INTERNATIONAL STANDARD

ISO 26462

IDF 214

First edition
2010-06-15

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

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

Reference numbers
ISO 26462:2010(E)
IDF 214:2010(E)

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. Neither the ISO Central Secretariat nor the IDF accepts any liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies and IDF national committees. In the unlikely event that a problem relating to it is found, please inform the ISO Central Secretariat at the address given below.

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established 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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

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.

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.

If the time between two consecutive measurements is 5 min or more, renew the buffer solution (4.1) in the mixing chamber of the apparatus.

8.2 Blank determination

Using a micropipette (5.2), add 20 µl of glucokinase enzyme solution (4.2.1) into the mixing chamber of the differential pH apparatus (5.5).

Dilute the enzyme solution with buffer solution (4.1) to a total volume of 1 200 µl and mix.

Fill the flow-through electrodes, E1 and E2 (see Figure A.1), of the pH apparatus (5.5) with the buffer and glucokinase mixture obtained. Measure the offset differential pH, D_1, between the two electrodes. The difference between the electrodes shall be within 0 mpH ± 150 mpH, where mpH is a milli-pH unit.

Using another micropipette, add 20 µl of β-galactosidase enzyme solution (4.2.2) to the buffer and glucokinase mixture in the mixing chamber and mix. Only fill E2 with the mixture of buffer, glucokinase, and β-galactosidase. Again, measure the offset differential pH, D_2, between the two electrodes.

Calculate the numerical value of the difference in pH for the blank, ΔD_0, using Equation (1):

$$\Delta D_0 = D_2 - D_1 \quad (1)$$

where

D_1 is the numerical value of the differential pH between the electrodes both filled with the buffer and glucokinase mixture;

D_2 is the numerical value of the differential pH between electrode E1 filled with the buffer/glucokinase mixture and electrode E2 with the buffer/glucokinase/β-galactosidase mixture.

The difference, ΔD_0, shall be in the range −20 mpH units to 4 mpH units, while the difference between two consecutive differential measurements shall be $\leqslant$ 1,0 mpH unit.

If these results are not obtained, check the buffer solution and repeat the above procedure. If the results still do not fulfil requirement(s), clean the electrodes (see 8.7) and restart the blank determination specified in the first four paragraphs of this subclause.

8.3 Calibration

8.3.1 Calibration solution pH difference

Add, with one micropipette (5.2), 20 µl of lactose standard solution (4.3) and, with another, 20 µl of glucokinase enzyme solution (4.2.1) to the mixing chamber of the differential pH apparatus (5.5). Dilute with buffer solution (4.1) to a total volume of 1 200 µl. Fill both E1 and E2 with the mixture of buffer, lactose standard, and glucokinase obtained. Measure the offset differential pH, D_3, between the two electrodes.

Using a micropipette, add 20 µl of β-galactosidase enzyme solution (4.2.2) to the mixture of buffer, lactose standard, and glucokinase in the mixing chamber and mix. Fill E2 with the mixture of buffer, lactose standard, glucokinase, and β-galactosidase. After completion of the enzymatic reaction, measure the offset differential pH, D_4, between the two electrodes.

Calculate the difference in pH for the calibration solution, ΔD_c, using Equation (2):

$$\Delta D_c = (D_4 - D_3) - \Delta D_0 \quad (2)$$

where

D_3 is the numerical value of the differential pH between the two electrodes when both are filled with the mixture of buffer, lactose standard, and glucokinase (8.3.1);

D_4 is the numerical value of the differential pH between the electrodes when one is filled with the mixture of buffer, lactose standard, and glucokinase and the other with the mixture of buffer, lactose standard, glucokinase, and β-galactosidase (8.3.1).

8.3.2 Slope of the calibration curve

Calculate the slope, s_c, expressed in millimoles per litre mpH unit, of the calibration curve using Equation (3):

$$s_c = \frac{c_L}{\Delta D_c} \quad (3)$$

where c_L is the concentration, in millimoles per litre, of the lactose standard solution (4.3).

8.4 Checking the calibration

Check the calibration by analysing 20 µl of lactose standard solution (4.3) in accordance with the procedure in 8.5. The results obtained shall be between 148,5 mmol/l and 151,5 mmol/l for the lactose determination. If these values are not met, repeat the calibration procedure.

8.5 Determination

Operate the instrument and introduce the test portion in accordance with the manufacturer's instructions.

Add, with one micropipette (5.2), 20 µl of test sample (Clause 7) and, with another, 20 µl of glucokinase enzyme solution (4.2.1) to the mixing chamber of the differential pH apparatus (5.5). Dilute with buffer solution (4.1) to a total volume of 1 200 µl. Fill E1 and E2 with the mixture of buffer, test portion, and glucokinase obtained. Measure the offset differential pH, D_5, between the two electrodes.

Using another micropipette, add 20 µl of β-galactosidase enzyme solution (4.2.2) to the mixture of buffer, test portion, and glucokinase in the mixing chamber and mix. Fill E2 with the mixture of buffer, test portion, glucokinase, and β-galactosidase.

After completion of the enzymatic reaction, measure the offset differential pH, D_6, between the two electrodes.

NOTE Completion is reached when the variation has not exceeded 1 mpH unit over the last 1 min.

8.6 Checking the stability

After analysing no more than 30 test portions and at the end of each analytical series, analyse two blank solutions to check the zero point and 20 µl of the lactose standard solution (4.3) using the determination procedure (8.5) to check the calibration.

The second zero value shall be within 0 mmol/l ± 1,5 mmol/l and the standard value in the range 148,5 mmol/l to 151,5 mmol/l. If the values obtained are out of range, repeat both the offset blank determination (8.2) and the calibration procedure (8.3).

8.7 Cleaning procedure

Wash the electrodes and the mixing chamber of the differential pH apparatus (5.5), replacing the buffer solution (see also Annex A) with the cleaning solution (4.4). If the equipment is in full operation, leave the electrodes in contact with the cleaning solution until the next use while renewing the cleaning solution every 120 min. When not in full operation, treat the electrodes according to the manufacturer's instructions.

9 Maintenance of the electrodes

9.1 Regeneration

At least once a week, wash the electrodes and the mixing chamber of the differential pH apparatus (5.5), replacing the buffer solution (see also Annex A) with the regenerating solution (4.5), then carry out the cleaning procedure in 8.7, this time replacing the regenerating solution with the cleaning solution (4.4).

9.2 Strong regeneration

At least every three months (or every 500 samples analysed for use at low frequency), or earlier if necessary, wash the electrodes and the mixing chamber of the differential pH apparatus (5.5), replacing the buffer solution (see also Annex A) with the strong regenerating solution (4.6).

After the strong regeneration, rinse the apparatus by replacing the strong regenerating solution with water before starting the cleaning procedure in 8.7 while then replacing the water in its turn by the cleaning solution (4.4). While the cleaning solution is running, add a test portion of milk to the mixing chamber and run three additional cleaning procedures in order to coat the electrodes.

10 Calculation and expression of results

10.1 Calculation

Calculate the lactose content of the test sample, $c_{L,t}$, expressed in millimoles per litre, using Equation (4):

$$c_{L,t} = s_c\left[\left(D_6 - D_5\right) - \Delta D_0\right] \quad (4)$$

where

D_5 is the numerical value for the differential pH between the two electrodes when both are filled with the buffer, test sample, and glucokinase mixture (8.5);

D_6 is the numerical value for the differential pH between the two electrodes when one is filled with the buffer, test portion, and glucokinase mixture and the other with the mixture of the buffer, test portion, glucokinase, and β-galactosidase (8.5).

10.2 Expression of results

Express the test results to two decimal places.

Table 1 shows a conversion table for the different bases on which lactose content can be expressed.

Table 1 — Conversion table

Type	Lactose content, $c_{L,t}$		
	As amount of substance concentration mmol/l	As mass concentration g/100 ml	As mass fraction g/100 g
Lactose monohydrate	c_L	$\rho = c_L \times 0{,}036\ 03$	$w = (c_L \times 0{,}036\ 03)/d$ [a]
Lactose anhydrous	$c_L \times 0{,}95$	$\rho = c_L \times 0{,}036\ 03 \times 0{,}95$	$w = (c_L \times 0{,}036\ 03 \times 0{,}95)/d$ [a]

[a] Where d is the relative density of the milk.

11 Precision

11.1 Interlaboratory test

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two individual single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 2,96 mmol of lactose monohydrate per litre.

11.3 Reproducibility

The absolute difference between two individual single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 3,13 mmol of lactose monohydrate per litre.

12 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard (ISO 26462 | IDF 214:2010);

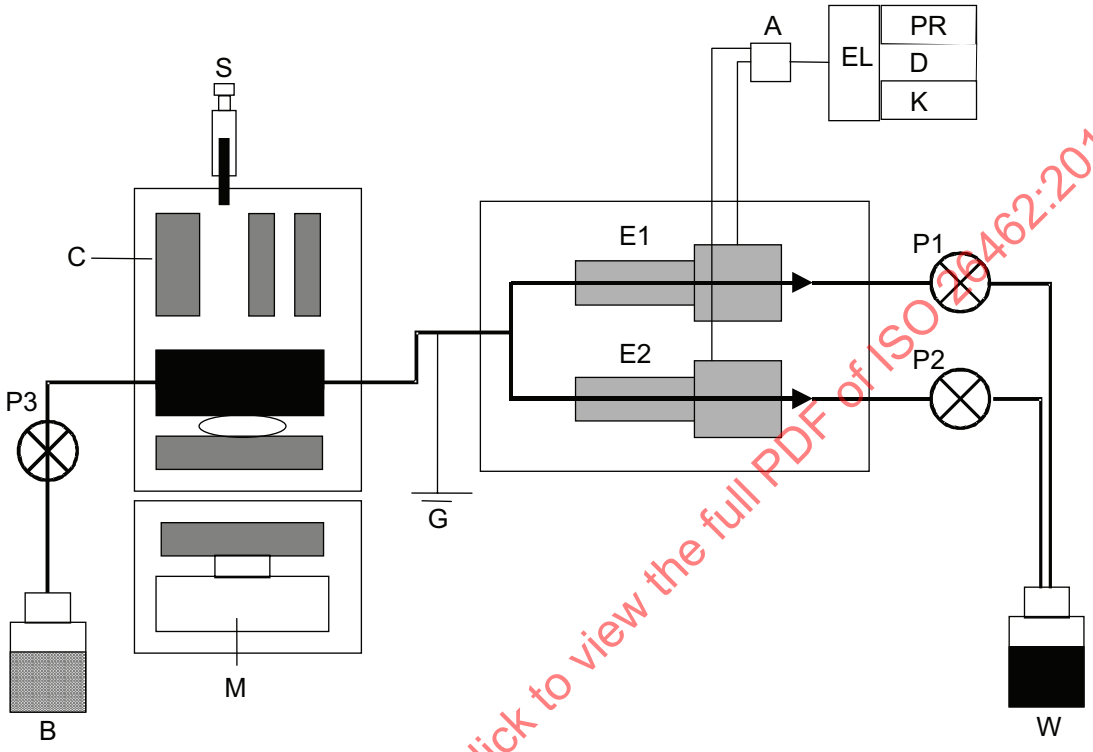
d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained;

f) if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Basic diagram of a differential pH apparatus



Key

A	differential amplifier
B	buffer solution
C	mixing chamber
D	display
E1	glass capillary electrode
E2	glass capillary electrode
EL	electronics
G	ground or earth
K	keyboard
M	magnetic stirrer
P1	peristaltic pump 1
P2	peristaltic pump 2
P3	peristaltic pump 3
PR	printer
S	micropipette for sample and enzyme suspension injection
W	waste

Figure A.1 — Differential pH apparatus