INTERNATIONAL STANDARD

ISO 1737

Third edition
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Evaporated milk and sweetened condensed milk — Determination of fat content — Gravimetric method (Reference method)

Lait concentré sucré et non sucré — Détermination de la teneur en matière grasse — Méthode gravimétrique (Méthode de référence)

Reference number
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Contents

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

International Standard ISO 1737 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations.

This third edition cancels and replaces the second edition (ISO 1737:1985), which has been technically revised.

Annexes A and B of this International Standard are for information only.

Evaporated milk and sweetened condensed milk — Determination of fat content — Gravimetric method (Reference method)

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

1 Scope

This International Standard specifies the reference method for the determination of the fat content of all types of evaporated milk and sweetened condensed milk (liquid sweetened and unsweetened concentrated milk).

2 Normative reference

The following normative document contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreement based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3889, *Milk and milk products — Determination of fat content — Mojonnier-type fat extraction flasks*.

3 Term and definition

For the purposes of this International Standard the following term and definition apply.

3.1
fat content of evaporated milk and sweetened condensed milk
mass fraction of substances determined by the procedure specified in this International Standard

NOTE The fat content is expressed as a mass fraction, in percent [formerly given as % (m/m)].

4 Principle

An ammoniacal ethanolic solution of a test portion is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined.

NOTE This is usually known as the Röse-Gottlieb principle.

5 Reagents

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

The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 9.2.2).

5.1 Ammonia solution, containing a mass fraction of NH_3 of approximately 25 % (ρ_{20} = 910 g/l).

NOTE If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2).

5.2 Ethanol (C_2H_5OH), or ethanol denatured by methanol, containing a volume fraction of ethanol of at least 94 %. (See A.5.)

5.3 Congo red solution

Dissolve 1 g of Congo red in water in a 100 ml one-mark volumetric flask (6.14). Dilute to the mark with water.

NOTE The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 9.4.3). Other aqueous colour solutions may be used provided that they do not affect the result of the determination.

5.4 Diethyl ether ($C_2H_5OC_2H_5$), free from peroxides (see A.3), containing no more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see 9.2.2, A.1 and A.4).

NOTE The use of diethyl ether could lead to hazardous situations. Due to expected changes in safety regulations studies are ongoing to replace diethyl ether by another reagent provided that it does not affect the end result of the determination.

5.5 Light petroleum, with any boiling range between 30 °C and 60 °C or, as equivalent, **pentane** ($CH_3[CH_2]_3CH_3$) with a boiling point of 36 °C and complying with the requirements for the blank test (see 9.2.2, A.1 and A.4).

NOTE The use of pentane is recommended because of its higher purity and constant quality.

5.6 Mixed solvent

Shortly before use, mix equal volumes of diethyl ether (5.4) and light petroleum (5.5).

6 Apparatus

WARNING — Since the determination involves the use of volatile flammable solvents, all electrical apparatus employed shall comply with legislation relating to the hazards in using such solvents.

Usual laboratory equipment and, in particular, the following.

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

6.2 Centrifuge, capable of holding the fat-extraction flasks or tubes (6.6) and capable of spinning at a rotational frequency of 500 min^{-1} to 600 min^{-1} to produce a radial acceleration of 80 *g* to 90 *g* at the outer end of the flasks or tubes.

NOTE The use of the centrifuge is optional but recommended (see 9.4.6).

6.3 Distillation or evaporation apparatus, for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from beakers and dishes (see 9.4.13) at a temperature not exceeding 100 °C.

6.4 Drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at a temperature of 102 °C ± 2 °C throughout its working space.

The oven shall be fitted with a suitable thermometer.

6.5 Water baths, capable of being maintained at a temperature of between 30 °C and 40 °C, and 40 °C and 60 °C.

6.6 Mojonnier-type fat-extraction flasks, as specified in ISO 3889.

NOTE It is also possible to use fat-extraction tubes, with siphon or wash-bottle fittings, but then the procedure is different. The alternative procedure is given in annex B.

The fat-extraction flasks shall be provided with good quality bark corks or stoppers of other material [e.g. silicone rubber or polytetrafluoroethylene (PTFE)] unaffected by the reagents used. Bark corks shall be extracted with the diethyl ether (5.4), kept in water at a temperature of 60 °C or more for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

6.7 Rack, for holding the fat-extraction flasks (or tubes) (6.6).

6.8 Wash bottle, suitable for use with the mixed solvent (5.6).

A plastics wash bottle shall not be used.

6.9 Fat-collecting vessels, such as boiling flasks (flat-bottomed), of capacities 125 ml to 250 ml, conical flasks, of capacity 250 ml, or metal dishes.

If metal dishes are used, they shall be of stainless steel, flat-bottomed with a diameter of 80 mm to 100 mm and a height of approximately 50 mm.

6.10 Boiling aids, fat-free, of non-porous porcelain or silicon carbide (optional when metal dishes are used).

6.11 Measuring cylinders, of capacities 5 ml and 25 ml.

6.12 Pipettes, graduated, of capacity 10 ml.

6.13 Tongs, made of metal, for holding flasks, beakers or dishes.

6.14 Volumetric flask, one-mark, of capacity 100 ml.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Store the samples at a temperature of between 2 °C and 6 °C from the time of sampling to the time of commencing the procedure. In the case of samples in sealed cans, store the closed cans at a temperature below 20 °C.

8 Preparation of test sample

8.1 Evaporated milk

Shake and invert the sample container. Open the sample container and pour the sample slowly into a second sample container (provided with an airtight lid). Mix by repeated transfer, taking care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the first container. Finally, transfer the product as completely as possible to the second container.

If necessary in the case of samples in sealed cans, condition the unopened container in the water bath (6.5) set at a temperature of between 40 °C and 60 °C. Remove and shake the can vigorously every 15 min. After 2 h, remove the can and allow it to cool to room temperature.

Remove the lid entirely and thoroughly mix the sample by stirring with a spoon or spatula. (If fat separates, do not test the sample.)

8.2 Sweetened condensed milk

Open the sample container and mix thoroughly with a spoon or spatula. Use an up-and-down rotary movement in such a way that the top layers and the content of the lower corners of the container are moved and mixed. Take care to incorporate in the sample any milk adhering to the wall and ends of the container. Transfer the sample as completely as possible to a second sample container (provided with an airtight lid). Close the second container.

If necessary, in the case of samples in sealed cans, condition the unopened can in the water bath (6.5) at a temperature of between 30 °C and 40 °C. Open the can, scrape out all milk adhering to the interior of the can, transfer to a dish large enough to permit stirring thoroughly and mix until the whole mass is homogeneous.

In the case of a sample in a collapsible tube, open the tube and transfer the contents to a jar. Then cut open the tube and scrape out all material adhering to the interior and add to the contents of the jar.

9 Procedure

NOTE An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see note in 6.6) is given in annex B.

9.1 Test portion

Mix the test sample (clause 8), by stirring in the case of sweetened condensed milk, or by gently inverting the bottle three or four times in the case of evaporated milk. Immediately weigh to the nearest 1 mg, directly or by difference, 4 g or 5 g of the test sample of evaporated milk, or 2,0 g to 2,5 g of the test sample of sweetened condensed milk in a fat-extraction flask (6.6).

Transfer the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

9.2 Blank tests

9.2.1 Blank test for method

Carry out a blank test simultaneously with the determination using the same procedure and same reagents, but replacing the test portion by 10 ml of water (see A.2).

If the value obtained in the blank test regularly exceeds 1,0 mg, check the reagents if this has not been recently done (9.2.2). Corrections of more than 2,5 mg should be mentioned in the test report.

9.2.2 Blank test for reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2.1. Additionally use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes. The reagents shall leave no residue greater than 1,0 mg (see A.1).

If the residue of the complete reagent blank test is greater than 1,0 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether (5.4) and light petroleum (5.5), respectively. Use an empty fat-collecting vessel, prepared for control purposes as described above, to obtain the real mass of residue which shall not exceed 1,0 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-

collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after the redistillation.

Replace unsatisfactory reagents, solvents, or redistil solvents.

9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the oven (6.4) set at 102 °C for 1 h.

NOTE 1 Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvents, especially when using glass fat-collecting vessels; their use is optional with metal dishes.

Protect the fat-collecting vessel from dust and allow it to cool to the temperature of the weighing room (glass fat-collecting vessel for at least 1 h, metal dish for at least 30 min).

NOTE 2 To avoid insufficient cooling or unduly long cooling times, the fat-collecting vessel should not be placed in a desiccator.

Use tongs to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

NOTE 3 Tongs should preferably be used to avoid, in particular, temperature variations.

9.4 Determination

9.4.1 Carry out the determination without delay.

Add water at a temperature of about 50 °C to the test portion in the fat-extraction flask (9.1) to obtain a total volume of 10 ml to 11 ml. Use the water to wash the test portion on to the bottom of the flask. Shake gently with slight warming at about 50 °C in the waterbath (6.5) until the test portion is completely dispersed. Cool in running water to room temperature.

9.4.2 Add 2 ml of ammonia solution (5.1) to the dispersed test portion in the fat-extraction flask (9.4.1), or an equivalent volume of a more concentrated ammonia solution (see note in 5.1). Mix thoroughly with the test portion in the small bulb of the fat-extraction flask.

9.4.3 Add 10 ml of ethanol (5.2). Mix gently but thoroughly by allowing the contents of the fat-extraction flask to flow backwards and forwards between the small and large bulb. Avoid bringing the liquid too near to the neck of the flask. If desired, add 2 drops of the Congo red solution (5.3).

9.4.4 Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a cork saturated with water or with a stopper of other material wetted with water (6.6). Shake the flask vigorously, but not excessively, for 1 min to avoid the formation of persistent emulsions.

While shaking, keep the fat-extraction flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid to run from the large bulb into the small bulb. If necessary, cool the flask in running water to about room temperature. Carefully remove the cork or stopper and rinse it and the neck of the flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask.

9.4.5 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the rewetted (by dipping into water) cork or stopper. Shake the flask gently again for 30 s as described in 9.4.3. Proceed with shaking as described in 9.4.4.

9.4.6 Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of 80 g to 90 g. If a centrifuge is not available, allow the closed flask to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the flask in running water to room temperature.

9.4.7 Carefully remove the cork or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask. If the interface is below the

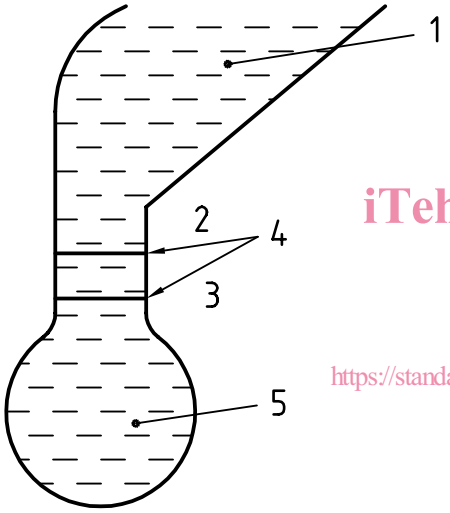
bottom of the stem of the flask, raise it slightly above this level by gently adding water down the side of the flask (see Figure 1) to facilitate the decanting of solvent.

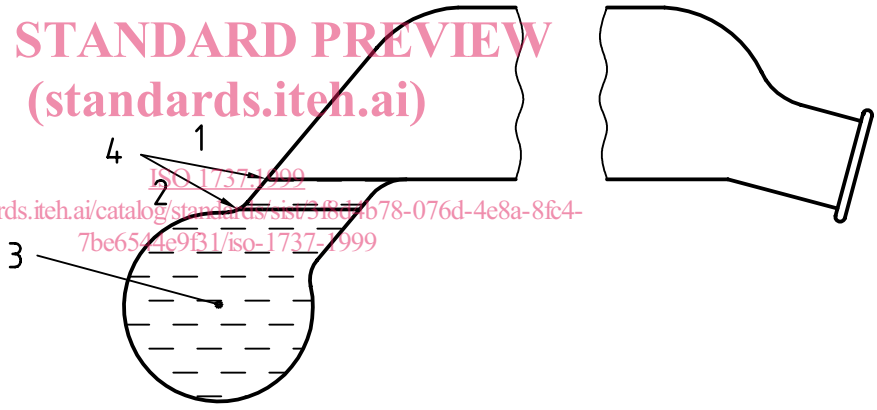
NOTE In Figures 1 and 2, one of the three types of fat-extraction flasks as specified in ISO 3889 has been chosen, but this does not imply any preference over other types.

9.4.8 Hold the fat-extraction flask by the small bulb and carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of a boiling or conical flask (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).

9.4.9 Rinse the outside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Collect the rinsings in the fat-collecting vessel. Take care that the mixed solvent does not spread over the outside of the fat-extraction flask. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as described in 9.4.13.

9.4.10 Add 5 ml of ethanol (5.2) to the contents of the fat-extraction flask. Using the ethanol, rinse the inside of the neck of the flask and mix as described in 9.4.3.





Key
1 Solvent
2 At second and third extraction
3 At first extraction
4 Interface
5 Aqueous layer

Figure 1 — Before decanting

Key
1 At second and third extraction
2 At first extraction
3 Aqueous layer
4 Interface

Figure 2 — After decanting

9.4.11 Carry out a second extraction by repeating the operations described in 9.4.4 to 9.4.8 inclusive. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask too.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

9.4.12 Carry out a third extraction without addition of ethanol by again repeating the operations described in 9.4.4 to 9.4.8 inclusive. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask again.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

NOTE The third extraction may be omitted for evaporated milk and sweetened condensed milk with a fat content of less than 1 %.

9.4.13 Remove the solvents (including the ethanol) as completely as possible from the fat-collecting vessel, by distillation if using a boiling or conical flask, or by evaporation if using a beaker or dish (6.3). Rinse the inside of the neck of the conical flask with a little mixed solvent (5.6) before commencing the distillation.

9.4.14 Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for 1 h in the drying oven (6.4) set at 102 °C. Remove the fat-collecting vessel from the oven and immediately verify whether or not the fat is clear. If the fat is not clear, fatty extraneous matter is presumed to be present and the whole procedure shall be repeated. If the fat is clear, protect the fat-collecting vessel from dust and allow the fat-collecting vessel to cool (preferably not in a desiccator) to the temperature of the weighing room (a glass fat-collecting vessel for at least 1 h, a metal dish for at least 30 min).

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

9.4.15 Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for a further 30 min in the drying oven (6.4) set at 102 °C. Cool and reweigh as described in 9.4.14. If necessary, repeat the heating and weighing procedures until the mass of the fat-collecting vessel decreases by 1,0 mg or less, or increases between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

10 Calculation and expression of results

10.1 Calculation

Calculate the fat content of the sample using the following equation:

$$w_f = \frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100\ \%$$

where

w_f is the mass fraction of fat in the sample, in percent;

m_0 is the mass of the test portion (9.1), in grams;

m_1 is the mass of the fat-collecting vessel and extracted matter, determined in 9.4.15, in grams;

m_2 is the mass of the prepared fat-collecting vessel (9.3), in grams;

m_3 is the mass of the fat-collecting vessel used in the blank test (9.2) and any extracted matter determined in 9.4.15, in grams;

m_4 is the mass of the fat-collecting vessel (9.3) used in the blank test (9.2), in grams.

10.2 Expression of results

Round the result to two decimal places.