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Milk — Determination of urea content — Enzymatic method using difference in pH (Reference method)

Lait — Détermination de la teneur en urée — Méthode enzymatique utilisant les fluctuations du pH (Méthode de référence)

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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 committee 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,

International Standard ISO 14637|IDF 195 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.

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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 IDF National Committees casting a vote,

International Standard ISO 14637|IDF 195 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.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Nitrogen compounds*, of the Standing Committee on *Main components of milk*, under the aegis of its project leader, Mr Ph. Trossat (FR).

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Milk — Determination of urea content — Enzymatic method using difference in pH (Reference method)

1 Scope

This International Standard specifies an enzymatic method for the determination of the urea content of milk by measurement of the difference in pH.

2 Terms and definitions

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

2.1
urea content
mass fraction of substances determined by the procedure specified in this International Standard

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NOTE The urea content is expressed in milligrams per litre.

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3 Principle

Urease is added to the test sample to split urea into ammonia and carbon dioxide. At pH 6,7, ammonia immediately hydrolyses thereby releasing hydroxyl ions, and carbon dioxide liberates protons that partly neutralize these hydroxyl ions. The balance between the ammonia and carbon dioxide hydrolysis and the resulting neutralization induces a change in pH. The pH change varies as a function of the urea content of the sample and is measured by using a differential pH analyser.

4 Reagents

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

4.1 Reagents for urea determination.

4.1.1 Buffer solution, pH 6,7.

Dissolve 1,777 g of potassium monohydrogenphosphate (K_2HPO_4), 1,388 g of potassium dihydrogen-phosphate (KH_2PO_4), 7,600 g of potassium chloride (KCl), 1,00 g of sodium azide (NaN_3), 0,010 g of acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide), 1,040 g of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 2 g of Triton X100, 1 g of Brij 35 and 20 ml of LM1[1)] in a 1 000 ml volumetric flask (5.5). Dilute to the mark with water and mix.

1) This detergent is available from Valetudo S.r.l., BG, Italy, and is an example of a suitable 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.

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The buffer solution may be kept for 6 months if stored at 4 °C.

4.1.2 Urease enzyme solution.

Dissolve 360 mg of lyophilized urease (EC 3.5.1.5) in 1 ml of a 50 % (volume fraction) aqueous solution of glycerol. The activity of the obtained urease enzyme solution shall be 2 100 units/ml ± 300 units/ml[2] .

The urease enzyme solution may be kept for 6 months if stored at 4 °C.

4.1.3 Urea standard solution.

Dissolve 1,000 g of dry urea (N_2H_4CO) (dried under vacuum in an oven at 90 °C ± 1 °C for 1 day), 7,45 g of potassium chloride (KCl) and 1,0 g of sodium azide (NaN_3) in a 1 000 ml volumetric flask (5.5). Dilute to the mark with water and mix.

The urea standard solution may be kept for 6 months if stored at 4 °C.

4.1.4 Zero milk.

Add 20 µl of urease solution (4.1.2) to 1 ml of raw milk. Mix and incubate the thus-prepared raw milk for 10 min in the water bath (5.3) set at 40 °C.

4.2 Reagents for cleaning and maintenance of electrodes.

4.2.1 Cleaning solution.

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Dissolve 1,742 g of potassium 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 Triton X100, 2 g of Brij 35 and 3 g of LM1[1] in a 1 000 ml volumetric flask (5.5). Dilute to the mark with water and mix.

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

4.2.2 Regenerating solution.

Use hydrochloric acid of concentration, c(HCl) = 0,1 mol/l.

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

4.2.3 Strong regenerating solution.

Dissolve 30 g of nitric acid (HNO_3) with a mass fraction of approximately 69 %, 30 g of hydrochloric acid (HCl) with a mass fraction of approximately 37 %, 30 g of sodium fluoride (NaF) and 1 g of Triton X100 in a 1 000 ml volumetric flask (5.5). Dilute to the mark with water and mix.

The strong regenerating solution may be kept for 1 year if stored 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.

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2) This unit (often called the International Unit or Standard Unit) is defined as the amount of enzyme which will catalyse the transformation of one micromole of substrate per minute under standard conditions.

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5.2 Micropipettes (positive displacement), of capacities 15 µl and 20 µl.

5.3 Water bath, capable of being maintained at 38 °C ± 1 °C and at 40 °C ± 1 °C.

5.4 Differential pH apparatus, generally operating according to the scheme shown in Annex A.

The arrangement and components used may be different.

The differential pH apparatus consists of peristaltic pumps to circulate liquids, a mixing chamber, two glass capillary flow-through electrodes (EL1 and EL2) and an electronic system for measurement.

5.5 One-mark volumetric flasks, of capacity 1 000 ml.

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not 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.

7 Preparation of test sample

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Heat the test sample in the water bath (5.3), set at 38 °C, to that temperature while mixing. Cool to 20 °C just before the preparation of the test portion.

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8 Procedure

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8.1 General

Since various types of differential pH apparatus (5.4) 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 (4.1.1) in the mixing chamber of the apparatus.

8.2 Blank determination

Fill the flow-through electrodes, EL1 and EL2, of the pH apparatus (5.4) with buffer solution (4.1.1). Measure the offset differential pH (D_1) between the electrodes. The difference between the two electrodes shall be between the limits ± 150 mpH (millipH) units.

Using a micropipette (5.2), add 15 µl of urease enzyme solution (4.1.2) to the mixing chamber of the apparatus and mix. Only fill the flow-through electrode EL2 with the buffer/enzyme mixture, Measure again the offset differential pH (D_2) between the two electrodes.

Calculate the difference in pH for the blank, ΔH_0, by using the following equation

$$\Delta H_0 = D_2 - D_1$$

where

ΔH_0 is the difference in pH units between the two offset differential pH measurements, D_1 and D_2, for the blank determination;

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