INTERNATIONAL STANDARD

ISO 14156

IDF 172

First edition
2001-12-01

Milk and milk products — Extraction methods for lipids and liposoluble compounds

Lait et produits laitiers — Méthodes d'extraction des lipides et des composés liposolubles

Reference numbers
ISO 14156 :2001(E)
IDF 172:2001(E)

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Printed in Switzerland

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14156|IDF 172 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.

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 National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Fat*, under the aegis of its project leader, Mr R.J. de Knegt (NL).

Milk and milk products — Extraction methods for lipids and liposoluble compounds

1 Scope

This International Standard specifies methods for the extraction or separation of a representative part of the fat, containing lipids and liposoluble compounds, from milk and milk products.

The method is applicable to pretreatment of samples for the methods described in ISO 15884 and ISO 15885.

It should be noted that free fatty acids are not part of extracted fat as described in methods for the fat determination in milk, condensed milk, dried milk products, cream and fermented milk.

2 Term and definition

For the purposes of this International Standard, the following term and definition apply.

2.1
lipids and liposoluble compounds
substances extracted or separated by the various procedures specified in this International Standard

3 Principle

The lipids and liposoluble compounds of milk and various milk products are extracted or separated for further analysis. In the case of butter and other high-fat products, the lipid fraction is physically separated. For other products, the lipids and related compounds are extracted with solvents after suitable preparation of the sample.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water free from chloride, or water of equivalent purity.

4.1 **Ammonia solution**, $c(NH_3)$ = 14 mol/l (ρ_{20} = 919 g/l).

4.2 **Ethanol** (C_2H_5OH), containing a volume fraction of 96 % ± 2 %.

4.3 **Diethyl ether** ($C_2H_5OC_2H_5$), free from peroxides.

4.4 **Anyhdrous sodium sulfate** (Na_2SO_4).

4.5 **Sodium sulfate solution**.

Dissolve 100 g of anhydrous sodium sulfate (Na_2SO_4) in water. Dilute to 1 litre with water.

4.6 ***n*-Pentane**.

4.7 **Sand**, free from organic material.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Oven, capable of being maintained at a temperature of 50 °C ± 5 °C.

5.2 Soxhlet extraction apparatus, provided with an extraction thimble.

5.3 Water baths, capable of being maintained at temperatures of between 40 °C and 60 °C, 30 °C and 40 °C, and 50 °C ± 2 °C.

5.4 Separating funnel, of capacity 500 ml.

5.5 Measuring cylinders, of capacities 100 ml and 250 ml.

5.6 Beakers, of capacity 100 ml.

5.7 Filter paper, medium porosity, of diameter about 15 cm.

5.8 Conical flask, of capacity 250 ml.

5.9 Round-bottom flask, of capacity 250 ml.

5.10 Rotary evaporator, with accessories.

5.11 Spoon or **spatula**.

6 Sampling

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

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

Store the sample in such a way that deterioration and change in its composition are prevented.

7 Preparation of test sample

7.1 Raw milk and raw cream

Bring the test sample in a water bath (5.3) to a temperature of 35 °C to 40 °C. Mix the sample by repeated inversion, then cool it quickly to 20 °C ± 2 °C.

7.2 Homogenized milk, homogenized cream and fermented milk

Bring the test sample to a temperature of 20 °C ± 2 °C. Mix or stir the sample thoroughly.

7.3 Evaporated milk

Shake and invert the container. Open the container, pour the test sample slowly into a second container (provided with an airtight lid) and mix by repeated transfer, taking care to incorporate in the sample any fat or other constituent adhering to the wall of the first container. Finally, transfer the milk as completely as possible to the second container. Close the container.

In the case of samples in sealed cans, condition the unopened can in a water bath (5.3) set at a temperature of between 40 °C and 60 °C, if necessary. Shake the can vigorously every 15 min. After 2 h, remove the can from the water bath and allow the can and its contents to cool to room temperature. Remove the lid and thoroughly mix the contents of the can by stirring with a spoon or spatula (5.11).

7.4 Sweetened condensed milk

Open the sample container and thoroughly mix the test sample with a spoon or spatula. Use an up and down rotary movement in such a way that the top layer and the contents of the lower corners of the container are moved and mixed. Take care to incorporate in the sample all milk adhering to the wall of the container. Transfer the test sample as completely as possible to a second container (provided with an airtight lid). Close this container.

In the case of samples in sealed cans, condition the unopened can in a water bath (5.3) set at a temperature of between 30 °C and 40 °C, if necessary. Open the can and transfer the complete test sample, by scraping out all the sample adhering to the interior of the can, into a dish which is large enough to permit thorough stirring. Mix the contents of the dish until the mass is homogeneous.

In the case of a test sample in a collapsible tube, open the tube and transfer its contents to a jar. Cut open the tube, scrape out all test sample adhering to the interior of the tube, and add that to the contents of the jar too. Mix the contents of the jar until the mass is homogeneous.

7.5 Dried milk products

Thoroughly mix the test sample by repeatedly rotating and inverting the container.

7.6 Cheese

Before analysis, remove the rind or any smear or mouldy surface layer of the cheese so as to give a test sample representative of the cheese as it is usually consumed.

8 Procedure

8.1 General

For test samples with a relatively high content of phospholipids and a relatively low content of simple lipids (e.g. buttermilk), the chosen extraction procedure will affect the fatty acid composition of the extracted fat. For these test samples the procedure described in 8.3 and the addition of approximately 1,5 g of sodium chloride to the test portion is recommended. The fat thus extracted will contain the phospholipids. The fatty acid composition of the phospholipids differs considerably from that of the other milk fat components.

8.2 Anhydrous milk fat, butteroil and butter

Melt 50 g to 100 g of test sample in the oven (5.1) set at a temperature of 50 °C. Place 0,5 g to 1,0 g of anhydrous sodium sulfate (4.4) in a folded filter paper. Filter the fat through the filter paper containing anhydrous sodium sulfate collecting the filtrate in a beaker (5.6) maintained in the oven (5.1) set at a temperature of 50 °C. When decanting the melted butter onto the filter paper (5.7), take care that no serum is transferred.

8.3 Raw milk and homogenized milk

Mix 100 ml of the test sample with 80 ml of ethanol (4.2) and 20 ml of ammonia solution (4.1) in a separating funnel (5.4).

Add 100 ml of diethyl ether (4.3) and shake the funnel vigorously for 1 min. Let it stand to achieve phase separation. Then add 100 ml of *n*-pentane (4.6) to the contents of the funnel and mix carefully. Let the funnel stand for a second phase separation, then discard the aqueous layer.

Add 100 ml of sodium sulfate solution (4.5) to the remaining contents of the funnel and mix carefully again. Let the funnel stand for a third phase separation and thereafter discard the aqueous layer. Add a second amount of 100 ml of the sodium sulfate solution to the remaining contents of the funnel and shake vigorously for 1 min. Let the funnel stand for phase separation and discard the aqueous layer again. Transfer the remaining organic layer to a conical flask (5.8). Add 5 g to 10 g of anhydrous sodium sulfate (4.4), stopper the flask and mix the contents of the flask.

Allow the flask to stand for 10 min and filter its contents into a round-bottom flask (5.9). Using the rotary evaporator (5.10), evaporate the contents of the flask under reduced pressure in a water bath (5.3) set at 50 °C until evaporation is visually completed.

Disconnect the flask from the rotary evaporator. Flush the flask contents with a stream of nitrogen for 1 min. Again connect the flask to the evaporator and continue evaporation for another period of 10 min. Despite this second evaporation step, the evaporation of the solvent will not be complete. The solvent residue will have a mass fraction of less than 2 %.

For some analyses of fat (e.g. fatty acid composition) complete removal of the solvent is not necessary. If, however, complete removal of the solvent is necessary, heat the contents of the flask in an oven set at 102 °C until a constant mass is obtained.

CAUTION — It should be noted that at a temperature of 102 °C decomposition of the fat (e.g. linolenic acid), or of other components of the fat, might occur.

Note that the fat extracted as above does not contain the free fatty acids.

8.4 Sweetened condensed milk, evaporated milk, cream and fermented milk

Dilute a suitable amount of test sample so as to obtain about 100 ml of test portion with a mass fraction of fat of approximately 4 %. Proceed as in 8.3.

8.5 Dried milk products

Prepare a test portion by reconstituting 10 g of the test sample in 100 ml of distilled water. Place the test portion in a water bath (5.3) set at 60 °C for 30 min, with shaking from time to time. Cool to room temperature and proceed as in 8.3.

8.6 Cheese

Grate or mash the cheese depending on its texture.

Transfer an amount of test sample containing approximately 4 g of fat into a mortar. Grind the sample well with a 1 + 1 mixture of sand (4.7) and sodium sulfate (4.4) to yield a dry test sample.

NOTE The amount of sodium sulfate/sand mixture required depends on the water content of the cheese.

Transfer the complete test portion into the extraction thimble and insert the latter (closed, for example, by a cotton wool plug) into the chamber of the Soxhlet extraction apparatus (5.2). Fill the round-bottom flask with 250 ml of *n*-pentane (4.6) and extract the sample for 6 h under reflux.

Using the rotary evaporator (5.10), evaporate the contents of the flask under reduced pressure in a water bath (5.3) set at 50 °C until evaporation is visually completed.

Disconnect the flask and flush its contents with a stream of nitrogen for 1 min. Connect the flask again to the rotary evaporator and continue evaporation for another period of 10 min. (See also CAUTION in 8.3.)

9 Report

The report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the extraction method used, together with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the result.