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**INTERNATIONAL STANDARD**



**2450**

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INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

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**Cream – Determination of fat content** <sup>R</sup> (reference method)

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## FOREWORD

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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.

International Standard ISO 2450 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*.

It was approved in November 1971 by the Member Bodies of the following countries :

Australia	Germany	New Zealand
Austria	Hungary	Poland
Belgium	India	Portugal
Brazil	Iran	South Africa, Rep. of
Chile	Israel	Spain
Czechoslovakia	Korea, Dem. P. Rep. of	Turkey
Egypt, Arab Rep. of	Malaysia	United Kingdom
France	Netherlands	U.S.S.R.

No Member Body expressed disapproval of the document.

NOTE – This International Standard has been developed jointly with the IDF (International Dairy Federation) and the AOAC (Association of Official Analytical Chemists, USA) for the purpose of being included in the FAO/WHO Code of Principles concerning Milk and Milk Products.

The text as approved by the above organizations was also published by FAO/WHO (Code of Principles, Standard No. B-15), by the IDF (IDF Standard 16A) and by the AOAC (Official Methods of Analysis, 12th Edition, No. 16.1).

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Delete the last line of the note, and substitute :

“Methods of Analysis, 12th Edition, No. 16.138).”

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# Cream – Determination of fat content (reference method)

## 1 SCOPE AND FIELD OF APPLICATION

### 1.1 Scope

This International Standard specifies a reference method for the determination of the fat content of cream.

### 1.2 Field of application

The method is suitable for raw cream, processed cream and cultured cream.

## 2 REFERENCE

ISO/R 707, *Milk and milk products – Sampling*.

## 3 DEFINITION

By the **fat of cream** is meant the substances extracted by the procedure described.

The fat content is expressed as a percentage by mass.

## 4 PRINCIPLE

Extraction of the fat from an ammoniacal ethanolic solution of cream with diethyl ether and light petroleum, evaporation of the solvents and weighing of the residue. (Commonly known as the Röse-Gottlieb method.)

## 5 REAGENTS

All reagents shall be of analytical reagent quality and shall leave no residue greater than that permitted for the blank test (see 8.2). If necessary, solvents may be redistilled in the presence of about 1 g of butterfat per 100 ml of solvent. Water used shall be distilled water or water of at least equivalent purity.

**5.1 Ammonia**, approximately 25 % (m/m) solution of  $\text{NH}_3$  ( $\rho_{20}$  approximately 0,91 g/ml), or a stronger solution of known concentration.

**5.2 Ethanol**, 94 to 97 % (V/V) or, if not available, ethanol denatured with one of the following: methanol, ethyl methyl ketone, benzene or light petroleum.

1) 1 mbar = 0,1 kPa.

**5.3 Diethyl ether**, peroxide-free.

### NOTES

1 To test for peroxides, add to 10 ml of the ether in a small glass-stoppered cylinder previously rinsed with the ether, 1 ml of freshly prepared 10 % potassium iodide solution. Shake and let stand for 1 min. No yellow colour should be observed in either layer.

2 Diethyl ether may be freed and maintained free from peroxides by adding wet zinc foil that has previously been immersed completely in dilute acidified copper sulphate solution for 1 min and then washed in water. Approximately 80 cm<sup>2</sup> of zinc foil should be used per litre and it should be cut in strips long enough to reach at least half-way up the container.

**5.4 Light petroleum** (petroleum ether), with any boiling range between 30 and 60 °C.

**5.5 Mixed solvent**, prepared shortly before use by mixing equal volumes of diethyl ether (5.3) and light petroleum (5.4).

NOTE Where mixed solvent is specified, the diethyl ether or the light petroleum may be used alone instead.

**5.6 Sodium chloride** (NaCl) solution, 5 g/l.

## 6 APPARATUS

**6.1 Analytical balance.**

**6.2 Suitable extraction apparatus**, provided with ground glass stoppers, good quality bark corks, or other closures unaffected by the reagents used.

Treat bark corks by extracting successively with diethyl ether and light petroleum. Then keep these corks for at least 20 min in water at 60 °C or above, and cool in the water so that they are saturated when used.

**6.3 Thin-walled, flat-bottomed flasks**, of 150 to 250 ml capacity.

**6.4 Drying oven**, well ventilated, thermostatically controlled and adjusted to operate at  $102 \pm 2$  °C,

or

**vacuum drying oven**, temperature 70 to 75 °C, pressure less than 66 mbar (50 mmHg).<sup>1)</sup>

**6.5 Material to facilitate boiling**, fat-free, non-porous, non-friable in use, for example glass beads or pieces of silicon carbide.

NOTE — The use of this material is optional (see 8.3).

**6.6 Centrifuge**, in which the extraction apparatus (6.2) can be spun at 500 to 600 rev/min.

NOTES

- 1 The use of a centrifuge is optional (see 8.5.2).
- 2 When a centrifuge not provided with a three-phase motor is used, sparks may occur and care is therefore necessary to avoid explosion or fire due to the occurrence of solvent vapour following possible breakage of apparatus.

**7 SAMPLING**

Carry out the sampling according to the appropriate method specified in ISO/R 707.

**8 PROCEDURE**

**8.1 Preparation of the sample**

Bring the sample to a temperature of 20 °C. Mix or stir the cream thoroughly, but not so vigorously as to cause frothing or churning. If the cream is very thick, warm to 30 to 40 °C to facilitate mixing and then cool the sample quickly to room temperature. In order to reduce evaporation of water to a minimum during mixing, the container should be uncovered for as short a time as possible.

Correct 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.

**8.2 Blank test**

At the same time as the determination of the fat content of the sample, perform a blank test on 10 ml of distilled water using the same type of extraction apparatus, the same reagents in the same amounts and the same procedure as described in 8.3 and 8.5. If the result of the blank determination exceeds 0,000 5 g the reagents shall be checked and the impure reagent or reagents shall be purified or replaced.

**8.3 Preparation of flask**

Dry a flask (6.3) (if desired, with some material (6.5) to promote gentle boiling during the subsequent removal of the solvent) in the oven (6.4) for 30 to 60 min. Allow the flask to cool to the temperature of the balance room and then weigh it to the nearest 0,000 1 g.

**8.4 Test portion**

Mix or stir the prepared sample and immediately weigh to the nearest 0,001 g directly in, or by difference into, the extraction apparatus (6.2) a quantity of the sample (depending on the fat content of the cream) to give 0,3 to 0,6 g of extracted fat.

**8.5 Determination**

**8.5.1** Add to the test portion 8 ml of sodium chloride solution (5.6) and mix carefully.

Add 1,5 ml of the ammonia solution (25 % *m/m*), or an equivalent volume of a stronger solution (5.1), and mix well.

Add 10 ml of the ethanol (5.2) and mix the liquids gently but thoroughly in the unclosed apparatus.

**8.5.2** Add 25 ml of the diethyl ether (5.3), close the apparatus with a moistened stopper and shake vigorously and invert repeatedly for 1 min. Cool, if necessary, in running water. Remove the stopper carefully and add 25 ml of the light petroleum (5.4), using the first few millilitres to rinse the stopper and the inside of the neck of the apparatus, and allowing the rinsings to run into the apparatus.

Close the apparatus by replacing the stopper, and shake and invert repeatedly for 30 s. Do not shake too vigorously if centrifuging is not be used. Allow the apparatus to stand until the upper liquid layer has become clear and is distinctly separated from the aqueous layer. Alternatively perform the separation by the use of a suitable centrifuge (6.6).

**8.5.3** Remove the stopper, rinse it and the inside of the neck of the apparatus with a few millilitres of mixed solvent (5.5) and allow the rinsings to run into the apparatus. Carefully transfer as much as possible of the supernatant layer by decantation or by means of a siphon into the flask (see 8.3 and section 10).

Rinse the outside and the inside of the neck of the apparatus, or the tip and the lower part of the siphon, with a few millilitres of the mixed solvent. Allow the rinsings from the outside of the apparatus to run into the flask and the rinsings from the inside of the neck or from the siphon to run into the extraction apparatus.

NOTE — When siphon tubes are used, the supernatant liquid may then be transferred, without further shaking, to the flask and the operations of rinsing and transference repeated.

**8.5.4** Make a second extraction by repeating the procedure described in 8.5.2 and 8.5.3 (including the rinsings) but using only 15 ml of the diethyl ether and 15 ml of the light petroleum.

**8.5.5** Make a third extraction by the procedure used for the second extraction (see 8.5.4) but omitting the final rinsings.

**8.5.6** Carefully evaporate or distil off as much solvent (including the ethanol) as possible. If the flask is of small capacity, some of the solvent will need to be removed in this manner after each extraction.

When there is no longer any solvent odour, heat the flask, placed on its side, for 1 h in the oven (6.4). Allow the flask to cool to the temperature of the balance room as before (see 8.3), and weigh to the nearest 0,000 1 g. Repeat the operations of heating for periods of 30 to 60 min, cooling and weighing until there is no further decrease in mass.

**8.5.7** Add 15 to 25 ml of the light petroleum in order to verify whether or not the extracted matter is wholly soluble. Warm gently and swirl the solvent until all the fat is dissolved.

**8.5.7.1** If the extracted matter is wholly soluble in the light petroleum, the mass of fat is the difference between the final mass of the flask containing the extract and its initial mass (see 8.3).

**8.5.7.2** If the extracted matter is not wholly soluble in the light petroleum, or in case of doubt and always in case of dispute, extract the fat completely from the flask by repeated washing with warm light petroleum, allowing the undissolved material to settle before each decantation. Rinse the outside of the neck of the flask three times. Heat the flask, placed on its side, for 1 h in the oven (6.4), allow to cool to the temperature of the balance room as before (see 8.3) and weigh to the nearest 0,000 1 g. The mass of fat is the difference between the mass of the flask containing the total extract and the final mass.

**8.5.8** Carry out two determinations on the same prepared sample.

## 9 EXPRESSION OF RESULTS

### 9.1 Method of calculation and formula

If A represents the flask used for the extraction of the fat from the cream, and

B represents the flask used for the blank test,

then the fat content of the sample, 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;

$m_1$  is the mass, in grams, of flask A and fat after heating to constant mass;

$m_2$  is the mass, in grams, of flask A after the first heating (see 8.3) or, in the case of undissolved material, after the final heating;

$m_3$  is the mass, in grams, of flask B after heating to constant mass;

$m_4$  is the mass, in grams, of flask B after the first heating (see 8.3) or, in the case of undissolved material, after the final heating.

Take as the result the arithmetic mean of two determinations, if the requirement of repeatability (see 9.2) is satisfied.

### 9.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 0,2 g of fat per 100 g of the product.

## 10 NOTE ON PROCEDURE

If the transfer is made by decantation it may be necessary to add a little water to raise the interface between the two layers in order to facilitate the decantation.

## 11 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The report shall include all details required for the complete identification of the sample.

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