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Butter, edible oil emulsions and spreadable fats — Determination of fat content (Reference method)

Beurre, émulsions d'huile alimentaire et matières grasses tartinables — Détermination de la teneur en matière grasse (Méthode de référence)

Reference numbers
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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

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Foreword

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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 17189 | IDF 194 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

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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 and AOAC International 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 IDF National Committees casting a vote.

International Standard ISO 17189│IDF 194 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Fat*, of the Standing Committee on *Main components in milk*, under the aegis of its project leader, Mr J.M. Evers (NZ).

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Butter, edible oil emulsions and spreadable fats — Determination of fat content (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 a method for the determination of the fat content of butter, edible oil emulsions (2.2) and spreadable fats (margarine, vegetable oil spreads, dairy spreads and blended spreads).

2 Terms and definitions

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

2.1
fat content
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.

2.2
edible oil emulsion
high fat product (> 75 % fat) having the same constituents as butter but a composition that does not meet the Codex definition for butter

NOTE Reduced fat butters (e.g. 3/4 fat, 1/2 fat) are considered to belong to the class of spreadable fats.

3 Principle

Fat is extracted from the test portion using a specified solvent. The solvent/fat phase is separated from the aqueous phase and transferred quantitatively to a fat-collecting vessel. The solvent is removed by distillation or evaporation and the mass of substances extracted is determined.

4 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 upon evaporation when the determination is carried out by the method specified (see 8.1.2).

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4.1 Extraction solvent, petroleum ether, with any boiling range between 30 °C and 60 °C, or, as equivalent, *n*-hexane [$CH_3(CH_2)_4CH_3$], with a boiling point of 69 °C, both complying with the requirements for the extraction solvent blank test (8.1.2).

4.2 Ethanol (C_2H_5OH), of concentration at least 94 % (volume fraction).

4.3 Congo-red solution

Dissolve 1 g of Congo-red in about 50 ml of water in a 100 ml one-mark volumetric flask. Make up to the mark with water.

WARNING — Take appropriate safety precautions when handling Congo-red solid as this chemical may be a carcinogen.

NOTE The use of this solution, which helps the analyst to better see the interface between the solvent layer and the aqueous layer, is optional (see 8.4.1). Other aqueous colour indicators may be used provided they do not affect the fat result.

5 Apparatus

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

Usual laboratory equipment and, in particular, the following.

5.1 Analytical balance, with a readability of 0,1 mg.

5.2 Drying oven, electrically heated, ventilated, thermostatically controlled, capable of maintaining a temperature of 102 °C ± 2 °C throughout its working space. The oven shall be fitted with a suitable thermometer.

5.3 Desiccator, containing a suitable drying agent, for example, freshly dried silica gel with hygrometric indicator.

If the method is used purely to obtain a routine result, that is, where high accuracy and precision are not required, then the fat-collecting vessels may be cooled to the temperature of the weighing room on the laboratory bench protected from dust.

5.4 Fat-collecting vessels, such as glass boiling flasks with ground necks, of capacity 125 ml, or metal dishes.

When using metal dishes, it is recommended to use dishes with relatively high walls (e.g. 6 cm). This will reduce the risk of fat loss through splashing of the solvent during solvent transfer from the centrifuge tube to the fat-collecting vessel, or through vigorous boiling during the evaporation of the solvent.

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

5.6 Tongs, made of metal, or **cotton gloves**, for holding the fat-collecting vessels (5.4).

5.7 Leak-proof centrifuge tubes, with screw cap, of capacity 50 ml, of plastic which is resistant to the solvent (4.1) for at least the duration of the test.

NOTE Tubes having a large opening (e.g. 25 mm to 35 mm) are preferred to facilitate the addition of the test portion.

5.8 Vortex mixer

5.9 Centrifuge, capable of holding the leak-proof centrifuge tubes (5.7) and capable of producing a radial acceleration of 50 *g* to 100 *g* at the outer end of the tubes.

NOTE The use of the centrifuge is optional but recommended (see 8.4.3).

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5.10 Automatic pipettor, or other suitable liquid-transfer apparatus (e.g. of capacity 5 ml), for quantitative transfer of the solvent/fat phase.

5.11 Distillation or evaporation apparatus (e.g. steam bath), for distilling or evaporating the solvent from the fat-collecting vessels (see 8.4.8).

5.12 Solvent dispenser or **measuring cylinders**, of capacity 10 ml and 20 ml.

6 Sampling

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

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

The test sample shall be received in an airtight container. Its capacity shall be such that one-half to two-thirds is filled by the sample. Store the samples in the closed container at a temperature of between 5 °C and 14 °C until commencing the preparation of the test sample.

7 Preparation of test sample

7.1 Warm the test sample in the original unopened container to a temperature at which the sample will be soft enough to facilitate thorough mixing to a homogeneous state (either by a mechanical shaker or by hand) without any rupture of the emulsion. The temperature shall normally not exceed 35 °C for samples of butter and edible oil emulsions, or 30 °C in the case of spreadable fat samples.

7.2 Where applicable, cool the test sample to ambient temperature with mixing until cooling is complete. As soon as possible after cooling, open the sample container and stir briefly for no longer than 10 s with a suitable device, e.g. a spoon or spatula, before weighing.

8 Procedure

8.1 Blank tests

8.1.1 Blank test for method

Simultaneously with the determination of the test portion (see 8.4), carry out a blank test using the same procedure for preparation of the fat-collecting vessel (see 8.2), but without weighing of the test portion (see 8.3) and the addition of the Congo-red solution (see 8.4.1) (i.e. add solvents only).

8.1.2 Blank test for extraction solvent

To test the quality of the extraction solvent (4.1), evaporate 60 ml of the solvent from a prepared empty fat-collecting vessel (see 8.2). Additionally, use another prepared empty fat-collecting vessel (see 8.2) for mass control purposes. The extraction solvent shall leave no residue greater than 1,0 mg (see Annex A). Replace or redistil unsatisfactory extraction solvents.

8.2 Preparation of fat-collecting vessel

8.2.1 Dry the empty fat-collecting vessel (5.4) with a few boiling aids (5.5) in the drying oven (5.2) set at 102 °C for at least 30 min.

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8.2.2 Allow the fat-collecting vessel to cool in the desiccator (5.3) to the temperature of the weighing room and weigh the vessel. Record the mass of the fat-collecting vessel with the boiling aids to the nearest 0,1 mg.

The length of the cooling time will depend on the number of fat-collecting vessels and the size of the desiccator used. Ensure that the length of the cooling time used for the empty fat-collecting vessel is practically the same as that for the fat-collecting vessel containing the extracted fat.

8.3 Preparation of test portion

8.3.1 Butter and edible oil emulsions

Weigh 4 g to 6 g of the test sample (see 7.2) into the leak-proof centrifuge tube (5.7). If gravity separation of the phases is used (see 8.4.3), weigh 2 g to 3 g of the test sample into the leak-proof centrifuge tube. In both cases record the mass of the test portion to the nearest 0,1 mg.

8.3.2 Spreadable fats

Weigh 1 g to 2 g of the test sample (see 7.2) into the leak-proof centrifuge tube (5.7). Record the mass of the test portion to the nearest 0,1 mg.

8.4 Determination

8.4.1 Add 20 ml of extraction solvent (4.1) and one drop of the Congo-red solution (4.3) to the test portion (see 8.3.1 or 8.3.2) in the leak-proof centrifuge tube. Firmly screw the cap on the centrifuge tube.

NOTE 1 The number of extractions and the volume of solvent required for the various extractions depend on the type of product and the means used to separate the phases (see Table 1).

NOTE 2 The use of the Congo-red solution (4.3) is optional, but is particularly useful for some spreadable fats which give a transparent aqueous phase.

8.4.2 Mix the contents of the closed centrifuge tube (see 8.4.1) by using the vortex mixer (5.8) until all lumps of the test portion have been dissolved.

8.4.3 Centrifuge the closed centrifuge tube until a clear extraction solvent phase is obtained. Take adequate safety precautions when centrifuging tubes containing ether.

NOTE The centrifuge speed depends on the type of centrifuge (5.9). A clear extraction solvent layer is usually obtained within 3 min to 5 min using a radial acceleration of 50 *g* to 100 *g*.

If a suitable centrifuge (5.9) is not available, allow the two phases to separate under gravity until the extraction solvent phase is clear and distinctly separated from the aqueous phase.

If, in the case of a test portion of spreadable fat and after separation of the phases, a cloudy solvent phase or a persistent emulsion is obtained, unscrew the cap and add 2 ml of ethanol (4.2) to the contents of the centrifuge tube. Close the centrifuge tube, mix its contents and centrifuge the tube as described above.

8.4.4 Unscrew the cap and check for evidence of leakage of the centrifuge tube by inspecting the outside of the rim of the tube for fat. Repeat the analysis if there is evidence of fat loss.

Using a pipettor (5.10), quantitatively transfer as much as possible of the extraction solvent phase to the corresponding fat-collecting vessel (see 8.2.2) without withdrawing any of the aqueous phase. Perform the solvent transfer over a fume bench or in a fume hood.

Do not immerse the pipette tip too deeply into the extraction solvent phase. Always place the tip just below the surface and move the tip down as the extraction solvent is removed.

NOTE 1 This technique will greatly reduce the quantity of fat remaining on the outside of the tip.

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Avoid cross-contamination of fat from one sample to the next. If an automatic pipettor is used for solvent transfer, use a different tip (numbered, if necessary) for each fat-collecting vessel. Upon completion of the transfer there may be some residual fat left on the outside of the tip. Place the tip in a position that avoids loss of this fat (i.e. place it horizontally on a rack or, alternatively, rest the tip at an angle in the corresponding fat-collecting vessel, but not in the collected extraction solvent extracts).

The rubber seals in pipettors (5.10) used for transferring the extraction solvent can deteriorate. Check that the pipettor can be used for transferring the solvent, or dedicate the pipettor to this method only.

NOTE 2 At the first extraction stage, the extraction solvent phase has a relatively high concentration of fat. Even small losses of the extraction solvent phase during transfer from the tube to the fat-collecting vessel can significantly affect (lower) the fat result.

8.4.5 Perform a second extraction by adding to the centrifuge tube a volume of fresh extraction solvent (4.1) as specified in Table 1, depending on the test sample and separation procedure. During addition of the solvent, rinse the inside and the outside lower end of the pipette tip.

Replace the screw cap, vortex for 15 s and centrifuge as described in 8.4.3. Repeat the extraction solvent transfer as described in 8.4.4, adding the extraction solvent phase to the previous extract in the fat-collecting vessel

8.4.6 Perform a third extraction by following the procedure described in 8.4.5 but taking a volume of solvent (4.1) as specified for the third extraction in Table 1, depending on the test sample and separation procedure. Add the third extraction solvent phase to the previous two extracts in the fat-collecting vessel.

8.4.7 If gravity separation of the phases is used, perform a fourth extraction, again using fresh extraction solvent (4.1) and following the procedure described in 8.4.5, but taking a volume of solvent depending on the test sample specified for the fourth extraction in Table 1. Add the fourth extraction solvent phase to the previous three extracts in the fat-collecting vessel.

8.4.8 Remove the extraction solvent as completely as possible from the fat-collecting vessel (see 8.4.6 or 8.4.7) by using the distillation or evaporation apparatus (5.11). While distilling or evaporating the extraction solvent, ensure that adequate safety precautions are taken and that the risk of fire is eliminated.

8.4.9 Place the fat-collecting vessel containing the fat (see 8.4.8) in the drying oven (5.2) set at 102 °C for 30 min. Cool in the desiccator (see 8.2.2). Weigh the vessel, recording its mass to the nearest 0,1 mg.

Repeat the oven drying and cooling process until the difference in mass between two consecutive weighings of the fat-collecting vessel, corrected for the blank, does not exceed 1,0 mg or until the mass increases. Use the lowest mass for the calculation.

NOTE The drying time may depend on the type of fat-collecting vessel used. For metal dishes, 30 min is usually sufficient, but for glass flasks a drying time of 1 h may be more suitable (to reduce the number of drying and cooling cycles).

If more than one analysis is performed, weigh the fat-collecting vessel used for the blank test for the method in parallel to those of the test portions until all fat-collecting vessels have attained constant mass.

If the fat-collecting vessels need a different number of weighings to attain constant mass, use for the calculation of the fat content the blank value corresponding to the lowest mass of the individual vessel.

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Table 1 — Number of extractions and volume of extraction solvent to be used per extraction for centrifugal separation and gravity separation of the phases for different types of test sample

Test sample	Extraction solvent (4.1) ml						
	Centrifugal separation			Gravity separation			
	Number of extractions			Number of extractions			
	1	2	3	1	2	3	4
Butter; edible oil emulsions	20	10	10	20	20	10	10
Spreadable fats	20	20	20	20	20	20	20
NOTE Reducing the volumes of extraction solvent below those shown in the table can result in an incomplete fat recovery.							

9 Calculation and expression of results

9.1 Calculation

Calculate the fat content of the sample, w, as a mass fraction in percent, using the following equation:

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

where

m_0 is the mass of the test portion (see 8.3.1 or 8.3.2), in grams;

m_1 is the mass of the fat-collecting vessel and extracted matter, determined in 8.4.9, in grams;

m_2 is the mass of the prepared fat-collecting vessel (see 8.2.2), in grams;

m_3 is the mass of the fat-collecting vessel used in the blank test and any extracted matter, determined in 8.4.9, in grams;

m_4 is the mass of the fat-collecting vessel used in the blank test (see 8.2.2), in grams.

9.2 Expression of results

Express the test result to two decimal places.

10 Precision

10.1 Interlaboratory tests

Details of the interlaboratory tests are presented in Annex B. The values are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

NOTE IDF 135 provides specific guidance for interlaboratory tests on methods of analysis for milk and milk products. It is based on ISO 5725.

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10.2 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 0,26 %.

10.3 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 0,45 %.

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, together with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incident that may have influenced the test result(s);

e) the test result(s) obtained and, if the repeatability has been checked, the final quoted results obtained.

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Annex A
(normative)

Blank test to check the extraction solvent

A.1 In this blank test (see 8.1.2), a fat-collecting vessel (5.4) shall be used for mass control purposes in order that changes in the atmospheric conditions or temperature effects of the fat-collecting vessel will not falsely suggest the presence or absence of non-volatile matter in the extraction solvent. This fat-collecting vessel may be used as a counterweight vessel in the case of a two-pan balance. The criterion for the solvent blank is that the change in apparent mass of the fat-collecting vessel from which the extraction solvent was evaporated, corrected for the apparent change in mass of the fat-collecting vessel for control purposes, shall show no increase greater than 1,0 mg.

A.2 Very occasionally, the extraction solvents (4.1) may contain volatile matter that is strongly retained in the fat. If there are indications of the presence of such substances, carry out blank tests on the solvent using a fat-collecting vessel with about 4 g of anhydrous butterfat. A fat-collecting vessel containing 4 g of anhydrous butterfat shall be used for mass control purposes so that oxidation of the milk fat, changes in the atmospheric conditions, or temperature effects of the fat-collecting vessel will be corrected for. If necessary, redistil the extraction solvent (4.1) in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the thus-obtained extraction solvent within a short time after redistillation.

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Annex B
(informative)

Results of interlaboratory trials

The results of five different studies were obtained in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table B.1. A meta-analysis of the five studies was conducted to obtain pooled estimates for repeatability and reproducibility using the following equation:

$$x_p^2 = \frac{\sum \nu_i x_i^2}{\sum \nu_i}$$

where

x_p is the pooled estimate for repeatability or reproducibility;

x_i is the ith estimate;

ν_i is the number of degrees of freedom associated with estimate x_i.

Table B.1 — Precision data

Samples	Bibliographic reference	No. of laboratories	Mean %[a]	r %[a]	R %[a]	CV(r)[b] %	CV(R)[c] %
Salted butter	[6]	4	81,51	0,11	0,17	0,05	0,07
Salted butter	[6]	4	81,53	0,14	0,23	0,06	0,10
Unsalted butter	[6]	4	83,16	0,22	0,30	0,09	0,13
Salted butter	[6]	4	81,49	0,09	0,26	0,04	0,11
Salted butter	[6]	4	82,57	0,08	0,14	0,04	0,06
Salted butter	[6]	4	80,88	0,18	0,24	0,08	0,10
EOE[d] (Russian-type butter)	[7]	11	77,3	0,36	0,48	0,17	0,22
EOE (white sauce blend)	[7]	11	77,5	0,27	0,43	0,12	0,20
High salt butter	[7]	11	81,0	0,11	0,34	0,05	0,15
Salted butter	[7]	10	81,3	0,28	0,75	0,12	0,33
Pastry butter	[7]	11	81,4	0,19	0,23	0,08	0,10
Unsalted butter	[7]	11	82,4	0,20	0,36	0,09	0,15
Lactic butter	[7]	11	82,7	0,10	0,34	0,04	0,14
Unsalted butter	[7]	11	82,7	0,23	0,48	0,10	0,21
Vegetable oil spread	[8]	5	23,0	0,21	0,62	0,32	0,95
Dairy spread	[8]	5	37,8	0,28	0,50	0,26	0,47
Dairy spread	[8]	5	38,0	0,24	0,31	0,22	0,29
Dairy spread	[8]	5	38,4	0,17	0,36	0,16	0,33
Vegetable oil spread	[8]	5	49,3	0,18	0,40	0,13	0,29
Vegetable oil spread	[8]	5	55,6	0,50	0,50	0,32	0,32

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Table B.1 (*continued*)

Samples	Bibliographic reference	No. of laboratories	Mean %[a]	r %[a]	R %[a]	CV(r)[b] %	CV(R)[c] %
Blend	[8]	5	60,2	0,34	0,34	0,20	0,20
Vegetable oil spread	[8]	5	60,2	0,23	0,25	0,14	0,15
Vegetable oil spread	[8]	5	64,5	0,20	0,49	0,11	0,27
Blend	[8]	5	82,2	0,24	0,51	0,10	0,22
Vegetable oil spread	[9]	11	22,91	0,19	0,33	0,30	0,51
Dairy spread	[9]	12	38,99	0,20	0,34	0,19	0,31
Vegetable oil spread	[9]	12	49,37	0,23	0,46	0,16	0,33
Blend	[9]	12	59,90	0,19	0,40	0,11	0,24
Vegetable oil spread	[9]	12	60,19	0,28	0,48	0,16	029
Vegetable oil spread	[9]	12	74,49	0,15	0,58	0,07	0,28
Margarine	[9]	12	75,60	0,26	0,43	0,12	0,20
Blend	[9]	12	82,27	0,33	0,48	0,14	0,21
Butter	[10]	3	82,59	0,35	0,45	0,15	0,19
Dairy spread	[10]	4	40,71	0,35	0,75	0,30	0,65
Margarine	[10]	4	80,30	0,20	0,41	0,09	0,18
Vegetable oil spread	[10]	4	39,50	0,35	0,59	0,31	0,53
Blend	[10]	4	59,44	0,43	1,06	0,26	0,63
Blend	[10]	4	40,71	0,26	0,48	0,22	0,42

[a] Mass fraction.

[b] CV(r) is the repeatability coefficient of variation.

[c] CV(R) is the reproducibilty coefficient of variation.

[d] EOE is edible oil emulsion.

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