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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)

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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**.

6.4 Sieve, made of copper-free material, with nominal size of aperture 0,5 mm.

6.5 Water baths, capable of operating at 20 °C ± 2 °C, at 40 °C ± 1 °C, at 45 °C ± 1 °C, at 60 °C ± 1 °C, at between 30 °C and 40 °C, at between 40 °C and 60 °C, at between 60 °C and 65 °C and at between 80 °C and 90 °C.

6.6 Micro gas burner.

6.7 Digestion flasks, (Kjeldahl flasks) with ground-glass stoppers, calibrated on the lower part of the neck at 50 ml.

6.8 Glass beads, kept in 10 % nitric acid (mass fraction), as for the glassware (6.1).

6.9 Graduated cylinders, of capacity 5 ml and 25 ml.

6.10 Graduated pipettes, of capacity 5 ml and 25 ml with 0,1 ml graduations, complying with ISO 835-1:1981, class A.

6.11 One-mark pipettes, capable of delivering 1 ml, 2 ml, 5 ml, 10 ml and 20 ml, complying with ISO 648:1977, class A.

6.12 One-mark volumetric flasks, of capacity 100 ml, 500 ml and 1 000 ml.

6.13 Spectrophotometer, capable of operating at 436 nm, equipped with cells of 10 mm optical path length.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not 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.

Take precautions to avoid copper contamination during sampling. Store glass sampling jars in 10 % nitric acid (mass fraction).

Store the test sample so that deterioration and change in composition are prevented.

8 Procedure

8.1 Preparation of test sample

WARNING — Take precautions to avoid contamination of copper during the procedure.

8.1.1 Milk and skimmed milk

Bring the test sample to 20 °C in the water bath (6.5) and mix carefully. If, in case of a milk sample, the fat is not evenly dispersed, heat the test sample slowly in the water bath (6.5) set at 40 °C while mixing gently by inversion only. Then cool the sample quickly in another water bath (6.5) set at 20 °C.

8.1.2 Buttermilk

Remove any butter granules, if necessary. Bring the test sample to 20 °C in the water bath (6.5). Mix carefully, immediately before weighing (see 8.2.1).

8.1.3 Cream

Bring the test sample to 20 °C in the water bath (6.5). Mix or stir thoroughly but not so vigorously as to cause frothing or churning. If the sample is very thick, or if the fat is not evenly dispersed, heat it slowly in the water bath (6.5) set at 40 °C to facilitate mixing. Then cool the sample quickly in another water bath (6.5) set at 20 °C.

NOTE Reliable results cannot be expected if adequate mixing of the sample is not achieved or if the sample shows any evidence of churning or any other signs of abnormality.

8.1.4 Evaporated milk

Shake the test sample container thoroughly with frequent inversion. Open the container and pour the test sample slowly into another container made of glass and provided with an airtight lid. Incorporate any fat or other constituents adhering to the wall of the original container in the test sample. Stir vigorously and close the container.

Heat the closed container in a water bath (6.5) set at between 40 °C and 60 °C. Remove and shake the container vigorously every 15 min. Remove the container after 2 h. Cool it in another water bath (6.5) set at 20 °C. Remove the lid and mix thoroughly by stirring the milk with a spoon or spatula.

NOTE Correct results cannot be expected If the fat separates.

8.1.5 Sweetened condensed milk

Open the container and thoroughly mix the test sample with a spoon or spatula, using an up and down rotary movement in such a way that the top and bottom layers are moved and mixed. Incorporate all material adhering to the wall and ends of the container in the test sample.

Transfer the test sample as completely as possible to a second container made of glass provided with an airtight lid. Close the container. Heat the closed container in a water bath (6.5) set at between 30 °C and 40 °C. Cool it in another water bath (6.5) set at 20 °C. Stir the sample in the container thoroughly. Mix until the whole mass is homogeneous and close the container again.

In the case of a collapsible tube, open it and transfer the contents to a glass container. Cut open the tube and transfer all material adhering to the interior as completely as possible to the container.

8.1.6 Whole and skimmed milk powder

Transfer the test sample to a container provided with an airtight lid of a capacity about twice the volume of the sample. Close the container immediately. Mix the sample thoroughly by repeatedly shaking and inverting the container.

8.1.7 Butter

8.1.7.1 If the test sample is not visibly inhomogeneous, cool it to 4 °C ± 2 °C before weighing to facilitate transfer to the digestion flask.

8.1.7.2 If the test sample is visibly inhomogeneous, warm the sample to a temperature at which it will be soft enough to facilitate thorough mixing to a homogeneous state without any rupture of emulsion. The temperature of mixing shall normally not exceed 35 °C.

Cool the sample in a water bath (6.5) to about 20 °C (or until rather firm) while mixing. As soon as possible after cooling, open the sample container and stir briefly for less then 10 s with a spoon or spatula. Proceed as in 8.1.7.1.

8.1.8 Butterfat

Before weighing, warm the test sample in the water bath (6.5) set at 40 °C. Keep it at this temperature for 5 min then mix gently.

8.1.9 Ice-cream

For test samples taken in small packages, remove the packaging and place the sample in a container provided with an airtight lid. Keep samples from bulk or from large packages in their sample containers. In either case, melt the sample by standing it in a water bath (6.5) set at 45 °C for just enough time to allow the sample to become fluid. Mix the sample by shaking. Cool it in another water bath (6.5) set at 20 °C while mixing until cooling is completed.

8.1.10 Cheese and processed cheese

Remove the rind or smear or mouldy surface layer of the test sample in such a way as to provide a sample representative of the cheese as it is usually consumed. Grind or grate the sample by means of an appropriate grinding or grating device (6.3). Quickly mix the whole ground or grated mass then preferably grind the mass again quickly.

If the test sample (e.g. soft cheese) cannot be ground or grated, mix the whole sample thoroughly. Transfer the pretreated sample, or a representative part of it, immediately to a container provided with an airtight lid.

Analyse the sample without delay as soon as possible after grinding. Ground or grated cheeses showing unwanted mould growth or beginning to deteriorate shall not be examined. Clean the device after grinding or grating each sample.

8.1.11 Caseins, caseinates and coprecipitates

8.1.11.1 If most of the sample is fine enough to pass through the sieve (6.4), it may be used without any grinding. Transfer about 50 g of the received test sample to a container, provided with an airtight lid, of capacity about twice the volume of the powder.

Close the container immediately. Mix the sample thoroughly by repeatedly shaking and inverting the container.

8.1.11.2 If most of the test sample is not fine enough to pass through the sieve (6.4), grind about 50 g of test sample until most of it does. Transfer all material to a container. Continue as in 8.1.11.1.

8.2 Weighing and pretreatment of the test portion

8.2.1 Milk, skimmed milk and buttermilk

Weigh, to the nearest 10 mg, 20 g of test sample (8.1.1, 8.1.2) in a digestion flask (6.7). Add 3 ml of nitric acid (5.5) and 2,5 ml of concentrated sulfuric acid (5.6.1). Continue as in 8.3.

8.2.2 Evaporated milk

Weigh, to the nearest 10 mg, 8 g of test sample (8.1.4) in a digestion flask (6.7). Add 3 ml of nitric acid (5.5) and 2,5 ml of concentrated sulfuric acid (5.6.1). Continue as in 8.3.

8.2.3 Sweetened condensed milk

Weigh, to the nearest 10 mg, 5 g of test sample (8.1.5) in a digestion flask (6.7). Add 10 ml of water (5.1) and 2,5 ml of concentrated sulfuric acid (5.6.1). Do not add nitric acid at this stage, as that would cause excessive foaming. Continue as in 8.3.

8.2.4 Whole and skimmed milk powder

Weigh, to the nearest 1 mg, 2 g of test sample (8.1.6) in a digestion flask (6.7). Add 5 ml of water (5.1) and mix well. Then add 3 ml of nitric acid (5.5) and 2,5 ml of concentrated sulfuric acid (5.6.1). Continue as in 8.3.

8.2.5 Cream, butter and butterfat

Weigh, to the nearest 10 mg, 20 g of test sample (8.1.3, 8.1.7, 8.1.8) in a digestion flask (6.7). Add, in the case of cream or butter, 8 ml of nitric acid (5.5) and, in the case of butterfat, 4 ml of water (5.1) and 8 ml of nitric acid (5.5). Heat the flask in a water bath (6.5) set at between 80 °C and 90 °C for 1 h. Shake thoroughly every 3 min in order to wash the fat with nitric acid (5.5). Cool in another water bath (6.5) set at 40 °C and remove the fat layer as far as possible by means of a pipette.

Add 15 ml of petroleum ether (5.4). Swirl carefully and remove the solvent as far as possible by means of a pipette. Repeat twice with fresh 15 ml portions of petroleum ether (5.4). Remove residual petroleum ether by warming in a water bath (6.5) set at between 80 °C and 90 °C. Cool to room temperature. Add 2,5 ml of concentrated sulfuric acid (5.6.1). Continue as in 8.3.

8.2.6 Ice-cream, cheese and processed cheese

Weigh, to the nearest 1 mg, 3 g of test sample (8.1.9, 8.1.10) in a digestion flask (6.7). Add 3 ml of nitric acid (5.5) and 2,5 ml of concentrated sulfuric acid (5.6.1). Continue as in 8.3.

8.2.7 Caseins, caseinates and coprecipitates

Weigh, to the nearest 1 mg, 1 g of test sample (8.1.11) in a digestion flask (6.7). Add 5 ml of water (5.1), 3 ml of nitric acid (5.5) and 2,5 ml of concentrated sulfuric acid (5.6.1). Continue as in 8.3.

8.3 Digestion

8.3.1 Add three glass beads (6.8) to the contents of the digestion flask (8.2.1 to 8.2.7). Operating under a well-ventilated fume hood, place the flask in an inclined position and heat with a micro gas burner (6.6). Control the height of the flame so as to limit the production of foam in the flask. Foaming into the neck of the flask is allowed but the foam shall not escape. Keep the mixture gently boiling. Avoid local overheating.

Carry out a blank test (see 8.5) simultaneously.

8.3.2 When the solution turns brown, carefully add 3 to 5 drops of nitric acid (5.5). Vigorously shake the solution again as soon as possible. Continue heating and adding the nitric acid, 5 to 20 drops at a time, while swirling the flask occasionally to remove any material adhering to the wall. Continue until the mixture remains colourless, then cool to room temperature.

8.3.3 Carefully add 2 ml of water (5.1) and 1 ml of hydrogen peroxide solution (5.7). Swirl and heat again until white fumes are emitted. If the solution becomes yellow, add a further 0,5 ml of hydrogen peroxide solution. Continue heating for 45 min after the beginning of the emission of white fumes. Cool to room temperature and add water to give a total volume of approximately 25 ml.

8.3.4 Add 5 ml of citrate/EDTA solution (5.9) and 0,1 ml of phenolphthalein solution (5.13). Add ammonia solution (5.8) while swirling occasionally until the mixture remains light red.

8.4 Colour development

During the colour development and the photometric determination, avoid unnecessary exposure to light stronger than an illuminance of 150 lx (subdued daylight). Add 5 ml of sodium diethyl dithiocarbamate solution (5.10), while swirling. Dilute to the 50 ml mark with water and pipette 4 ml of amyl acetate (5.11) into the 50 ml solution. Close the flask with the stopper and mix. Heat the closed flask for 10 min in a water bath (6.5) set at between 60 °C and 65 °C. Then shake the flask vigorously with repeated inversion for 1 min while ensuring the stopper remains in position. Cool the closed flask immediately under running tap water for at least 10 min.

In the case of using xylene instead of amyl acetate, shake the flask for 2 min.

Eliminate the turbidity appearing in the amyl acetate layer by tilting the flask cautiously from the vertical to a horizontal position a number of times. Continue cautiously tilting the flask if turbidity persists. Keep the flask in the dark for 1 h.

8.5 Blank test

Simultaneously with the analysis of the test portion, carry out a blank test using all reagents and 20 ml of water (5.1) instead of the test portion. During the digestion period, use the same amount of nitric acid (5.5) and hydrogen peroxide solution (5.7) as for the digestion of the test portion.

8.6 Photometric measurements

Transfer the amyl acetate (upper) layer by means of a pipette into the 10 mm cell of the spectrophotometer (6.13). Measure the absorbances of the amyl acetate layers of the test solution (8.4) and the reagent blank solution (8.5) against that of water at a wavelength of 436 nm. Subtract the value for the reagent blank solution from that of the test solution.

The absorbance value of the blank test shall correspond to less than 0,4 µg of Cu per 50 ml. If the obtained value is higher, check all reagents.

Bismuth and tellurium may be assumed to be absent if the amyl acetate layer turns colourless after shaking with potassium cyanide solution (5.14). Otherwise, remove them by washing the amyl acetate layer with sodium hydroxide solution (5.15).

8.7 Number of determinations

Carry out the determinations, including the blank test (8.5), in duplicate.

8.8 Calibration curve

8.8.1 Pipette 0 ml (blank), 0,5 ml, 1 ml, 2 ml, 3 ml, 4 ml and 6 ml of the copper(II) working solution (5.12.2) into a series of seven separate digestion flasks (6.7). Dilute with water (5.1) to about 25 ml. Add 2,5 ml of concentrated sulfuric acid (5.6.1) and mix well. Add 5 ml of citrate/EDTA solution (5.9) and 0,1 ml of phenolphthalein solution (5.13) and mix again. Add ammonia solution (5.8) while swirling occasionally until the mixture remains light red.

8.8.2 Carry out the procedure described in 8.4.

8.8.3 Transfer each amyl acetate (upper) layer by means of a pipette into the 10 mm cell of the spectrophotometer (6.13). Measure the absorbance of the amyl acetate layers against that of water at a wavelength of 436 nm. Subtract the blank value from the values found for the other solutions.

8.8.4 Plot these absorbance against the amounts of copper added.

8.8.5 Check the calibration curve monthly.

9 Calculation and expression of results

9.1 Calculation

Calculate the copper content of the sample, w, in milligrams per kilogram, by using the following equation:

$$w = \frac{m_2}{m_1}$$