INTERNATIONAL STANDARD

ISO 5738

IDF 76

Second edition
2004-09-01

Milk and milk products — Determination of copper content — Photometric method (Reference method)

Lait et produits laitiers — Détermination de la teneur en cuivre — Méthode photométrique (Méthode de référence)

Reference numbers
ISO 5738:2004(E)
IDF 76:2004(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

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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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ISO 5738|IDF 76 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition of ISO 5738|IDF 76 cancels and replaces the first edition of ISO 5738 (ISO 5738:1980), which has been technically revised.

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 and AOAC International 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 National Committees casting a vote.

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All work was carried out by Joint ISO/IDF/AOAC Action Team on *Minor compounds,* of the Standing Committee on *Minor components and characterization of physical properties*, under the aegis of its project leader, Dr G. Ellen (NL).

This edition of ISO 5738 | IDF 76 cancels and replaces the first edition of IDF 76 (IDF 76A:1980), which has been technically revised.

Milk and milk products — Determination of copper content — Photometric method (Reference method)

1 Scope

This International Standard specifies a reference method for the determination of the copper content of milk and milk products.

The method is applicable to

a) milk, skimmed milk and buttermilk,

b) evaporated milk and sweetened condensed milk,

c) whole and skimmed milk powder,

d) cream and butter,

e) butterfat,

f) ice-cream,

g) hard, semi-hard and soft cheeses of various ages, and processed cheese, and

h) caseins, caseinates and coprecipitates.

The method is suitable for determining copper contents as low as 0,05 mg/kg in test samples of butter and butterfat.

NOTE See IDF 68 for details of butterfat.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 648:1977, *Laboratory glassware — One-mark pipettes*

ISO 835-1:1981, *Laboratory glassware — Graduated pipettes — Part 1: General requirements*

3 Terms and definitions

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

3.1
copper content
mass fraction of substances determined by the procedure described in this International Standard

NOTE The copper content is expressed as milligrams per kilogram.

4 Principle

The organic material in the test sample is digested with a mixture of nitric and sulfuric acids (preceded in the case of cream, butter and butterfat by removal of the fat). The solution is neutralized with ammonia solution followed by complexing the copper as a salt of diethyl dithiocarbamic acid. The copper(II) salt is extracted with amyl acetate. The absorbance of the yellow solution is measured photometrically.

The presence of bismuth and/or tellurium interferes with the determination of copper. See the check for absence and the method of removal specified in 8.6.

5 Reagents

All reagents shall be of analytical grade and, with the exception of the copper(II) sulfate standard solutions (5.12), shall be free from copper.

5.1 Water, double distilled, with the final distillation being carried out in a copper-free distillation unit.

5.2 Ethanol (CH_3CH_2OH), with a volume fraction of about 96 %.

Distil the ethanol, if necessary, in a copper-free distillation unit.

5.3 Diethyl ether $[(C_2H_5)_2O]$

Distil the diethyl ether, if necessary, in a copper-free distillation unit.

5.4 Light petroleum (petroleum ether), with boiling range between 40 °C and 60 °C.

Distil the light petroleum, if necessary, in a copper-free distillation unit.

5.5 Nitric acid, concentrated, $\rho_{20}(HNO_3)$ = 1,42 g/ml.

Distil in a copper-free distillation unit. Discard the first 50 ml.

5.6 Sulfuric acid

5.6.1 Sulfuric acid, concentrated, $\rho_{20}(H_2SO_4)$ = 1,84 g/ml.

5.6.2 Sulfuric acid, dilute, $c(H_2SO_4)$ = 0,5 mol/l).

5.7 Hydrogen peroxide solution, $\rho_{20}(H_2O_2)$ = 1,099 g/ml to 1,103 g/ml.

5.8 Ammonia solution, concentrated, $\rho_{20}(NH_3)$ = 0,91 g/ml.

Purify the ammonia solution, if necessary, by vacuum distillation in a copper-free distillation unit.

5.9 Citrate/EDTA solution

Dissolve 400 g of ammonium citrate $[(NH_4)_3C_6H_5O_7]$ and 100 g of EDTA disodium salt dihydrate [(ethylenedinitrilo)-tetraacetic acid disodium salt dihydrate] $(Na_2C_{10}H_{14}N_2O_8{\cdot}2H_2O)$ in water (5.1) in a 1 000 ml one-mark volumetric flask (6.12). Dilute to the mark with water.

Purify the citrate/EDTA solution, if necessary, as follows.

Add three drops of phenolphthalein solution (5.13) to the citrate/EDTA solution. Then add sufficient ammonia solution (5.8) until the solution remains pale pink. Add 10 mg of sodium diethyl dithiocarbamate (5.10). Warm the solution in a water bath (6.5) set at 60 °C for about 10 min to completely dissolve the sodium diethyl dithiocarbamate. Cool to ambient temperature.

Extract the solution in a 2 l separation funnel five times with 15 ml of amyl acetate (5.11). Repeat the whole purification procedure until the last 15 ml portion of amyl acetate remains colourless.

5.10 Sodium diethyl dithiocarbamate solution [$(C_2H_5)_2NCSSNa$]

Dissolve 400 mg of sodium diethyl dithiocarbamate trihydrate [$(C_2H_5)_2NCSSNa \cdot 3H_2O$] in 90 ml of water (5.1) in a 100 ml one-mark volumetric flask (6.12). Dilute to the mark with ammonia solution (5.8).

Store the solution in the dark in a refrigerator at between 0 °C and 8 °C. Renew the solution every week.

5.11 Amyl acetate

Dry 1 l of amyl acetate on 15 g of anhydrous sodium sulfate for 24 h. Distil in a copper-free distillation unit. Collect the fraction distilled at between 136 °C and 140 °C.

Instead of amyl acetate, xylene distilled in a copper-free distillation unit may be used.

5.12 Copper(II) sulfate standard solutions

5.12.1 Copper stock solution

Dissolve 196,5 mg of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in an amount of water in a 1 000 ml one-mark volumetric flask (6.12). Carefully add 5 ml of dilute sulfuric acid (5.6.2) and mix. Dilute to the mark with water (5.1).

5.12.2 Copper working solution

Prepare this solution on the day of use.

Use a one-mark pipette (6.11) to add 10 ml of copper stock solution (5.12.1) to 5 ml of dilute sulfuric acid (5.6.2) previously added to a 500 ml one-mark volumetric flask (6.12) and mix. Dilute to the mark with water.

NOTE 1 ml of the copper working solution contains 1 µg of Cu.

5.13 Phenolphthalein solution

Dissolve 1 g of phenolphthalein in 100 ml of 90 % ethanol (volume fraction).

5.14 Potassium cyanide solution (KCN), with a mass fraction of 5 % KCN.

WARNING — Take safety precautions as KCN is poisonous. It is the responsibility of the user of this standard to establish safety and health practices and to determine the applicability of regulatory limitations prior to use.

5.15 Sodium hydroxide solution, c(NaOH) = 1 mol/l.

6 Apparatus

6.1 Glassware

Keep the clean glassware in 10 % nitric acid (mass fraction). Before use, rinse three times with distilled water and then three times with double-distilled water. Dry, if necessary, by successively rinsing with ethanol and diethyl ether.

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

6.3 Appropriate grinding or **grating device**.