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**Milk products and milk-based foods —
Determination of fat content by the
Weibull-Berntrop gravimetric method
(Reference method) —**

**Part 3:
Special cases**

*Produits laitiers et produits à base de lait — Détermination de la teneur
en matière grasse par la méthode gravimétrique Weibull-Berntrop
(Méthode de référence) —*

Partie 3: Cas particuliers



Reference numbers
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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 2.

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 8262-3|IDF 124-3 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 8262-3|IDF 124-3 cancels and replaces ISO 8262-3:1987, of which it constitutes a minor revision.

ISO 8262|IDF 124 consists of the following parts, under the general title *Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method)*:

- *Part 1: Infant foods*
- *Part 2: Edible ices and ice-mixes*
- *Part 3: Special cases*

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 8262-3|IDF 124-3 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 IDF and ISO.

All work was carried out by the Joint ISO-IDF Group of Experts on *Fat determination* (E 31), under the aegis of its chairman, Mr J. Eisses (NL).

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Introduction

ISO 8262|IDF 124 has been prepared within the framework of producing a series of reference methods, which are harmonized to the greatest possible extent, for the gravimetric determination of the fat content of milk, milk products and milk-based foods. These methods are based on the Röse-Gottlied (RG) method, or the Weibull-Berntrop (WB) method, or the Schmid-Bondzynski-Ratzlaff (SBR) principle.

For this part of ISO 8262|IDF 124, dealing with milk-based and with liquid, concentrated or dried milk products in poor condition and/or containing insoluble non-milk ingredients, a method based on the WB principle has been chosen for the following reasons:

- a) the RG procedure is not suitable when a distinct quantity of free fatty acids is present, or when the product contains lumps and/or non-milk ingredients insoluble in ammonia, since the extraction of fat is incomplete;
- b) the SBR procedure is not suitable owing to a considerable lactose content, which gives rise to some ether-extractable compounds in the digestion with acid and thus gives too high values for the fat content;
- c) the WB procedure, although it also includes acid digestion, is not adversely affected by the ether-extractable compounds, since the acid digest is filtered and washed, and the dried residue on the filter does not contain compounds that are extractable by light petroleum;
- d) the method described is already used for this purpose in many countries.

The original Weibull method was designed for bread; a considerably modified method, as specified in this International Standard, was developed by Berntrop. This version has found wide application for the determination of fat in many types of food product.

Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method) —

Part 3: Special cases

1 Scope

This part of ISO 8262 | IDF 124 specifies the reference method for the determination of the fat content of milk-based and of liquid, concentrated or dried milk products to which the Röse-Gottlieb method is not applicable; i.e. those containing distinct quantities of free fatty acids or those which are not completely soluble in ammonia owing to the presence of lumps or non-milk ingredients, such as custards, porridges or certain milk-based products for bakery purposes.

NOTE 1 Reference Röse-Gottlieb methods for the determination of the fat content of milk, of cream, of evaporated and sweetened condensed milk, and of dried milk products are specified in ISO 1211, ISO 2450, ISO 1737 and ISO 1736 respectively.

The method is also applicable to fresh cheese types, such as cottage cheese and quarg, as well as to fresh cheeses with added fruit, syrup, “muesli”, etc. for which the SBR method is not suitable owing to the higher carbohydrate contents and/or extreme inhomogeneity.

NOTE 2 A reference Schmid-Bondzynski-Ratzlaff method for the determination of the fat content of cheese and processed cheese products having lactose contents below 5 % (mass fraction) of the non-fat solids is specified in ISO 1735.

2 Terms and definitions

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

2.1

fat content

all the substances determined by the method specified in this part of ISO 8262 | IDF 124

NOTE It is expressed as a mass fraction in percent.

3 Principle

A test portion is digested by boiling with dilute hydrochloric acid. The hot digest is filtered through a wetted filter paper to retain fatty substances, then the fat is extracted from the dried filter paper using *n*-hexane or light petroleum. The solvent is removed by distillation or evaporation and the substances are extracted and weighed. (This is usually known as the Weibull-Berntrop principle.)

4 Reagents and materials

Use only reagents of recognized analytical grade that leave no appreciable residue when the determination is carried out by the method specified. Use distilled or deionized water, or water of at least equivalent purity.

4.1 Dilute hydrochloric acid, containing approximately 20 % (mass fraction) of HCl, ρ_{20} approximately 1,10 g/ml.

Dilute 100 ml of concentrated hydrochloric acid ($\rho_{20} = 1,18$ g/ml) with 100 ml of water and mix.

4.2 Extraction solvent, free from water: *n*-hexane or light petroleum having any boiling range between 30 °C and 60 °C.

To test the quality of the extraction solvent, distil 100 ml of it from an extraction flask (5.4) prepared as specified in 7.4. Use an empty extraction flask, prepared in the same way, to check the mass (see 10.1). The solvent shall leave no residue greater than 1,0 mg.

Replace or distil the solvent if it does not meet this requirement.

4.3 Filter papers, of diameter 150 mm, pleated, medium grade, preferably defatted.

To test the quality of the filter paper, carry out a blank test as specified in 7.3, using a solvent satisfying the requirement of 4.2. Use an empty extraction flask (5.4), prepared as specified in 7.4, to check the mass (see 10.1). The paper shall leave no residue greater than 2,5 mg.

Replace unsatisfactory filter papers.

4.4 Blue litmus paper.

4.5 Diatomaceous earth (optional; see 7.5.3).

4.6 Pure lactose (optional; see 7.5.3).

4.7 Cotton wool, defatted by extraction with the solvent (4.2) for 1,5 h and dried.

5 Apparatus

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

Usual laboratory equipment and, in particular, the following.

5.1 Analytical balance.

5.2 Blender, for homogenizing the laboratory sample, if necessary. For example, use a food chopper or a high-speed blender with a blender jar, of capacity 1 litre, fitted with a lid.

5.3 Extraction apparatus, continuous or semi-continuous. For example, use a Soxhlet type, consisting of an extraction flask (flat-bottomed, short-necked) of capacity 150 ml, an extractor with a siphoning volume of 40 ml to 60 ml, and an efficient reflux condenser fitted with a drying tube or plug of cotton wool.

5.4 Extraction flasks, of capacity 150 ml, flat-bottomed and short-necked.

5.5 Extraction thimbles, made of defatted filter paper, glass, alumina or polytetrafluoroethylene (PTFE), contributing no appreciable residue in the blank test, or made of cellulose, single thickness, of internal diameter 22 mm and external length 80 mm, for use with the extraction apparatus (5.3).

5.6 Water baths, capable of being maintained at the following temperatures:

— 40 °C to 60 °C (see 7.1.1);

— 30 °C to 40 °C (see 7.1.2).

5.7 Heating apparatus, for the extraction apparatus. For example, use a water bath, sand bath or a thermostatically controlled hotplate.

5.8 Boiling aids, fat-free, such as glass beads or pieces of non-friable, non-porous porcelain or silicon carbide.

5.9 Conical flask, of capacity 250 ml, fitted with a reflux condenser, preferably of the Liebig type.

5.10 Heating apparatus, for heating a conical flask fitted with a condenser. For example, use a wire gauze and gas burner, an electric hotplate or a sand bath.

5.11 Filter funnel, suitable for use with the pleated filter paper (4.3).

5.12 Beakers with spouts, of capacities 100 ml and 250 ml.

5.13 Distillation apparatus, to enable the solvent to be gently distilled from the flasks at a temperature not exceeding 100 °C.

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

The oven shall be fitted with a suitable thermometer.

5.15 Measuring cylinders, of capacities 50 ml, 100 ml and 250 ml.

5.16 Tongs, made of metal, suitable for holding flasks or beakers.

5.17 Tweezers, flat-tipped, for holding filter papers and thimbles.

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 part of ISO 8262|IDF 124. A recommended sampling method is given in ISO 707|IDF 50.

All liquid, viscous or pasty laboratory samples shall be kept at a temperature of 2 °C to 4 °C from the time of sampling to the time of commencing the procedure. In the case of a sealed can or bottle, store it unopened at a temperature below 20 °C.

7 Procedure

7.1 Preparation of test sample

7.1.1 Liquid products

Shake and invert the container. Open the container, pour the product slowly into a second container (provided with an airtight lid) and 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. If the product still contains lumps or pieces of ingredients, homogenize it in an appropriate blender (5.2). Finally, transfer the product as completely as possible to the second container. Close this container.

If necessary, condition the unopened container in the water bath (5.6) at 40 °C to 60 °C. Remove and shake the container vigorously every 15 min. After 2 h, remove the container, dry the outside with a tissue and allow to cool to room temperature. Remove the lid or cap entirely and thoroughly mix the contents by stirring with a spoon or spatula. (If fat separates out, do not test the sample.) Transfer the product as completely as possible to the second container. Close this container.

7.1.2 Viscous or pasty products

Open the container and thoroughly mix the contents with a spoon or spatula. If possible, use an up-and-down rotary movement in such a way that the top layers and the contents of the lower corners of the container are moved and mixed. Take care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the container. If the product still contains lumps or pieces of ingredients, homogenize in an appropriate blender (5.2). Transfer the product as completely as possible to a second container (provided with an airtight lid). Close this container.

If necessary, condition the unopened container in the water bath (5.6) at 30 °C to 40 °C. Remove the container, dry the outside with a tissue and open it. Scrape out all product adhering to the interior of the container, transfer to a dish large enough to permit thorough stirring, and mix until the whole mass is homogeneous. Transfer the product as completely as possible to a second container as above. Close this container.

7.1.3 Dried products

Mix thoroughly by repeatedly rotating and inverting the container. If necessary, transfer the laboratory sample to a suitable airtight container of adequate capacity to allow this operation to be carried out.

If the product still contains lumps or pieces of ingredients, homogenize it in an appropriate blender (5.2).

7.2 Test portion

Mix the test sample (7.1) by stirring (in the case of viscous, pasty or dried products) or by gently inverting the container three or four times (in the case of liquid products) and immediately weigh into a conical flask (5.9), directly or by difference, to the nearest 1 mg, 3 g to 20 g of the test sample, corresponding to 3,0 g to 3,5 g of dry matter. The test portion shall contain not more than 1,0 g of fat; to meet this requirement, it may be necessary to take a smaller test portion.

The test portion shall be delivered as completely as possible onto the bottom of the conical flask (5.9).

7.3 Blank test

Carry out a blank test simultaneously with the determination, using the same procedure and same reagents, but replacing the diluted test portion (see 7.5.1) by 25 ml of water (see 10.2).

7.4 Preparation of extraction flask

Dry a flask (5.4), containing a few boiling aids (5.8) to promote gentle boiling during the extraction and subsequent removal of solvent, in the oven (5.14) set at 102 °C, for 1 h.

Allow the flask to cool (protected from dust) for at least 0,5 h to the temperature of the weighing room.

To avoid insufficient cooling or unduly long cooling times, the flask should not be placed in a desiccator.

Using tongs (5.16) (to avoid, in particular, temperature variations), place the flask on the balance and weigh to the nearest 0,1 mg.

7.5 Determination

7.5.1 Add water at 30 °C to the test portion (7.2) to give a total volume of 25 ml (in order to obtain a 4 mol/l hydrochloric acid solution in 7.5.2) and shake gently.

NOTE For the optional addition of lactose (4.6), see the Note to 7.5.3.

7.5.2 Add 50 ml of the hydrochloric acid solution (4.1) to the diluted test portion, rinsing the walls of the conical flask during the addition, and mix gently by swirling. Connect the flask to the reflux condenser, heat the flask until the contents start to boil and then boil gently for 30 min, swirling the contents occasionally.

7.5.3 Rinse the inside of the condenser with about 75 ml of a portion of 150 ml of hot water (at least 80 °C), remove the conical flask from the condenser and add the remainder of the hot water to the flask so as to rinse the inside of the neck and wall. Add, if desired (particularly recommended in the case of a low non-fat solids content), 1 g of diatomaceous earth (4.5) or approximately 100 cm² of defatted filter paper, torn into pieces, to promote rapid filtration.

NOTE The filtration can also be improved by adding 1 g of pure lactose (4.6) to the diluted test portion in 7.5.1.

7.5.4 Immediately filter the contents of the flask, pouring the liquid down a glass rod, through a pleated filter paper (4.3) thoroughly wetted with hot water, placed in the filter funnel (5.11). Thoroughly rinse the flask three times with hot water, transferring the rinsings, with the aid of the glass rod, quantitatively to the filter paper, and finally wash the filter paper at least three times with hot water until the washings are acid-free as indicated by the litmus paper (4.4). Do not use more than 400 ml of water. Allow the filter paper to drain well.

7.5.5 Remove the filter paper from the funnel using the tweezers (5.17), and insert it in an extraction thimble (5.5) so that the top edge of the paper is at least 20 mm below the rim. Place the thimble in a 100 ml beaker (5.12).

7.5.6 Heat the beaker and its contents, and the conical flask with glass rod, in the drying oven (5.14), set at 102 °C, for 1 h to 1,5 h to dry them thoroughly. Remove the beaker and flask with glass rod from the oven and allow to cool.

The filter paper should be thoroughly dry, since otherwise the fat tends to be incompletely extracted. In the case of a very wet filter paper and a continuous extractor, with water-soluble compounds, drops of water may enter the extract, thus causing a dark colour of the extract and high values for the fat content.

7.5.7 Holding the thimble with the tweezers (5.17), loosely plug it with defatted cotton wool (4.7) and then place it in the extractor. Measure 100 ml of *n*-hexane or light petroleum (4.2) in a measuring cylinder; use portions of the solvent to rinse the tips of the tweezers, the inside of the beaker and the conical flask and glass rod, collecting the rinsings in the prepared extraction flask (see 7.4). Add the remainder of the solvent to the extraction flask so as to rinse the inside of the neck of the flask.

7.5.8 Connect the extraction flask to the extractor containing the thimble, connect the extractor to the reflux condenser and heat the flask for approximately 4 h in such a way that the thimble and its contents are extracted with at least 1 000 ml of the solvent (20 siphonings).

7.5.9 Remove the extraction flask from the extraction apparatus, and rinse the inside of the neck of the flask and the tip of the condenser with a little solvent. Then cautiously distil all the solvent from the flask. If a water bath is used, wipe the outside of the flask carefully to remove any adhering water.

7.5.10 Heat the extraction flask (placed on its side to allow solvent vapour to escape) in the drying oven (5.14), set at 102 °C, for 1 h. Remove the flask from the oven, allow to cool (not in a desiccator, but protected from dust) to the temperature of the balance room (for at least 0,5 h) and weigh to the nearest 0,1 mg. Do not wipe the flask immediately before weighing. Place the flask on the balance using tongs (to avoid, in particular, temperature variations).

7.5.11 Repeat the operations described in 7.5.10 until the mass of the flask decreases by 1,0 mg or less, or increases, between two successive weighings. Record the minimum mass as the mass of the flask and extracted matter.

8 Calculation and expression of results

The fat content, w , expressed as a mass fraction in percent, is equal to

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

where

m_0 is the mass, in grams, of the test portion (7.2);

m_1 is the mass, in grams, of the extraction flask and extracted matter determined in 7.5.11;

m_2 is the mass, in grams, of the prepared flask (see 7.4);

m_3 is the mass, in grams, of the extraction flask used in the blank test (7.3) and any extracted matter determined as in 7.5.11;

m_4 is the mass, in grams, of the prepared flask (see 7.4) used in the blank test (7.3).

Report the result to the nearest 0,01 % (mass fraction).

9 Precision

9.1 Interlaboratory test

The values for repeatability and reproducibility are expressed at the 95 % probability level and were derived from the results of an interlaboratory trial carried out in accordance with ISO 5725¹⁾.

9.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than the following values:

- for products having a fat content of more than 20 % (mass fraction):
1 % of the fat content;
- for products having a fat content of more than 5 % and up to and including 20 % (mass fraction):
0,2 g of fat per 100 g of product;
- for products having a fat content of 5 % (mass fraction) or less:

1) ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn).

0,1 g of fat per 100 g of product;

— for liquid products having a fat content of more than 5 % (mass fraction):

1 % of the fat content;

— for liquid products having a fat content of 5 % (mass fraction) or less:

0,05 g of fat per 100 g of product.

9.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the following values:

— for products having a fat content of more than 20 % (mass fraction):

2 % of the fat content;

— for products having a fat content of more than 5 % and up to and including 20 % (mass fraction):

0,4 g of fat per 100 g of product;

— for products having a fat content of 5 % (mass fraction) or less:

0,2 g of fat per 100 g of product;

— for liquid products having a fat content of more than 5 % (mass fraction):

2 % of the fat content;

— for liquid products having a fat content of 5 % (mass fraction) or less:

0,1 g of fat per 100 g of product.

10 Notes on procedure

10.1 Blank tests to check the solvent and filter papers

In these blank tests, a vessel for mass control purpose shall be used in order that changes in the atmospheric condition of the balance room or temperature effects of the fat-collecting vessel will not falsely suggest the presence or absence of non-volatile matter in the extract of the reagent. This vessel may be used as a counterweight vessel in the case of a two-pan balance. Otherwise, deviations of the apparent mass $[(m_3 - m_4)]$ in the formula in Clause 8] of the control vessel shall be considered when checking the mass of the fat-collecting vessel used for the blank test. Hence the change in apparent mass of the fat-collecting vessel, corrected for the apparent change in mass of the control vessel, shall not exceed 0,5 mg.

Very occasionally, the solvent may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out a blank test using a fat-collecting vessel with about 1 g of fresh anhydrous butterfat. If necessary, distil the solvent in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Only use the solvent shortly after distillation.

10.2 Blank test carried out simultaneously with the determination

The value obtained in the blank test, carried out simultaneously with the determination, enables the apparent mass of substances extracted from a test portion ($m_1 - m_2$) to be corrected for the presence of any non-volatile matter derived from the reagents and the filter paper and also for any change in atmospheric conditions of the balance room and any temperature difference between the fat-collecting vessel and the balance room at the two weighings (7.4 and 7.5.11).

Under favourable conditions (low value in the blank test on solvent and filter paper, equable temperature of the balance room, sufficient cooling time for fat-collecting vessel), the value will usually be less than 3 mg. Slightly higher values, up to 5 mg, are also often encountered. After correction for these values, the results will still be accurate. When corrections for a value of more than 5 mg are applied, this fact should be mentioned in the test report (Clause 11).

If the value obtained in this blank test regularly exceeds 3 mg, the solvent and filter paper should be checked (if this has not been recently done) and should be replaced or purified (see 4.2 and 4.3).

11 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this part of ISO 8262 | IDF 124;
- d) all operating details not specified in this part of ISO 8262 | IDF 124, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the test result(s) obtained or, if the repeatability has been checked, the final quoted result obtained;
- f) the blank value [$(m_3 - m_4)$, see Clause 8] if it exceeds 5 mg.

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