UDC 637.127.6

Ref. No. : ISO/R 1211-1970 (E)

ISO

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION

TC 34

ISO RECOMMENDATION

R 1211

MILK

DETERMINATION OF FAT CONTENT

(REFERENCE METHOD)

1st EDITION

November 1970

Printed in Switzerland

Also issued in French and Russian. Copies to be obtained through the national standards organizations.

BRIEF HISTORY

The ISO Recommendation R 1211, *Milk – Determination of fat content* (*Reference method*), was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, the Secretariat of which is held by the Magyar Szabványügyi Hivatal (MSZH).

Work on this question led to the adoption of Draft ISO Recommendation No. 1211, which was circulated to all the ISO Member Bodies for enquiry in April 1967. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

Argentina	Iran	Romania
Australia	Iraq	South Africa, Rep. of
Belgium	Ireland	Switzerland
Brazil	Israel	Thailand
Bulgaria	Italy	Turkey
Colombia	Korea, Rep. of	U.A.R.
Czechoslovakia	Netherlands	United Kingdom
France	New Zealand	U.S.S.R.
Greece	Norway	Yugoslavia
Hungary	Poland	
India	Portugal	

No Member Body opposed the approval of the Draft.

This Draft ISO Recommendation was then submitted by correspondence to the ISO Council, which decided to accept it as an ISO RECOMMENDATION.

NOTE. – This ISO Recommendation has been developed jointly with the IDF (International Dairy Federation) and the AOAC (Association of Official Analytical Chemists; U.S.A.) for the purpose of being included in the FAO/WHO Code of Principles concerning Milk and Milk Products and Associated Standards.

The text as approved by the above organizations was also published by FAO/WHO (Code of Principles, Standard No. B-6), by the IDF (IDF Standard 1A) and by the AOAC (Official Methods of Analysis, 11th Edition, 16.052).

ISO Recommendation | R 1211 | November 1970

MILK

DETERMINATION OF FAT CONTENT

(REFERENCE METHOD)

1. SCOPE AND FIELD OF APPLICATION

1.1 Scope

This ISO Recommendation describes a reference method for the determination of the fat content of milk.

1.2 Field of application

The method is suitable for raw and processed liquid milk, partially skimmed milk and skimmed milk.

2. DEFINITION

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

The fat content is expressed as a percentage by mass.

3. PRINCIPLE

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

4. REAGENTS

All reagents should be of analytical reagent quality and should leave no residue greater than that permitted for the blank test (see clause 7.2). If necessary, solvents may be redistilled in the presence of about 1 g of butterfat per 100 ml of solvent. Water used should be distilled water or water of at least equivalent purity.

4.1 *Ammonia*, approximately 25 % (m/m) solution of NH_3 (ρ_{20} approximately 0.91 g/ml), or a stronger solution of known concentration.

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

4.3 *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 minute. 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 minute and then washed in water. Approximately 80 cm^2 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.

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

4.5 *Mixed solvent*, prepared shortly before use by mixing equal volumes of diethyl ether (4.3) and light petroleum (4.4).

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

5. APPARATUS

5.1 *Analytical balance.*

5.2 *Suitable extraction apparatus*, 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 these corks for at least 20 minutes in water at 60 °C or above, and cool in the water so that they are saturated when used.

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

5.4 *Drying oven*, well ventilated, thermostatically controlled and adjusted to operate at 102 ± 2 °C,
or

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

5.5 *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 clause 7.3).

5.6 *Centrifuge* in which the extraction apparatus (5.2) can be spun at 500 to 600 rev/min.

NOTES

1. The use of a centrifuge is optional (see clause 7.5.2).
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.

6. SAMPLING

Carry out the sampling according to the appropriate method described in ISO Recommendation R 707, *Milk and milk products – Sampling.*

7. PROCEDURE

7.1 Preparation of the sample

Bring the sample to a temperature of 20 °C. Mix thoroughly to ensure a homogeneous mixture of the fat throughout the sample. Do not agitate so vigorously as to cause frothing of the milk or separation of the fat.

If it is found difficult to disperse the cream layer, warm slowly to 35 to 40 °C with careful mixing and incorporation of any cream adhering to the container. Cool the sample quickly to room temperature. If desired, a homogenizer may be used to assist the dispersion of the fat.

Correct results cannot be expected if the sample contains separated liquid fat or separate visible irregularly shaped white particles adhering to the walls of the sample bottle.

7.2 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 clauses 7.3 and 7.5. If the result of the blank determination exceeds 0.0005 g, the reagents should be checked and the impure reagent or reagents purified or replaced.

7.3 Preparation of flask

Dry a flask (5.3) (if desired, with some material (5.5) to promote gentle boiling during the subsequent removal of the solvents) in the oven (5.4) for 30 to 60 minutes. Allow the flask to cool to the temperature of the balance room and then weigh it to the nearest 0.0001 g.

7.4 Test portion

Invert the bottle containing the prepared sample three or four times and immediately weigh to the nearest 0.001 g directly in, or by difference into, the extraction apparatus (5.2), 10 to 11 g of the well mixed sample.

7.5 Determination

7.5.1 Add to the test portion 1.5 ml of ammonia solution, 25 % (m/m), or an equivalent volume of a stronger solution (4.1), and mix well.

Add 10 ml of ethanol (4.2) and mix the liquids gently but thoroughly in the unclosed apparatus.

7.5.2 Add 25 ml of diethyl ether (4.3), close the apparatus with a moistened stopper and shake vigorously and invert repeatedly for 1 minute. Cool, if necessary, in running water. Remove the stopper carefully and add 25 ml of light petroleum (4.4), 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 seconds. 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 (5.6).

7.5.3 Remove the stopper, rinsing it and the inside of the neck of the apparatus with a few millilitres of mixed solvent (4.5) 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 clauses 7.3 and 9.1).

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.

7.5.4 Make a second extraction by repeating the procedure described in clauses 7.5.2 and 7.5.3 (including the rinsing(s)) but using only 15 ml of diethyl ether and 15 ml of light petroleum.

7.5.5 Make a third extraction by the procedure used for the second extraction (see clause 7.5.4) but omitting the final rinsing(s) (see clause 9.2).

7.5.6 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 hour in the oven. Allow the flask to cool to the temperature of the balance room as before (see clause 7.3), and weigh to the nearest 0.0001 g. Repeat the operations of heating, for periods of 30 to 60 minutes, cooling and weighing until there is no further decrease in mass.

7.5.7 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.

7.5.7.1 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 clause 7.3).

7.5.7.2 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 hour in the oven, allow to cool to the temperature of the balance room as before (see clause 7.3) and weigh to the nearest 0.0001 g. The mass of fat is the difference between the mass of the flask containing the total extract and the final mass.

7.5.8 Carry out two determinations on the same prepared sample.

8. EXPRESSION OF RESULTS

8.1 Method of calculation and formula

If A represents the flask used for the 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 equal to

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

where

m_0 is the mass, in grammes, of the test portion;

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

m_2 is the mass, in grammes, of flask A after the first heating (see clause 7.3) or, in the case of undissolved material, after the final heating;

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

m_4 is the mass, in grammes, of flask B after the first heating (see clause 7.3) 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 is satisfied (see clause 8.2).

8.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.03 g of fat per 100 g of the product.

9. NOTES ON PROCEDURE

9.1 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.

9.2 It is not essential to carry out the third extraction (see clause 7.5.5) in the case of machine-skimmed milk.

10. TEST REPORT

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

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

Date of the first printing :
December 1970

Price based on 4 pages