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Heat-treated milk — Determination of lactulose content — Method using high-performance liquid chromatography

Lait traité thermiquement — Détermination de la teneur en lactulose — Méthode par chromatographie liquide à haute performance

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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 11868 | IDF 147 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This edition of ISO 11868 | IDF 147 cancels and replaces ISO 11868:1997, of which it constitutes a minor revision.

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 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 the IDF National Committees casting a vote.

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ISO 11868 | IDF 147 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by a former Joint ISO/IDF/AOAC Group of Experts on *Characterization of milk and milk products according to heat treatment* (E704), under the aegis of its project leader, Mr M.A.J.S. van Boekel (NL).

This edition of ISO 11868 | IDF 147 cancels and replaces IDF 147A:1994, of which it constitutes a minor revision.

Heat-treated milk — Determination of lactulose content — Method using high-performance liquid chromatography

1 Scope

This International Standard specifies a method for the determination of the lactulose content of heated milk, skimmed, partially skimmed or whole milk, by high-performance liquid chromatography, in order to distinguish milk sterilized by ultra-heat treatment (UHT) from in-bottle sterilized milk.

The method has been tested over a lactulose content range of 200 mg/l to 1 500 mg/l and is applicable to all heat-treated milks.

The method described in this International Standard is to be used in cases of dispute.

2 Terms and definitions

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

2.1
lactulose content of skimmed, partially skimmed or whole milk
mass of substances determined by the procedure specified in this International Standard

NOTE The lactulose content is expressed as milligrams per litre of sample.

3 Principle

Fat and protein are removed from a sample of milk, which is then filtered. The lactulose content of the filtrate is determined by high-performance liquid chromatography (HPLC). The result obtained for the sample is evaluated by reference to standard samples consisting of lactulose-free skimmed milk with known amounts of added lactulose.

4 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and double-distilled water or water of equivalent purity.

4.1 Lactose monohydrate.

4.2 Lactulose, at least 99 % pure.

4.3 Sample pretreatment solution.

Dissolve 91,0 g of zinc acetate dihydrate, $Zn(CH_3COO)_2{\cdot}2H_2O$, 54,6 g of phosphotungstic acid tetracosahydrate, $H_3[P(W_3O_{10})_4]{\cdot}24H_2O$, and 58,1 ml of glacial acetic acid in water in a 1 000 ml volumetric flask and dilute to the mark with water.

4.4 Eluent.

Filter the water, HPLC grade, through a membrane filter with a 0,45 µm pore diameter (5.8) and, prior to use, boil to remove dissolved air.

To remove dissolved air, other methods giving the same results (e.g. helium sparging) may be used instead of boiling water.

NOTE These alternatives are usually more expensive.

4.5 Standard samples.

4.5.1 Lactulose standard solution.

Weigh, to the nearest 0,1 mg, about 75 mg of lactulose (4.2) in a 100 ml volumetric flask (5.6). Dissolve in water and dilute to the mark with water.

4.5.2 Pasteurized skimmed milk, lactulose free, as determined using the method specified below.

Use identical pasteurized skimmed milk samples containing approximately 250 mg, 500 mg, 750 mg and 1 000 mg of lactulose per litre, obtained by the addition of 5 ml, 10 ml, 15 ml and 20 ml, respectively, of the lactulose standard solution (as described in 8.2) to the pasteurized skimmed milk.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Analytical balance, capable of weighing to the nearest 0,1 mg.

5.2 Glass funnels, of diameter about 7 cm.

5.3 Filters.

5.3.1 Filter papers, medium grade, of diameter about 12,5 cm.

5.3.2 Cellulose acetate membranes, with 0,45 µm pore diameter.

5.4 Measuring cylinder, of capacity 25 ml.

5.5 Graduated pipette, of capacity 10 ml, graduated in 0,1 ml.

5.6 One-mark volumetric flasks, of capacity 50 ml, 100 ml and 1 000 ml.

5.7 One-mark pipettes, capable of delivering 5 ml, 10 ml, 15 ml and 20 ml.

5.8 Glass filtration equipment, with 0,45 µm pore diameter filter.

5.9 Glass flasks, of capacity 20 ml, with stopcock.

5.10 Ultrasonic water bath.

5.11 Water vacuum pump.

5.12 HPLC equipment, as follows.

5.12.1 Magnetic stirrer and **heater**, for keeping the eluent at a temperature of 90 °C ± 2 °C before it is transported to the precolumn for analysis.

5.12.2 Pump, capable of delivering a volume flow rate of between 0,3 ml/min and 0,6 ml/min, with a pulsation of less than 1 % of the pressure drop over the column (1,5 MPa to 4 MPa).

5.12.3 HPX-87 P column (Bio-Rad, 30 cm × 0,78 cm)[1)], or an equivalent column packed with sulfonic ion exchanger in the lead form, based on a polystyrene divinylbenzene 8 % crosslinked polymer. The pre-column consists of the Bio-Rad de-ashing system[1)] (a cartridge, 3 cm × 0,46 cm, packed with a cation-exchange resin in the hydrogen form and a cartridge, 3 cm × 0,46 cm, packed with an anion-exchange resin in the carbonate form) or a system of equivalent effectiveness.

The pre-columns extend both the life and the length of the analytical column, minimizing separation problems and substantially reducing quantization errors. When the HPLC system begins to lose resolution, replace the spent pre-column before contamination extends to the main column.

5.12.4 Thermostatic column oven, capable of being maintained at a temperature of 75 °C ± 1 °C.

The pre-columns should be placed outside the oven. The inlet tubing to the main column should have a length of 10 cm to 15 cm in the oven to equilibrate the eluent temperature to 75 °C, otherwise peak distortion may occur.

5.12.5 Refractive index detector, highly sensitive, with a noise level of less than 5×10^{-9} refractive index units (RIU), measured in water.

The internal thermostat should be set at a temperature above room temperature, sufficient to obtain a stable baseline. A temperature of 35 °C to 40 °C is advisable in most cases.

NOTE Highly sensitive monitoring of the refractive index is hampered by baseline drift due to thermal changes. To minimize the baseline drift, it is advisable to locate the HPLC equipment in a conditioned room to avoid temperature changes.

5.12.6 Integrator, capable of peak height measurements.

The integration control parameters should be carefully chosen (e.g. peak width, slope drift, peak threshold). The integrator should be forced to drop a perpendicular between the lactose and the lactulose peaks. (Skimming leads to inaccuracy due to the presence of varying amounts of glucose in the milk.) The integrator shall be inhibited against finding the baseline between the lactose and the lactulose peaks, unless the valley reaches the baseline at all lactulose concentrations.

Many integrators automatically vary peak integration parameters during the run. If possible, this feature should be disconnected in order to obtain more repeatable results.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have 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 | IDF 50.

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

1) The HPX-87 P column (Bio-Rad, 30 cm × 0,78 cm) and Bio-Rad de-ashing system are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.