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

## Part 1 :
## Infant foods

*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 1 : Aliments pour enfants en bas âge*



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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.

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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 8262-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, in collaboration with the International Dairy Federation (IDF) and the Association of Analytical Chemists (AOAC) and will also be published by these organizations.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.





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

# Part 1 : Infant foods



## 0 Introduction

This International Standard 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 either the Röse-Gottlieb (RG), or the Weibull-Berntrop (WB) or the Schmid-Bondzynski-Ratzlaff (SBR) principle.

For this part of ISO 8262, dealing with milk-based and other types of infant foods containing more than 5 % ($m/m$) (in the dry matter) of starch or dextrin, or vegetable, fruit, meat, etc., a method based on the WB principle has been chosen because

a) owing to the high level of the above ingredients, which causes incomplete extraction of the fat and thus gives too low values for the fat content, the RG procedure is not suitable;

b) owing to the generally high content of carbohydrates, which gives rise to ether-extractable compounds in the digestion with acid and thus to too high values for the fat content, the SBR procedure is not suitable;

c) the WB procedure, although also applying an acid digestion, is not adversely affected by these ether-extractable compounds, as 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 and is recommended by the Codex Committee on Methods of Analysis and Sampling.

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 products.

## 1 Scope and field of application

This part of ISO 8262 specifies the reference method for the determination of the fat content of infant foods to which the Röse-Gottlieb method is not applicable, viz. those milk-based and other types of infant foods that contain more than 5 % ($m/m$) (in the dry matter) of starch or dextrin, or vegetable, fruit, meat, etc.

NOTE — Other milk-based infant foods should be examined by the method utilizing the RG principle given in ISO 8381 [1]. (Malto-dextrins without higher molecular dextrins, which are often present in infant foods, do not disturb the RG extraction even when present in high percentages.)

The method is also applicable if the product contains free fatty acids in significant quantities or if hard lumps that do not dissolve completely in ammonia are present in the product.

## 2 Reference

ISO 707, *Milk and milk products — Methods of sampling*.

## 3 Definition

**fat content**: All the substances determined by the method specified in this part of ISO 8262.

It is expressed as a percentage by mass.

## 4 Principle

Digestion of a test portion by boiling with dilute hydrochloric acid, filtration of the hot digest through a wetted filter paper to retain fatty substances, extraction of the fat from the dried filter paper using *n*-hexane or light petroleum, removal of the solvent by distillation or evaporation and weighing of the substances extracted. (This is usually known as the Weibull-Berntrop principle.)

1) ISO 8381, *Milk-based infant foods — Determination of fat content — Röse-Gottlieb gravimetric method (Reference method).*

## 5 Reagents and materials

All reagents shall be of recognized analytical grade and shall leave no appreciable residue when the determination is carried out by the method specified. The water used shall be distilled water or water of at least equivalent purity.

**5.1 Hydrochloric acid** solution, containing approximately 20 % ($m/m$) of HCl, $\varrho_{20}$ approximately 1,10 g/ml.

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

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

To test the quality of the extraction solvent, distil 100 ml of it from an extraction flask (6.4) prepared as specified in 8.4. Use an empty extraction flask, prepared in the same way, for mass control purposes (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.

**5.3 Filter papers**, of diameter 150 mm, pleated, medium grade, preferably de-fatted.

To test the quality of the filter paper, carry out a blank test as specified in 8.3, using a solvent satisfying the requirement of 5.2. Use an empty extraction flask (6.4), prepared as specified in 8.4, for mass control purposes (see 10.1). The paper shall leave no residue greater than 2,5 mg.

Replace unsatisfactory filter papers.

**5.4 Blue litmus paper.**

**5.5 Diatomaceous earth** (optional; see 8.5.3).

**5.6 Pure lactose** (optional; see 8.5.3).

**5.7 Cotton wool**, de-fatted by extraction with the solvent (5.2) for 1,5 h and dried.

## 6 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

**6.1 Analytical balance.**

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

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

**6.4 Extraction flasks**, of capacity 150 ml, flat-bottomed, short-necked.

**6.5 Extraction thimbles**, made of de-fatted filter paper, glass, alumina or PTFE[1], 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 (6.3).

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

40 to 60 °C (see 8.1.1);

30 to 40 °C (see 8.1.2).

**6.7 Heating apparatus**, for the extraction apparatus; for example a water bath, a sand bath or a thermostatically controlled hotplate.

**6.8 Boiling aids**, fat-free: glass beads or pieces of non-friable, non-porous porcelain or silicon carbide.

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

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

**6.11 Filter funnel**, suitable for use with the pleated filter paper (5.3).

**6.12 Beakers with spout**, of capacities 100 and 250 ml.

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

**6.14 Drying oven**, electrically heated, with ventilation port(s) fully open, capable of being maintained at a temperature of 102 ± 2 °C throughout the working space. The oven shall be fitted with a suitable thermometer.

**6.15 Measuring cylinders**, of capacities 50, 100 and 250 ml.

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

1) Polytetrafluoroethylene.

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

## 7 Sampling

See ISO 707.

All liquid, viscous or pasty laboratory samples shall be kept at a temperature of 2 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.

## 8 Procedure

### 8.1 Preparation of the test sample

#### 8.1.1 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 in an appropriate blender (6.2). 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 (6.6) at 40 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.

#### 8.1.2 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 (6.2). 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 (6.6) at 30 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.

#### 8.1.3 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 in an appropriate blender (6.2).

### 8.2 Test portion

Mix the test sample (8.1) 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 (6.9), directly or by difference, to the nearest 1 mg, 3 to 20 g of the test sample, corresponding to 3,0 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 (6.9).

### 8.3 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 8.5.1) by 25 ml of water (see 10.2).

### 8.4 Preparation of extraction flask

Dry a flask (6.4) containing a few boiling aids (6.8), to promote gentle boiling during the extraction and subsequent removal of solvent, in the oven (6.14), controlled at 102 ± 2 °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.

NOTE — The flask should not be placed in a desiccator, to avoid insufficient cooling or unduly long cooling times.

Using tongs (6.16) (to avoid, in particular, temperature variations), place the flask on the balance and weigh to the nearest 0,1 mg.

### 8.5 Determination

**8.5.1** Add water at 30 °C to the test portion (8.2) to give a total volume of 25 ml (in order to obtain a 4 mol/l hydrochloric acid solution in 8.5.2) and shake gently.

NOTE — For the optional addition of lactose, see the note to 8.5.3.

**8.5.2** Add 50 ml of the hydrochloric acid solution (5.1) 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.

**8.5.3** Rinse the inside of the condenser with about 75 ml of a portion of 150 ml of hot water (at 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 (5.5) or

approximately 100 $cm^2$ of de-fatted filter paper, torn into pieces, to promote rapid filtration.

NOTE — The filtration can also be improved by adding 1 g of pure lactose (5.6) to the diluted test portion in 8.5.1.

**8.5.4** Immediately filter the contents of the flask, pouring the liquid down a glass rod, through a pleated filter paper (5.3) thoroughly wetted with hot water, placed in the filter funnel (6.11). 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 (5.4). Use not more than 400 ml of water. Allow the filter paper to drain well.

**8.5.5** Remove the filter paper from the funnel using the tweezers (6.17), and insert it in an extraction thimble (6.5) so that the top edge of the paper is at least 20 mm below the rim. Place the thimble in a 100 ml beaker (6.12).

**8.5.6** Heat the beaker and its contents, and the conical flask with glass rod, in the drying oven (6.14), controlled at 102 ± 2 °C, for 1 to 1,5 h to dry them thoroughly. Remove the beaker and flask with glass rod from the oven and allow to cool.

NOTE — 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, drops of water with water-soluble compounds may enter the extract, thus causing a dark colour of the extract and high values for the fat content.

**8.5.7** Holding the thimble with the tweezers (6.17), loosely plug it with de-fatted cotton wool (5.7) and then place it in the extractor. Measure 100 ml of *n*-hexane or light petroleum (5.2) 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 8.4). Add the remainder of the solvent to the extraction flask so as to rinse the inside of the neck of the flask.

**8.5.8** 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).

**8.5.9** 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.

**8.5.10** Heat the extraction flask (placed on its side to allow solvent vapour to escape) in the drying oven (6.14), controlled at 102 ± 2 °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).

**8.5.11** Repeat the operations described in 8.5.10 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.

## 9 Expression of results

### 9.1 Method of calculation and formula

The fat content, expressed as a percentage by mass, is equal to

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

where

$m_0$ is the mass, in grams, of the test portion (8.2);

$m_1$ is the mass, in grams, of the extraction flask and extracted matter determined in 8.5.11;

$m_2$ is the mass, in grams, of the prepared flask (see 8.4);

$m_3$ is the mass, in grams, of the extraction flask used in the blank test (8.3) and any extracted matter determined as in 8.5.11;

$m_4$ is the mass, in grams, of the prepared flask (see 8.4) used in the blank test (8.3).

Report the result to the nearest 0,01 %.

### 9.2 Precision

NOTE — The values for repeatability and reproducibility are expressed at the 95 % probability level and were derived from the results of an inter-laboratory trial carried out on infant foods in accordance with ISO 5725[1].

#### 9.2.1 Repeatability

The difference between two single results found on identical test material by one analyst within a short time interval should not exceed the following values:

— for products having a fat content of more than 5 % (*m*/*m*):

0,2 g of fat per 100 g of product

— for products having a fat content of 5 % (*m*/*m*) or less:

0,1 g of fat per 100 g of product

— for liquid products:

0,05 g of fat per 100 g of product

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