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Milk fat — Determination of peroxide value

Matière grasse laitière — Détermination de l'indice de peroxyde

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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.

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 3976 | IDF 74 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.

This edition of ISO 3976 | IDF 74 cancels and replaces ISO 3976:1977, which has been technically revised. A comparison of the results using the new reagent (methanol/1-decanol/*n*-hexane mixture) with those found using chloroform/methanol is given in Annex C.

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 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 IDF National Committees casting a vote.

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ISO 3976 | IDF 74 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 the Joint ISO-IDF Action Team on *Fat*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr A. van Reusel (BE).

This edition of ISO 3976 | IDF 74 cancels and replaces IDF 74A:1991, which has been technically revised. A comparison of the results using the new reagent (methanol/1-decanol/*n*-hexane mixture) with those found using chloroform/methanol is given in Annex C.

Milk fat — Determination of peroxide value

WARNING — The use of this International Standard may involve the use of hazardous materials, operations, and equipment. This International Standard does not purport to address all the safety risks associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of local regulatory limitations prior to use.

1 Scope

This International Standard specifies a method for the determination of the peroxide value of anhydrous milk fat.

The method is suitable for anhydrous milk fat having a peroxide value up to 1,3 mmol of oxygen per kilogram.

NOTE For milk fat samples with peroxide values between 0,5 mmol and 1,3 mmol of oxygen per kilogram, an extended procedure (see Annex A) is used. For milk fat samples with peroxide values of more than 1,3 mmol of oxygen per kilogram, an iodine/thiosulfate method can be used (e.g. AOAC 920.160).

2 Terms and definitions

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

2.1
peroxide value
amount of substance determined by the procedure specified in this International Standard

NOTE The peroxide value is expressed as millimoles of oxygen per kilogram.

3 Principle

A test portion is dissolved in a mixture of methanol/1-decanol/*n*-hexane, then iron(II) chloride and ammonium thiocyanate are added. The peroxides oxidize the iron(II) which forms a red iron(III) complex with the ammonium thiocyanate. The amount of substance is calculated from a photometric determination of the red iron(III) complex, after a fixed period of reaction.

4 Reagents

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

4.1 Methanol/1-decanol/*n*-hexane mixture, in ratio 3:2:1 (volume fraction).

Mix 2 volume parts of 1-decanol with 1 volume part of *n*-hexane. Add 3 volume parts of anhydrous methanol to that mixture and mix again.

The mixture is flammable and has an unpleasant odour. Therefore, it is recommended to work in a fume cupboard and to wear gloves.

Petroleum ether with a boiling range at between 60 °C and 80 °C may be used instead of *n*-hexane.

4.2 Iron(II) chloride ($FeCl_2$) solution, $c(Fe^{2+})$ ~1 mg/ml.

Prepare the iron(II) chloride solution in indirect, dimmed light.

Dissolve approx. 0,4 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) in about 50 ml water. Then dissolve approx. 0,5 g of iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in about 50 ml water. Slowly pour the barium chloride solution, with constant stirring, into the iron(II) sulfate solution. Add about 2 ml of hydrochloric acid solution I (4.5) and mix again.

Allow the precipitate of barium sulfate to settle or centrifuge the mixture until the upper liquid layer is clear. Decant the thus-obtained clear solution into a brown bottle. Do not store the solution for more than 1 week.

Alternatively, the iron(II) chloride solution may be prepared by dissolving approximately 0,35 g of iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) in about 100 ml water. Add 2 ml of hydrochloric acid solution I (4.5) and mix.

4.3 Ammonium thiocyanate solution.

Dissolve approx. 30 g of ammonium thiocyanate (NH_4SCN) in water. Dilute with water to 100 ml. If the solution is not colourless, wash the solution several times with small amounts (e.g. 5 ml portions) of iso-amyl alcohol (3-methylbutan-1-ol).

4.4 Iron(III) chloride ($FeCl_3$) standard solution, $c(Fe)$ = 10 µg/ml.

Dissolve 0,500 g of iron powder in about 50 ml of hydrochloric acid solution I (4.5) in a 500 ml one-mark volumetric flask. Add 1 ml to 2 ml of hydrogen peroxide solution (4.7). Remove the excess of hydrogen peroxide by boiling for 5 min. Cool to room temperature. Dilute to the 500 ml mark with water and mix.

The iron(III) chloride solution containing 1 g/l of Fe may also be prepared from standardized chemicals available commercially.

Transfer, using a pipette, 1 ml of the obtained solution to a 100 ml one-mark volumetric flask. Dilute to the 100 ml mark with methanol/1-decanol/*n*-hexane mixture (4.1) and mix.

4.5 Hydrochloric acid solution I, approx. $c(HCl)$ = 10 mol/l.

4.6 Hydrochloric acid solution II, approx. $c(HCl)$ = 0,2 mol/l.

Dilute 2 ml of hydrochloric acid solution I (4.5) with water to 100 ml.

4.7 Hydrogen peroxide solution (H_2O_2), of mass fraction approx. 30 %.

4.8 Dilute nitric acid (HNO_3), of mass fraction approx. 10 %.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Glassware.

Clean all glassware by soaking in dilute nitric acid (4.8) for 24 h. Rinse the glassware four times with tap water and four times with distilled or equivalent water before drying it in the oven (5.10) set at 100 °C for 1 h.

The cleanliness of the glassware is of utmost importance. Other cleaning procedures may also be used if they give the same result.

5.2 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

5.3 Dispenser device, capable of accurately delivering 9,9 ml, 9,6 ml, 9,4 ml, 8,9 ml, 8,4 ml and 7,9 ml of methanol/1-decanol/*n*-hexane mixture (4.1).

5.4 Dispenser device, capable of accurately delivering 0,5 ml, 1,0 ml, 1,5 ml and 2,0 ml of iron(III) chloride standard solution (4.4).

5.5 Micropipettes, capable of accurately delivering 0,05 ml of ammonium thiocyanate solution (4.3), of iron(II) chloride solution (4.2) and of hydrochloric acid solution II (4.6) respectively.

5.6 Photometer, capable of measuring at a wavelength near to 500 nm.

5.7 Cells with caps, suitable for the photometer (5.6), resistant to all reagents used in the procedure.

5.8 Glass test tubes, provided with ground glass stoppers.

5.9 Oven, electrically heated, capable of operating at between 40 °C and 45 °C.

5.10 Oven, electrically heated, capable of operating at 100 °C ± 2 °C.

5.11 Centrifuge, capable of producing a radial acceleration of at least 350 g, with a swing-out rotor (e.g. a so-called Gerber centrifuge).

5.12 Centrifuge tubes, suitable for using in the centrifuge (5.11).

5.13 Glass funnels, with folded filter paper (medium grade).

5.14 Bottles, suitable for using with the reagents.

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 | IDF 50.

7 Preparation of test sample

7.1 General

Carry out all preparations in indirect subdued light.

7.2 Anhydrous milk fat, anhydrous butteroil, butteroil, ghee

If necessary, completely liquefy the test sample (see IDF 68A for details) by warming the unopened container at the lowest temperature necessary to achieve liquefaction. Mix the liquefied sample, while avoiding the inclusion of air in the sample as far as possible.

Proceed with the determination immediately and while the test sample is still liquid.

7.3 Butter

Add an appropriate quantity of test sample to a centrifuge tube (5.12). Melt the sample in the oven (5.9) set at between 40 °C and 45 °C. Separate the fat by centrifuging at a radial acceleration of at least 350 g for 5 min.

Filter the warm separated butterfat through a glass funnel (5.13) with a folded dry filter paper in the oven (5.9) set at between 40 °C and 45 °C. The filtered butterfat shall be clear and visibly free from water and non-fatty compounds.

Proceed with the determination without any delay while the test sample is still liquid.

8 Procedure (see Annex A)

8.1 Precautions to avoid oxidation and disturbed recording of extinction

8.1.1 Avoid any exposure of the test sample to light. Carry out the test in indirect light, subdued as much as is practicable.

8.1.2 Perform all optical extinction measurements at the wavelength of maximal extinction of the red iron(III) complex, i.e. near to 500 nm.

8.1.3 Perform all optical extinction measurements in cells (5.7) which are closed immediately after filling. From the moment of closure, allow the closed cells stand for 10 min to obtain equilibrium in the mixture before reading the extinction.

NOTE Evaporation of the solvent might produce condensation on the upper walls of the cells. By reintegrating the bulk liquid, this condensation creates a diffraction of the light beam by the different solvent streaks resulting in a fluctuating extinction. The 10-min waiting time is needed to obtain equilibrium between the solvent and the vapour phase.

8.2 Reagent blank

8.2.1 Add, using the dispenser (5.3), 9,90 ml of methanol/1-decanol/*n*-hexane mixture (4.1) to the test tube (5.8).

8.2.2 Add, using a micropipette (5.5), 0,05 ml of ammonium thiocyanate solution (4.3) to the mixture in the test tube and mix.

8.2.3 Add, using a micropipette (5.5), 0,05 ml of iron(II) chloride solution (4.2) to the mixture in the test tube and mix again.

8.2.4 Transfer the obtained reagent blank mixture to a photometer cell (5.7). Close the cell with a cap and allow it stand for 10 min to obtain equilibrium in the mixture.

Measure the extinction, E_1, of the reagent blank against the methanol/1-decanol/*n*-hexane mixture (4.1). Perform the reagent blank determination at least four times.

8.2.5 The obtained results (E_1) shall be within a range of 0,010 extinction units. The mean reagent blank extinction (E_m) shall not exceed 0,030 units. If the above requirements are not fulfilled, check the photometric procedure, the glassware and the reagents. Correct the procedure or replace what is necessary.

8.3 Test sample blank

8.3.1 Weigh, to the nearest 1 mg, approx. 0,33 g of prepared test sample (see 7.2 or 7.3) in a test tube (5.8).

8.3.2 Without any delay, add, using the dispenser (5.3), 9,60 ml of methanol/1-decanol/*n*-hexane mixture (4.1) to the test sample in the tube. Mix gently to dissolve the sample fat.

8.3.3 Add, using a micropipette (5.5), 0,05 ml of ammonium thiocyanate solution (4.3) and mix.

8.3.4 Transfer the test sample blank mixture to a photometer cell (5.7). Close the cell with a cap and allow it stand for 10 min to obtain equilibrium in the mixture. Measure the extinction (E'_0) of the sample blank against the methanol/1-decanol/*n*-hexane mixture (4.1).

8.3.5 Correct the obtained test sample blank extinction (E'_0) in 8.3.4 for the differences in mass of the test portions of the test sample blank and of the test sample by using the following equation:

$$E_0 = E'_0 \times \frac{m}{m_0}$$

where

E_0 is the numerical value of the corrected sample blank extinction;

E'_0 is the numerical value of the sample blank extinction (8.3.4);

m_0 is the mass of the test sample blank (8.3.1);

m is the mass of the test sample (8.4.1).

8.4 Test portion

8.4.1 Weigh, to the nearest 1 mg, approximately 0,33 g of prepared test sample (see 7.2 or 7.3) in a test tube (5.8).

8.4.2 Without any delay, add, using the dispenser (5.3), 9,60 ml of methanol/1-decanol/*n*-hexane mixture (4.1) to the test portion in the tube. Mix gently to dissolve the sample fat.

8.4.3 Add, using a micropipette (5.5), 0,05 ml of ammonium thiocyanate solution (4.3) to the mixture in the tube and mix.

8.4.4 Add, using a micropipette (5.5), 0,05 ml of iron(II) chloride solution (4.2) to the mixture in the tube and mix again.

8.4.5 Transfer the test portion mixture to a photometer cell (5.7) Close the cell with a cap and let it stand for 10 min to obtain equilibrium in the mixture. Measure the extinction (E_2) of the test portion against the methanol/1-decanol/*n*-hexane mixture (4.1).

8.4.6 The procedures described in 8.3 and 8.4 may be performed in one run directly by using a single photometer cell of suitable size. Proceed as described in 8.3.1 to 8.3.3 inclusive. Measure the test blank extinction, E_0, against the methanol/1-decanol/*n*-hexane mixture as in 8.3.4. Then proceed as described in 8.4.4 by adding and mixing 0,05 ml of iron(II) chloride solution (4.2) directly to the photometer cell and measure the sample extinction (E_2) as in 8.4.5.

8.5 Extinction coefficient of the red iron(III) complex

Add, using the dispenser device (5.4), 0,5 ml, 1,0 ml, 1,5 ml and 2,0 ml of iron(III) chloride standard solution (4.4), respectively, to four test tubes (5.8) to obtain a series of solutions containing 5 µg, 10 µg, 15 µg and 20 µg of Fe^{3+} respectively.

Add, using the dispenser device (5.3), 9,4 ml, 8,9 ml, 8,4 ml and 7,9 ml of methanol/1-decanol/*n*-hexane mixture (4.1), respectively, to the four tubes to obtain a 9,9 ml mixture in each tube.

Add, using the micropipettes (5.5), 0,05 ml of ammonium thiocyanate solution (4.3) and 0,05 ml of hydrochloric acid solution II (4.6) to each of the four tubes and mix.