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Milk — Determination of lactose content — Enzymatic method using difference in pH

Lait — Détermination de la teneur en lactose — Méthode enzymatique par pH-métrie différentielle

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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 26462 | IDF 214 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.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have 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.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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ISO 26462 | IDF 214 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 the ISO-IDF Joint Project Group on *Enzymatic determination of lactose* of the Standing Committee on *Analytical methods for composition* under the aegis of its project leader, Mr. P. Trossat (FR).

Milk — Determination of lactose content — Enzymatic method using difference in pH

1 Scope

This International Standard specifies an enzymatic method for the determination of the lactose content of milk and reconstituted milk by measurement of the difference in pH (differential pH measurement).

2 Terms and definitions

For the purpose of this International Standard, the following terms and definitions apply.

2.1
lactose content in milk
amount of substance concentration of compounds determined by the procedure specified in this International Standard

NOTE The lactose content of milk is expressed in millimoles per litre. For conversion of the result into other units, see Table 1.

2.2
unit of enzyme activity
international unit
standard unit
U

amount of enzyme which catalyses the transformation of one micromole of substrate per minute under standard conditions

3 Principle

β-Galactosidase is added to cleave lactose into glucose and galactose. At pH 7,8, glucose is phosphorylated by glucokinase, thereby releasing protons that induce a change in pH. The pH change varies as a function of the lactose content of the sample and is measured by using a differential pH analyser.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

4.1 **Buffer solution**, pH 7,8

Dissolve 0,242 g of tris(hydroxymethyl)methylamine (tris), 0,787 g of adenosine 5′-triphosphate disodium salt (ATP), 0,304 g of trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$), 0,009 g of sodium hydroxide (NaOH), 0,203 g of

magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 2 g of octylphenoxypolyethoxyethanol [e.g. Triton X100[1)]], 0,820 g of potassium chloride (KCl) and 0,010 g of 2-bromo-2-nitropropan-1,3-diol [e.g. Bronopol[1)]] in a 100 ml beaker containing 50 ml of water under continuous stirring. Adjust the final pH to 7,8 ± 0,1, if needed. Transfer to a 100 ml one-mark volumetric flask (5.4), make up to the mark with water and mix.

The buffer solution can be kept for 2 months if stored at 4 °C.

4.2 Enzyme solutions

4.2.1 Glucokinase enzyme solution

Dissolve 2,57 mg of lyophilized glucokinase-1 (GK1; 1 mg = 350 U; EC 2.7.1.2) in 3 ml of glycerol with a volume fraction of 50 %. The activity of the glucokinase solution obtained shall be 290 U/ml ± 30 U/ml (see 2.2).

The glucokinase enzyme solution can be kept for 6 months if stored at 4 °C.

4.2.2 β-Galactosidase enzyme solution

Dilute a concentrated β-galactosidase (EC 3.2.1.23) extract purified from contaminating enzymes with glycerol with a volume fraction of 50 %. The activity of the β-galactosidase solution obtained shall be 1 500 U/ml ± 200 U/ml.

The β-galactosidase enzyme solution can be kept for 6 months if stored at 4 °C.

4.3 Lactose standard solution (150 mmol/l)

Before use, determine the water content of lactose monohydrate powder by a Karl Fischer titration method, in order to correct for the quantity of lactose monohydrate used for the lactose standard solution. The correction should be based on the percentage of the determined water content in order to prepare a lactose standard solution containing 5,404 g lactose monohydrate per 100 ml.

Dissolve 5,404 g lactose monohydrate powder, 0,745 g of potassium chloride (KCl) and 0,01 g of 2-bromo-2-nitropropan-1,3-diol [e.g. Bronopol[1)]] in the buffer solution at pH 7,8 (4.1) in a 100 ml one-mark volumetric flask (5.4). Make up to the mark with water and mix.

The lactose standard solution can be kept for 6 months if stored at 4 °C.

4.4 Cleaning solution

Dissolve 1,742 g of dipotassium monohydrogenphosphate (K_2HPO_4), 1,361 g of potassium dihydrogenphosphate (KH_2PO_4), 7,455 g of potassium chloride (KCl), 1,00 g of sodium azide (NaN_3), 2 g of octylphenoxypolyethoxyethanol, 2 g of polyoxyethyleneglycol dodecylether [e.g. Brij 35[1)]] and 3 g of lauryl maltoside [e.g. LM[1)]] in a 1 000 ml one-mark volumetric flask (5.4). Make up to the mark with water and mix.

The cleaning solution can be kept for 1 year if stored at room temperature.

4.5 Regenerating solution

Use a 0,1 mol/l hydrochloric acid (HCl) solution as regenerating solution.

The regenerating solution can be kept for 1 year if stored at room temperature.

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1) Example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of this product.

4.6 Strong regenerating solution

DANGER — The use of sodium fluoride (NaF) alone and in combination with HCl may cause health problems due to inhalation and/or skin absorption. This International Standard does not purport to address all the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

Dissolve 30 g of nitric acid (HNO_3) with a mass fraction, $w(HNO_3) \approx 69$ %, 30 g of hydrochloric acid (HCl) with a mass fraction, $w(HCl) \approx 37$ %, 30 g of sodium fluoride (NaF), and 1 g of octylphenoxypolyethoxyethanol in a 1 000 ml one-mark volumetric flask (5.6). Make up to the mark with water and mix.

The strong regeneration solution can be kept for 1 year if stored in non-corroding material at room temperature.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

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

5.2 Micropipettes, capacity 20 µl, ISO 7550[5], with positive displacement.

5.3 Water bath, capable of maintaining a temperature of 38 °C ± 1 °C.

5.4 One-mark volumetric flasks, capacities 100 ml and 1 000 ml, ISO 1042[2] class A.

5.5 Differential pH apparatus, shown schematically in Figure A.1.

The differential pH apparatus consists of peristaltic pumps to circulate liquids, a mixing chamber, two glass capillary flow-through electrodes (E1 and E2), and an electronic system for measurement.

5.6 One-mark volumetric flasks, capacity 1 000 ml and of material capable of storing the extremely corrosive strong regenerating solution (4.6).

6 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707|IDF 50[1].

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

7 Preparation of test sample

Warm the test sample to 38 °C in the water bath (5.3) while mixing. Cool the sample to 20 °C, before preparing the test portion.

8 Procedure

8.1 General

Since the various types of differential pH apparatus (5.5) available differ in design and handling, the operator shall carefully follow the instrument manufacturer's instructions for setting up, calibration, and operation of the instrument. Switch the instrument on and allow its operating conditions to stabilize.

