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**INTERNATIONAL STANDARD**



**1735**

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

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**Cheese and processed cheese products — Determination of fat content (Reference method)**

*Fromages et fromages fondus — Détermination de la teneur en matière grasse (Méthode de référence)*

First edition — 1975-12-15

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UDC 637.32 : 543.85

Ref. No. ISO 1735-1975 (E)

**Descriptors** : agricultural products, animal products, dairy products, cheeses, chemical analysis, determination of content, fats.

## FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO Member Bodies). The work of developing International Standards is carried out through ISO Technical Committees. Every Member Body interested in a subject for which a Technical Committee has been set up 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.

Prior to 1972, the results of the work of the Technical Committees were published as ISO Recommendations; these documents are now in the process of being transformed into International Standards. As part of this process, Technical Committee ISO/TC 34 has reviewed ISO Recommendation R 1735 and found it technically suitable for transformation. International Standard ISO 1735 therefore replaces ISO Recommendation R 1735-1970 to which it is technically identical.

ISO Recommendation R 1735 was approved by the Member Bodies of the following countries :

Australia	Hungary	Portugal
Belgium	India	Romania
Brazil	Iran	South Africa, Rep. of
Chile	Israel	Sweden
Colombia	Korea, Rep. of	Switzerland
Czechoslovakia	Netherlands	Thailand
Egypt, Arab Rep. of	New Zealand	Turkey
France	Norway	United Kingdom
Germany	Peru	U.S.S.R.
Greece	Poland	

No Member Body expressed disapproval of the Recommendation.

No Member Body disapproved the transformation of ISO/R 1735 into an International Standard.

# Cheese and processed cheese products — Determination of fat content (Reference method)

## 1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the fat content of cheese and of processed cheese products.

## 2 REFERENCE

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

## 3 DEFINITION

For the purpose of this International Standard, the following definition applies :

**fat content of cheese and of processed cheese products :**  
The substances extracted by the procedure specified.

The fat content is expressed as a percentage by mass.

## 4 PRINCIPLE

Digestion of cheese with hydrochloric acid, addition of ethanol and subsequent extraction of the fat by means of diethyl ether and light petroleum, evaporation of the solvents and weighing of the residue (commonly known as the Schmid-Bondzynski-Ratzlaff 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 Hydrochloric acid** solution, approximately 25 % (m/m) ( $\rho_{20}$  1,125 g/ml).

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

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

### NOTES

1 To test for peroxides, add to 10 ml of the diethyl 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 been completely immersed 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.

## 6 APPARATUS

### 6.1 Analytical balance

**6.2 Suitable extraction tubes or flasks**, provided with ground glass stoppers, bark corks of good quality, or other closures unaffected by the solvents used.

Treat bark corks by extracting successively with diethyl ether and light petroleum. Then keep 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, thermostically controlled and adjusted to operate at  $102 \pm 2$  °C,

or

**vacuum drying oven**, temperature 70 to 75 °C, and pressure less than 66 mbar (50 mmHg).

**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 Water bath.**

**6.7 Sheets of cellulose film**, unlacquered, soluble in hydrochloric acid, 0,03 to 0,05 mm thick and about 50 mm X 75 mm in area. The cellulose films shall not affect the result of the analysis.

**6.8 Appropriate grinding device**, easy to clean, for grinding the sample of cheese.

**6.9 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 using a centrifuge not provided with a three-phase motor, sparks may occur and care is therefore necessary to avoid explosion or fire due to the occurrence of solvent vapour following breakage of apparatus.

## 7 SAMPLING<sup>1)</sup>

See ISO/R 707.

## 8 PROCEDURE

### 8.1 Preparation of the sample<sup>1)</sup>

Before the analysis, remove the rind or smear or mouldy surface layer of the cheese in such a way as to obtain a sample representative of the cheese as it is usually consumed.

Grind the sample by means of the appropriate device (6.8). Quickly mix the ground mass and, if possible, grind it a second time and again mix thoroughly. Clean the device after grinding each sample. If the sample cannot be ground, mix it thoroughly by intensive kneading.

Keep the prepared sample in an air-tight container until the time of the analysis, which should be carried out on the same day. If delay is unavoidable, take every precaution to ensure proper conservation of the sample and to prevent condensation of moisture on the inside surface of the container.

### 8.2 Blank test

At the same time as the determination of the fat content of the sample, perform a blank determination 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 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 solvents) 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

Weigh to the nearest 0,001 g directly in, or by difference into, the extraction apparatus (6.2) or a 100 ml beaker or flask, 1 to 3 g of the prepared sample of cheese (3 g for cheeses with fat content up to 30 % by mass in the product; 1 to 3 g for cheeses with higher fat content, yielding 750 to 1 000 mg of fat). The test portion may also be weighed on a sheet of cellulose film (6.7), which is subsequently folded and introduced into the vessel of the type chosen.

### 8.5 Determination

**8.5.1** Add 8 to 10 ml, depending on the form of the extraction apparatus, of the hydrochloric acid (5.1). Gently move the vessel in a boiling-water bath or over a flame until the cheese is completely dispersed. Let the vessel stand for 20 min in the boiling-water bath and then cool, for example in running water.

**8.5.2** If the digestion of the cheese has been carried out in the extraction flask, add 10 ml of ethanol (5.2) and mix the contents gently but thoroughly in the unclosed apparatus.

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, allowing the rinsings to run into the apparatus. Close by replacing the stopper and shake and invert repeatedly for 30 s.

If the digestion of cheese has been carried out in a vessel other than the extraction flask, pour the contents of the vessel into the extraction flask. Rinse the vessel successively with 10 ml of ethanol (5.2), 25 ml of diethyl ether (5.3) and 25 ml of light petroleum (5.4), each time pouring the solvent into the extraction flask. Mix after each addition, and shake the extraction flask as indicated above.

Do not shake too vigorously if centrifuging is not to 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 (6.9).

1) The technique to be used for each particular type of cheese should be indicated in the individual international cheese standards being prepared under the FAO/WHO Code of Principles concerning Milk and Milk Products.

**8.5.3** Remove the stopper, rinsing 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 clause 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 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 rinsing(s)) but using only 15 ml of diethyl ether and 15 ml of 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 rinsing(s).

**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 light petroleum in order to verify that 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 a 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, 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 extraction of the fat, and B represents the flask used for the blank test, then the fat content of the sample, expressed as a percentage by mass, is given by the formula

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

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

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