INTERNATIONAL STANDARD 3433

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

Cheese — Determination of fat content — Van Gulik method

Fromages — Détermination de la teneur en matière grasse — Méthode Van Gulik

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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.

International Standard ISO 3433 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and circulated to the Member Bodies in March 1974.

It has been approved by the Member Bodies of the following countries :

Austria	Hungary	Spain
Belgium	India	Thailand
Bulgaria	Iran	Turkey
Canada	Ireland	United Kingdom
Czechoslovakia	Israel	U.S.S.R.
Ethiopia	Netherlands	Yugoslavia
France	Poland	
Germany	South Africa, Rep. of	

No Member Body expressed disapproval of the document.

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AMENDMENT

Foreword (Inside front cover)

The ISO Member Body for the Arab Republic of Egypt has now approved this International Standard. The Arab Republic of Egypt should therefore be included in the list of countries whose Member Bodies have approved the document.

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Cheese — Determination of fat content — Van Gulik method

1 SCOPE AND FIELD OF APPLICATION

This International Standard describes the Van Gulik method for the determination of the fat content of cheese.

This method is applicable to all types of cheese. However, it may not give completely satisfactory results when it is applied to cheeses with an internal mould (blue-veined cheeses). See note in 8.3.11.

2 REFERENCES

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

ISO/R 1735, *Cheese and processed cheese products — Determination of fat content (Reference method).*

ISO 2446, *Milk — Determination of fat content — Gerber method.* [1]

ISO 3432, *Butyrometer for determination of the fat content of cheese by the Van Gulik method.*

3 DEFINITION

Van Gulik method : An empirical procedure which, when applied to a cheese, gives a value for fat content, expressed in grams per 100 g of cheese, that is equivalent to that obtained by the reference method (ISO/R 1735).

4 PRINCIPLE

Dissolution of the protein with sulphuric acid, followed by separation of the fat of the cheese in a Van Gulik butyrometer by centrifuging, the separation being assisted by the addition of a small quantity of amyl alcohol.

Direct reading of the fat content on the butyrometer scale.

5 REAGENTS

5.1 Sulphuric acid.

The sulphuric acid shall have a density at 20 °C of 1,522 ± 0,005 g/ml, which corresponds to 61,72 to 62,63 % (*m/m*) H_2SO_4. The acid shall be colourless or not darker in colour than pale amber, and shall not contain any impurity likely to affect the result.

5.2 Amyl alcohol.

5.2.1 *Composition*

At least 98 % (*V/V*) of the amyl alcohol shall consist of the primary alcohols 3-methylbutan-1-ol and 2-methylbutan-1-ol, the only permissible major impurities being 2-methylpropan-1-ol and butan-1-ol. It shall be free from secondary pentanols, 2-methylbutan-2-ol, 2-furaldehyde, gasoline (petrol) and derivatives of benzene. Not more than a trace of water shall be present.

5.2.2 *Physical appearance*

The amyl alcohol shall be clear and colourless.

5.2.3 *Density*

The amyl alcohol shall have a density at 20 °C of 0,808 to 0,818 g/ml.

5.2.4 *2-Furaldehyde and other organic impurities*

When 5 ml of the amyl alcohol is added to 5 ml of the sulphuric acid (5.1), no more than a yellow or light brown colour shall develop.

5.2.5 *Distillation range*

When the amyl alcohol is distilled at a pressure of 1 013 mbar*, not less than 98 % (*V/V*) shall distil below 132 °C and not more than 5 % (*V/V*) below 128 °C. There shall be no solid residue after distillation.

NOTE — If the atmospheric pressure during the distillation is lower or higher than 1 013 mbar, the specified temperatures should be respectively decreased or increased by 0,03 °C/mbar.

5.2.6 *Suitability test*

An amyl alcohol may satisfy the requirements of 5.2.1 to 5.2.5 yet be unsuitable for the Van Gulik method. Therefore, check the suitability of the amyl alcohol before use by means of the following comparative test with a standard amyl alcohol.

1) At present at the stage of draft.

* 1 mbar = 0,1 kPa.

5.2.6.1 STANDARD AMYL ALCOHOL

Distil an amyl alcohol satisfying the requirements of 5.2.1 to 5.2.5, using a suitable fractionation column, and collect a fraction within a boiling range of 2 °C between 128,0 and 131,5 °C (see note to 5.2.5). Apply the following tests to the fraction :

a) When analysed by gas-liquid chromatography, at least 99 % (*V*/*V*) shall consist of 3-methylbutan-1-ol and 2-methylbutan-1-ol. Only traces of impurities other than 2-methylpropan-1-ol and butan-1-ol shall be present.

b) When fractionally distilled, the first 10 % and the last 10 % collected, when compared using the procedure described in 5.2.6.2, shall give values for the fat content of milk that do not differ by more than 0,015 % fat.

If the fraction satisfies both these tests it can be regarded as standard amyl alcohol. The standard amyl alcohol can be used for several years, provided that it is kept in the dark in a cool place.

5.2.6.2 COMPARISON PROCEDURE

Determine in duplicate the fat content of four samples of whole milk with average fat content by the Gerber method described in ISO 2446, using butyrometers whose scale errors have been determined, and sulphuric acid of suitable quality. In one of each pair of duplicates use 1 ml of the amyl alcohol under test and in the other use 1 ml of the standard amyl alcohol (5.2.6.1).

Keep the butyrometers in a random order from the shaking stage onwards. Take the readings to the nearest 0,02 % fat (read by at least two persons) and correct for the scale errors of the butyrometers.

The mean fat content of the four milk samples obtained with the amyl alcohol under test shall not differ by more than 0,015 % fat from the mean value obtained using the standard amyl alcohol.

NOTE – Instead of the specified amyl alcohol an artificial amyl alcohol or an amyl alcohol substitute, coloured if desired, may be used, provided that it is found to be satisfactory when tested by the procedure described in 5.2.6.2.

6 APPARATUS

6.1 Van Gulik butyrometers, complying with ISO 3432.

6.2 Weighing device (see ISO 3432) which can be fitted to the large stopper of the butyrometer. Alternatively, a dish, a capsule or plastics sheet may be used.

6.3 Pipette or automatic measure to deliver sulphuric acid (5.1).

6.4 Pipette or automatic measure, to deliver 1 ± 0,05 ml of amyl alcohol (5.2).

6.5 Analytical balance.

6.6 Centrifuge, in which the butyrometers can be spun, provided with a speed indicator which indicates the number of revolutions per minute with a maximum tolerance of ± 50 rev/min, and preferably of the vertical-loading type rather than the horizontal-loading type.

The centrifuge shall be capable of producing within 2 min, when fully loaded, a relative centrifugal acceleration of 350 ± 50 *g* at the outer end of the butyrometer stopper. This acceleration is produced by centrifuges with the following effective radius (horizontal distance between the centre of the centrifuge spindle and the outer end of the butyrometer stopper) operated at the speed indicated :

Effective radius	Revolutions per minute
mm	± 70 rev/min
240	1 140
245	1 130
250	1 120
255	1 110
260	1 100
265	1 090
270	1 080
275	1 070
300	1 020
325	980

NOTE – The relative centrifugal acceleration produced in a centrifuge is given by the following formula :

$$1{,}12\,RN^2 \times 10^{-6}$$

where

R is the effective horizontal radius, in millimetres;

N is the speed, in revolutions per minute.

6.7 Water bath for butyrometers, capable of being maintained at 65 ± 2 °C and such that the butyrometers (6.1) can be supported in a vertical position with their scales completely immersed.

6.8 Thermometer, suitable for insertion in the water bath (6.7).

6.9 Grater, or other device for grinding the cheese.

7 SAMPLING

See ISO/R 707.

8 PROCEDURE

8.1 Preparation of the test sample[1)]

Prior to analysis, remove the rind or smear or mouldy surface layer of the cheese, to provide a sample which is

1) Special requirements for the preparation of the sample of any type or variety of cheese could be made in national standards.

representative of the cheese as usually consumed. Grind the sample by means of an appropriate device (6.9). Mix the ground mass quickly, and if possible grind a second time and again mix thoroughly. If the sample cannot be ground, mix it thoroughly by intensive stirring and kneading.

Transfer the test sample to an air-tight container to await analysis, which should be carried out on the same day. If delay is unavoidable, take all precautions to ensure proper preservation of the sample and to prevent condensation of moisture on the inside surface of the container.

Clean the device after grinding each sample.

8.2 Test portion

Weigh, to the nearest 0,005 g, 3 g of the test sample (8.1) into the weighing device (6.2) fitted to a suitable stopper, or into a capsule, or on plastics sheet.

8.3 Determination

8.3.1 If a stopper with a weighing device is used, close the neck of the butyrometer (6.1) with this stopper, including weighing device and test portion, and add sulphuric acid (5.1) to the small opening until the acid level reaches a height of about two-thirds of the body of the butyrometer and the weighing device is completely surrounded with sulphuric acid.

If no weighing device in the large stopper is used, close the small opening of the butyrometer with the small stopper and let sulphuric acid run into the butyrometer through the neck until the acid level reaches a height of about half the body of the butyrometer.

Transfer the cheese to the butyrometer. If plastics sheet is used, transfer the cheese with the sheet. Close the neck with the large stopper, invert the butyrometer and remove the small stopper.

8.3.2 Place the butyrometer with its neck (i.e. large opening) downwards for 5 min in the water bath (6.7) at 65 ± 2 °C.

8.3.3 Remove the butyrometer from the water bath and shake it thoroughly for 10 s.

8.3.4 Repeat the operations specified in 8.3.2 and 8.3.3 until the protein is completely dissolved. One hour is usually needed. Repeat the procedure for 15 min after the protein has been dissolved.

NOTE — Mechanical shaking devices may be used provided that their use produces the same results as the manual procedure specified above.

8.3.5 Remove the butyrometer from the water bath and add, after thorough shaking of the butyrometer, 1 ml of amyl alcohol (5.2) to the small opening. Immediately shake the butyrometer for at least 3 s.

8.3.6 Add sulphuric acid to the small opening until the level reaches the 35 % graduation mark. Close immediately with the small stopper and invert the butyrometer.

8.3.7 As soon as the fat has ascended into the body, shake the butyrometer thoroughly for 10 s. Invert again so that the acid drains out of the stem. Repeat shaking and inverting twice.

8.3.8 Place the butyrometer neck downwards for 5 min in the water bath; the water level should be above the top of the fat column in the butyrometer.

8.3.9 Remove the butyrometer from the water bath, adjust the large stopper to bring the fat column on the scale and centrifuge the butyrometer for 10 min at a relative centrifugal acceleration of 350 ± 50 *g*.

8.3.10 Place the butyrometer neck downwards in the water bath for 5 min. Maintain the water level above the top of the fat column in the butyrometer.

8.3.11 Remove the butyrometer from the water bath and carefully adjust the large stopper to bring the bottom of the fat column, with the minimum movement of the column, to a graduation mark, preferably a main graduation mark. This should preferably be done by slightly withdrawing the stopper and not by forcing it further into the neck. Note the scale reading coincident with the bottom of the fat column and then, taking care that the fat column does not move, as quickly as possible note the scale reading coincident with the lowest point of the fat meniscus at the top of the fat column; this reading shall be taken to the nearest half a smallest scale division (0,25 %).

While readings are being taken, the butyrometer shall be held vertically and the eye shall be level with the point of reading.

NOTE — If the fat is turbid or dark in colour of if there is white or black material at the bottom of the fat column, the value for the fat content will not be accurate.

9 EXPRESSION OF RESULTS

9.1 Method of calculation

The fat content of the cheese, expressed in grams per 100 g of cheese, is equal to

$$B - A$$

where

A is the reading obtained at the bottom of the fat column;

B is the reading obtained at the top of the fat column.

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 a value corresponding to one smallest scale division (0,5 %).

9.3 Correction of results

If the results obtained by this method are corrected to make them correspond to the results obtained by the reference method (see ISO/R 1735), this shall be clearly indicated in the test report.

10 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention all 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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