INTERNATIONAL STANDARD 3976

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Anhydrous milk fat — Determination of peroxide value (Reference method)

Matière grasse de lait déshydratée — Détermination de l'indice de peroxyde (Méthode de référence)

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FOREWORD

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 3976 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in September 1975.

It has been approved by the member bodies of the following countries :

Australia	Germany	New Zealand
Austria	Ghana	Poland
Belgium	Hungary	Portugal
Bulgaria	India	Romania
Canada	Iran	South Africa, Rep. of
Czechoslovakia	Israel	Spain
Egypt, Arab Rep. of	Mexico	Turkey
France	Netherlands	Yugoslavia

The member body of the following country expressed disapproval of the document on technical grounds :

United Kingdom

NOTE – The method specified in this International Standard has been developed by a Joint Group of Experts of the IDF (International Dairy Federation), the AOAC (Association of Official Analytical Chemists, U.S.A.) and ISO. The method will also be included in the FAO/WHO Code of Principles concerning Milk and Milk Products and Associated Standards.

Printed in Switzerland

Anhydrous milk fat — Determination of peroxide value (Reference method)

1 SCOPE AND FIELD OF APPLICATION

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

The method is applicable to anhydrous milk fat, anhydrous butter oil (anhydrous butterfat), butter oil (butterfat) or ghee having peroxide values not exceeding 1,0.

NOTE — These products are defined in IDF Standard 68 : 1971.

It is not applicable to products containing gallates as antioxidants.

2 REFERENCE

ISO/R 707, *Milk and milk products — Sampling.*

3 DEFINITION

peroxide value : The number of milliequivalents of oxygen per kilogram of anhydrous milk fat, determined by the procedure described.

4 PRINCIPLE

Dissolution of a test portion in a mixture of chloroform and methanol and addition of iron(II) chloride and ammonium thiocyanate. After a fixed reaction time, photometric determination of the red iron(III) complex.

5 REAGENTS

All reagents shall be of analytical reagent quality. The water used shall be distilled water or water of at least equivalent purity.

5.1 Chloroform/methanol mixture.

Mix 70 volumes of chloroform (trichloromethane) and 30 volumes of anhydrous methanol.

5.2 Iron(II) chloride solution.

This solution shall be prepared in indirect, dimmed light.

Dissolve approximately 0,4 g of barium chloride dihydrate ($BaCl_2.2H_2O$) in about 50 ml of water.

Dissolve approximately 0,5 g of iron(II) sulphate heptahydrate ($FeSO_4.7H_2O$) in about 50 ml of water.

Slowly pour the barium chloride solution, with constant stirring, into the iron(II) sulphate solution and add about 2 ml of approximately 10 N hydrochloric acid.

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

NOTE — The iron(II) chloride solution can also be prepared by dissolving approximately 0,35 g of iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$) in about 100 ml of water and adding 2 ml of approximately 10 N hydrochloric acid.

5.3 Ammonium thiocyanate solution.

Dissolve approximately 30 g of ammonium thiocyanate (NH_4SCN) in water and dilute to 100 ml. If the solution is not colourless, remove the colour by extracting the solution several times with small amounts (for example 5 ml portions) of iso-amyl alcohol (3-methyl-butan-1-ol).

5.4 Iron(III) chloride, standard solution corresponding to 10 μg of Fe per millilitre.

Dissolve 0,500 g of iron powder or iron wire in about 50 ml of 10 N hydrochloric acid and 1 to 2 ml of about 30 % (*m/m*) hydrogen peroxide solution.

Remove the excess of hydrogen peroxide by boiling for 5 min. Cool to room temperature and dilute with water to 500 ml in a volumetric flask. Transfer, by means of a pipette, 1 ml of this solution to a 100 ml volumetric flask, dilute to the mark with the mixture of chloroform and methanol (5.1) and mix.

5.5 Hydrochloric acid, approximately 0,2 N solution.

Dilute 2 ml of approximately 10 N hydrochloric acid with water to 100 ml.

6 APPARATUS

6.1 Analytical balance.

6.2 Burettes, of 10 ml capacity, graduated in 0,02 ml, complying with class A of ISO/R 385.

6.3 Graduated pipettes, of 1 ml capacity, graduated in 0,05 ml, complying with class A of ISO/R 835.

NOTE – Alternatively, pipettes of smaller capacity (not covered by ISO/R 835) may be used.

6.4 Photometer, suitable for measuring at a wavelength of 500 nm and with appropriate (preferably round) cells with an optical path length of at least 15 mm and a capacity of at least 15 ml.

7 SAMPLING

See ISO/R 707, "General instructions".
The laboratory sample should be received in a securely closed air-tight container at least three-quarters filled and protected from light. Note and report any laboratory sample not complying with these requirements.

8 PROCEDURE

8.1 Preparation of the test sample

Carry out the preparation as far as practicable in indirect subdued light.

Completely liquefy the laboratory sample, if necessary, by warming the unopened container at the lowest temperature necessary to achieve liquefaction. Mix the liquefied sample, taking care to avoid the inclusion of air in the sample as far as possible. Proceed with the determination without delay and while the test sample is still liquid.

8.2 Precautions

In order to eliminate lipid oxidation, the following precautions shall be observed.

8.2.1 Avoid exposure of the sample to light.

8.2.2 Take care that the procedure, from 8.3.1 to 8.3.5 inclusive, with inclusion of a reaction time of 5 min, is completed within 10 min.

8.2.3 Carry out the test in indirect light, subdued as much as is practicable.

8.3 Determination

8.3.1 Weigh, to the nearest 0,001 g, in a photometer cell (see 6.4) approximately 0,3 g of the prepared test sample (8.1). Note the time (see 8.3.5).

8.3.2 Without delay, add 9,60 ml of the mixture of chloroform and methanol (5.1) to the cell by means of a burette (6.2); mix gently to dissolve the test portion.

NOTE – For a number of simultaneous determinations it may be advantageous to carry out the analysis in cylindrical photometer cells fitted with ground glass stoppers.

8.3.3 Add from a graduated pipette (6.3) 0,05 ml of the ammonium thiocyanate solution (5.3) and mix.

8.3.4 Measure the extinction (fat blank extinction E_0) at 500 nm against the mixture of chloroform and methanol contained in a similar cell.

8.3.5 Add from a graduated pipette (6.3) 0,05 ml of the iron(II) chloride solution (5.2), mix and start an alarm-clock or stop-watch for a waiting time of 5 min. Then measure the extinction (E_2) at 500 nm against the mixture of chloroform and methanol. This operation shall be completed within 10 min of the time noted in 8.3.1.

8.3.6 Carry out a reagents blank test by transferring 9,90 ml of the mixture of chloroform and methanol to a photometer cell (but omitting the test portion) and proceeding as described in 8.3.3 and 8.3.5.

(The extinction observed is the reagents blank extinction E_1.)

8.4 Calibration curve

Transfer from a burette (6.2) to four cells 0,25 – 0,5 – 1 and 2 ml respectively of the standard iron(III) chloride solution (5.4) so as to obtain a series containing 2,5 – 5 – 10 and 20 μg of Fe^{3+}.

Add from a burette (6.2) to these four cells 9,65 – 9,4 – 8,9 and 7,9 ml respectively of the mixture of chloroform and methanol (5.1). Add from a graduated pipette (6.3) to each of the four cells 0,05 ml of the ammonium thiocyanate solution (5.3) and from another graduated pipette 0,05 ml of the hydrochloric acid solution (5.5) and mix. Note for each cell the time at which this stage is reached.

After a reaction time of 5 min for each cell, measure the extinction at 500 nm against the chloroform and methanol mixture contained in a similar cell.

Plot the measured extinctions against the masses of Fe^{3+} expressed in micrograms.

Construct the best-fitting straight line through the points.

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formulae

9.1.1 Calculate from the difference of extinction

$$E_2 - (E_0 + E_1)$$

by means of the calibration curve, or by means of the factor calculated from the calibration curve, the content (m) of Fe^{3+}, in micrograms.

E_0 is the extinction measured as described in 8.3.4.

E_1 is the extinction measured as described in 8.3.6.

E_2 is the extinction measured as described in 8.3.5.

9.1.2 The peroxide value of the fat, expressed as milli-equivalents of oxygen per kilogram, is equal to

$$\frac{m}{55{,}84\ m_0}$$

where

m is the mass, in micrograms, of Fe^{3+} calculated as described in 9.1.1;

m_0 is the mass, in grams, of the test portion.

Express the result to the nearest 0,01 unit of peroxide value.

9.2 Repeatability

The difference between the results of two determinations, carried out simultaneously or in rapid succession by the same analyst, using the same apparatus, shall not exceed 0,05 unit of peroxide value.

10 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The test report shall include all details required for the complete identification of the sample.

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