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Dried milk — Determination of content of lactic acid and lactates

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Foreword

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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.

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ISO 8069|IDF 69 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 8069|IDF 69 cancels and replaces ISO 8069:1986, which has been technically revised.

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 8069 | IDF 69 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 Joint ISO-IDF Action Team on *Lactose and lactate determination*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr J Romero (US).

This edition of ISO 8069 | IDF 69 cancels and replaces IDF 69:1987, which has been technically revised.

Dried milk — Determination of content of lactic acid and lactates

1 Scope

This International Standard specifies an enzymatic method for the determination of the lactic acid and lactates content of all types of dried milk.

2 Terms and definitions

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

2.1
lactic acid and lactates content
mass of substances determined by the procedure specified in this International Standard

NOTE It is expressed as milligrams of lactic acid per 100 g of non-fat solids.

3 Principle

A test portion of dried milk is dissolved in warm water. The fat and proteins are precipitated then filtered. The filtrate is treated with the following enzymes and biochemical substances, added simultaneously, but acting in sequence:

a) L-lactate dehydrogenase (L-LDH) and D-lactate dehydrogenase (D-LDH), in the presence of nicotinamide adenine dinucleotide (NAD), to oxidize lactate to pyruvate and to convert NAD to its reduced form NADH;

b) glutamate pyruvate transaminase (GPT), in the presence of L-glutamate, to transform pyruvate into L-alanine and to convert L-glutamate to α-ketoglutarate.

The amount of NADH produced is determined by spectrophotometric measurement at a wavelength of 340 nm, and is proportional to the lactic acid and lactates content.

4 Reagents

Use only reagents recognized analytical grade. The water used in the preparation of the enzyme solutions shall be of at least doubly glass-distilled purity and the water used for other purposes shall be glass-distilled or of at least equivalent purity.

4.1 Potassium hexacyanoferrate(II) solution, $c(K_4[Fe(CN)_6]\cdot 3H_2O)$ = 35,9 g/l.

Dissolve 35,9 g of potassium hexacyanoferrate(II) trihydrate in water. Dilute with water to 1 000 ml and mix.

4.2 Zinc sulfate solution, $c(ZnSO_4\cdot 7H_2O)$ = 71,8 g/l.

Dissolve 71,8 g of zinc sulfate heptahydrate in water. Dilute with water to 1 000 ml and mix.

4.3 Sodium hydroxide solutions

4.3.1 Sodium hydroxide solution I, c(NaOH) = 10 mol/l.

Dissolve 400 g of sodium hydroxide in water. Dilute with water to 1 000 ml and mix.

4.3.2 Sodium hydroxide solution II, c(NaOH) = 0,1 mol/l.

Dissolve 4,0 g of sodium hydroxide in water. Dilute with water to 1 000 ml and mix.

4.4 Glycerol solution ($C_3H_8O_3$), with a volume fraction of 50 % glycerol.

4.5 Ammonium sulfate solution, $c[(NH_4)_2SO_4]$ = 3,2 mol/l.

Dissolve 422,84 g of ammonium sulfate in water. Dilute with water to 1 000 ml and mix.

4.6 Buffer solution, pH 10.

Dissolve 7,92 g of glycylglycine ($C_4H_8N_2O_3$) and 1,47 g of L-glutamic acid ($C_5H_9NO_4$) in about 80 ml of water. Adjust the pH to 10,0 ± 0,1 at 20 °C with sodium hydroxide solution I (4.3.1). Dilute with water to 100 ml and mix.

This solution may be kept for 3 months if stored in a refrigerator at between 0 °C and +5 °C.

4.7 Nicotinamide adenine dinucleotide solution (NAD).

Dissolve 350 mg of nicotinamide adenine dinucleotide ($C_{21}H_{27}N_7O_{14}P_2$) in 10 ml of water.

This solution may be kept for 4 weeks if stored in a refrigerator at between 0 °C and +5 °C. When the solution is being used, keep the vessel immersed in crushed ice.

4.8 L-Lactate dehydrogenase (L-LDH), from hog muscle suspension.

Dissolve 10 mg of L-lactate dehydrogenase suspension in 1 ml of glycerol solution (4.4). The pH of the obtained suspension should be about 7. The specific activity of the L-lactate dehydrogenase (L-LDH, EC 1.1.1.27) suspension shall be at least 5 500 units/ml at 25 °C. If not, prepare another L-LDH suspension.

The L-LDH suspension may be kept for 12 months if stored in a refrigerator at between 0 °C and +5 °C. When the suspension is being used, keep the vessel immersed in crushed ice.

4.9 D-Lactate dehydrogenase (D-LDH), from *Lactobacillus leichmannii* suspension.

Dissolve 5 mg of D-LDH suspension in 1 ml of ammonium sulfate solution (4.5). The pH of the obtained suspension should be about 6. The specific activity of the D-lactate dehydrogenase (D-LDH, EC 1.1.1.28) suspension shall be at least 1 500 units/ml at 25 °C. If not, prepare another D-LDH suspension.

The D-LDH suspension may be kept for 12 months if stored in a refrigerator at between 0 °C and +5 °C. When the suspension is being used, keep the vessel immersed in crushed ice.

4.10 Glutamate pyruvate transaminase (GPT), from pig heart suspension.

Dissolve 20 mg of GPT suspension in 1,0 ml of ammonium sulfate solution (4.5). The pH of the obtained suspension should be about 7. The specific activity of the glutamate pyruvate transaminase (GPT, EC 2.6.1.2) suspension shall be at least 1 600 units/ml at 25 °C. If not, prepare another GPT suspension.

Add 1,0 ml of ammonium sulfate solution (4.5) to the 1 ml suspension with 20 mg of GPT and mix. Centrifuge this 2,0 ml suspension containing 10 mg of GPT/ml at a radial acceleration of 4 000 g for 10 min. Transfer 1,0 ml of the clear supernatant liquid and discard the remaining solution and pellet.

The suspension may be kept for 12 months if stored in a refrigerator at between 0 °C and +5 °C. When the suspension is being used, keep the vessel immersed in crushed ice.

4.11 Lithium L-lactate solution

Dissolve 50 mg of lithium L-lactate ($C_3H_5O_3Li$) in water. Dilute with water to 500 ml and mix.

4.12 Lithium D-lactate solution

Dissolve 50 mg of lithium D-lactate ($C_3H_5O_3Li$) in water. Dilute with water to 500 ml and mix.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

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

5.2 Glass beaker, of capacity 50 ml.

5.3 Graduated cylinder, of capacity 50 ml.

5.4 One-mark volumetric flasks, of capacity 100 ml.

5.5 Pipettes, capable of delivering 0,02 ml, 0,05 ml, 0,2 ml, 1,0 ml and 2,0 ml.

5.6 Graduated pipettes, capable of delivering 5 ml and 10 ml, graduated in 0,1 ml divisions.

5.7 Glass filter funnel, of diameter about 7 cm.

5.8 Filter paper, medium fast grade, of diameter about 15 cm, free from lactic acid and lactates.

5.9 Glass rod.

5.10 Plastic paddles, capable of mixing the sample-enzyme mixture in the spectrometric cell.

5.11 Spectrophotometer, capable of measuring at 340 nm, equipped with cells of optical path length 1 cm.

5.12 Parafilm[TM1)].

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.

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1) Parafilm[TM] is an example of a product 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 this product.

7 Preparation

7.1 Preparation of test sample

Transfer the test sample to a container with capacity about twice the volume of the sample and provided with an airtight lid. Close the container immediately. Mix the sample thoroughly by repeatedly shaking and inverting the container.

During preparation, avoid exposure of the test sample to the atmosphere in order to minimize adsorption of water.

7.2 Test portion

Weigh, to the nearest 1 mg, 1,0 g of the test sample in a 50 ml glass beaker (5.2).

7.3 Blank test

Carry out a blank test by proceeding as specified in 7.4 and 8.2, using all reagents but omitting the test portion.

7.4 Preparation of solution and deproteination

7.4.1 Dissolve the test portion (7.2) in about 20 ml of water preheated to between 40 °C and 50 °C, while stirring with the glass rod (5.9) or suitable means. Transfer the contents of the glass beaker quantitatively to a 100 ml one-mark volumetric flask (5.4) by rinsing the beaker with water. Cool the contents of the flask to about 20 °C.

7.4.2 Add to the solution (7.4.1), in the following order, 5,0 ml of potassium hexacyanoferrate(II) solution (4.1), 5,0 ml of zinc sulfate solution (4.2) and 10,0 ml of sodium hydroxide solution II (4.3.2), swirling thoroughly after each addition. Dilute with water to the 100 ml mark. Mix thoroughly and allow the mixture to stand at room temperature for 30 min.

7.4.3 Filter through a filter paper (5.8), discarding the first fraction of the filtrate.

Use of a centrifuge is a suitable alternative to filtration.

8 Procedure

CAUTION — Avoid contamination, especially with perspiration.

8.1 Test to check the activity of reagents

8.1.1 Whenever a new batch of reagents (4.6 to 4.10 inclusive) is prepared, or when such reagents have been kept in a refrigerator without being used for more than 2 weeks, or when restarting analytical work after a period of analytical inactivity, or whenever other conditions may justify it, perform the following test for the recovery of lactates.

8.1.2 Pipette 10 ml of lithium L-lactate solution (4.11) into each of two 100 ml one-mark volumetric flasks (5.4). Pipette 10 ml of lithium D-lactate solution (4.12) into each of two other 100 ml one-mark volumetric flasks (5.4). Determine the L-lactic acid and lactates content and the D-lactic acid and lactates content of the solutions in the two pairs of 100 ml flasks, proceeding as specified in 7.4.2, 7.4.3 and 8.2.

8.1.3 Calculate the lithium lactate content, w_L, expressed in milligrams per litre, using one of the following equations:

a) for the L-lactate solution:

$$w_L = 341 \times A$$

b) for the D-lactate solution:

$$w_L = 346 \times A$$

where

A is the numerical value of the absorbance at 340 nm, calculated in accordance with 8.2.1 and 8.2.2;

341 is the numerical value of the factor after substituting the molecular mass of lithium L-lactate (M_r = 96,1) and the final volume (V_1 = 2,24 ml) in 9.1 when L-lactate recoveries are evaluated;

346 is the numerical value of the factor after substituting the molecular mass of lithium D-lactate (M_r = 96,1) and the final volume (V_1 = 2,27 ml) in 9.1 when D-lactate recoveries are evaluated.

8.1.4 Taking into consideration the purity of the lithium L-lactate and lithium D-lactate used to prepare the solutions, the recovery of lithium L- or D-lactate from any of the flasks (8.1.2) shall be within the range 100 % ± 5 %. If the recoveries are not within this range, check the reagents, the operating technique, the accuracy of the pipettes and the condition of the spectrophotometer. Take the required action to obtain appropriate results. Repeat the test until satisfactory results are obtained.

8.2 Determination

8.2.1 Transfer using the required pipette (5.5) into the 1 cm cell of the spectrophotometer (5.11) according to the scheme in Table 1.

Table 1 — Procedure scheme

Pipette into spectrophotometer cells	Blank	D-Lactate standard	L-Lactate standard	Sample
Distilled water	1,000 ml	—	—	—
Standard (8.1.2)	—	1,000 ml	1,000 ml	—
Sample filtrate (7.4.3)	—	—	—	1,000 ml
Buffer solution, pH 10 (4.6)	1,000 ml	1,000 ml	1,000 ml	1,000 ml
NAD solution (4.7)	0,200 ml	0,200 ml	0,200 ml	0,200 ml
GPT suspension (4.10)	0,020 ml	0,020 ml	0,020 ml	0,020 ml
Mix the contents of the cell using a plastic paddle (5.10) or cover the 1 cm cell with parafilm (5.12) and invert several times. After mixing, leave the cell and its contents for 5 min before measuring the absorbance (A_{b0} and A_{s0}) against water at a wavelength of 340 nm.				
L-LDH suspension (4.8)	0,020 ml	—	0,020 ml	0,020 ml
D-LDH suspension (4.9)	0,050 ml	0,050 ml	—	0,050 ml
Exactly 45 min after mixing, measure the absorbance of the test solution (A_{b45} and A_{s45}) against water at a wavelength of 340 nm. Leave the cell again and after exactly 60 min after mixing measure the absorbance of the test solution (A_{b60} and A_{s60}) again against water at a wavelength of 340 nm.				

The L- or D-lactic acid and lactates content may be determined separately by adding either L-LDH (4.8) or D-LDH (4.9).

When only L-lactic acid and lactate are measured, the absorbance may be measured after 30 min and 45 min, respectively, after mixing.