determined according to 7.2	Δb_N $(b_i - b_0)$	Ratio $\Delta b_N / b(C_{16})_{i,\text{ref}}$
Basic fat (blank)		b_0		
Reference fat A	$b(C_{16})_{1,25}$	$b_{1,25}$	Δb_A	$[\Delta b_A / b(C_{16})_{1,25}]_A$
Reference fat B	$b(C_{16})_{1,00}$	$b_{1,00}$	Δb_B	$[\Delta b_B / b(C_{16})_{1,00}]_B$
Reference fat C	$b(C_{16})_{0,75}$	$b_{0,75}$	Δb_C	$[\Delta b_C / b(C_{16})_{0,75}]_C$
Reference fat D	$b(C_{16})_{0,50}$	$b_{0,50}$	Δb_D	$[\Delta b_D / b(C_{16})_{0,50}]_D$

To recapitulate the quantities shown in Table C.1:

a) column 2 lists the levels of palmitic acid in reference fats A, B, C, and D;

b) column 3 lists the BDI values of reference fats A, B, C, and D, determined according to the titration procedure (7.2);

c) column 4 lists the values Δb_A, Δb_B, Δb_C, and Δb_D, calculated by subtracting the BDI value of the basic fat, b_0, from the BDI values of reference fats A, B, C, and D.

For each of the reference fat samples, calculate the ratios: $\Delta b_N / b(C_{16})_{i,\text{ref}}$, where N is A, B, C, and D, and i is 1,25, 1,00, 0,75, and 0,50, respectively.

Calculate also the residual standard deviation, $s_{b(C_{16})_p, b}$, of the regression Equation (C.5):

$$b\left(C_{16}\right)_p = \alpha + \beta b \tag{C.5}$$

where

$b(C_{16})_p$ represents the predicted palmitic acid level in the reference fat samples (C.1.1);

b represents the BDI value of the reference fat samples (C.1.2).

If either one or more of the ratios $\Delta b_N / b(C_{16})_{i,\text{ref}}$ are out of the range: 1,00 ± 0,05 (i.e. if results deviate more than 5 % from the predicted values) or if the residual standard deviation $s_{b(C_{16})_p, b} > 0{,}02$ mmol / 100 g fat, check the titration solution (3.6), the titration device (Annex B) and the titration procedure (7.2).