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Milk — Determination of alkaline phosphatase

Lait — Détermination de la phosphatase alcaline

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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 3356|IDF 63 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.

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This second edition of ISO 3356|IDF 63 cancels and replaces the first edition of ISO 3356:1975, which has been technically revised.

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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 at 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 IDF National Committees casting a vote.

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All work was carried out by the Joint ISO-IDF Action Team on *Heat treatment* of the Standing Committee on *Minor components and characterization of physical properties* under the aegis of its project leader, Mrs. M. Nicolas (FR).

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Milk — Determination of alkaline phosphatase

WARNING — The use of this International Standard may involve hazardous materials, operations and reagents. Persons using this International Standard should be familiar with normal laboratory practice. 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.

1 Scope

This International Standard specifies a method for the determination of alkaline phosphatase activity in milk.

The method applies to alkaline phosphatase activities not less than 1 µg of phenol per millilitre.

The method is also suitable for the determination of alkaline phosphatase activity in milk powder, buttermilk and buttermilk powder, whey and whey powder.

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2 Terms and definitions

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

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2.1
alkaline phosphatase activity
ALP activity
〈alkaline phosphatase activity in milk〉 quantity of phenol liberated by the sample determined according to the procedure specified in this International Standard

NOTE The alkaline phosphatase activity is expressed as the quantity of phenol, in micrograms, liberated by 1 ml of the sample or of reconstituted sample, under the conditions specified in this International Standard. Other International Standards (e.g. ISO 11816-1|IDF 155-1[6], ISO 22160|IDF 209[7]) express alkaline phosphatase activity in milliunits per litre. The literature gives information on the equivalence of the different units used to express the alkaline phosphatase activity.

3 Principle

The sample is diluted with a buffer at pH 10,6 and incubated at a temperature of 37 °C for 1 h. Under these conditions, any alkaline phosphatase present in the sample liberates phenol from the disodium phenylphosphate added. The phenol liberated reacts with a quinoneimide (dibromoquinonechlorimide) to produce dibromoindophenol (blue colour) which is measured photometrically at 610 nm.

4 Reagents

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

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4.1 Barium borate-hydroxide buffer solution

Dissolve 25,0 g of barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$], carbonate free, in water in a 500 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

Dissolve 11,0 g of boric acid (H_3BO_3) in water in another 500 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

Warm both solutions to 50 °C. Add one to the other and mix by stirring. Cool the solution obtained rapidly to about 20 °C. Adjust the pH of the solution, if necessary, to 10,6 ± 0,1 with an additional amount of barium hydroxide solution. Filter the solution through filter paper (5.10).

Store the filtered barium borate-hydroxide buffer solution in a tightly stoppered container. Before use, dilute the buffer solution with an equal volume of water.

4.2 Colour development buffer solutions

4.2.1 Colour buffer solution I

Dissolve 6,0 g of sodium metaborate ($NaBO_2$) or 12,6 g of $NaBO_2 \cdot 4H_2O$, and 20,0 g of sodium chloride (NaCl) in water in a 1 000 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

4.2.2 Colour buffer solution II

Transfer 10 ml of buffer solution I (4.2.1) to a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

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4.3 Buffer substrate solution

4.3.1 Disodium phenylphosphate dihydrate ($Na_2C_6H_5PO_4 \cdot 2H_2O$), containing no more than 0,01 % mass fraction phenol.

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4.3.2 Dissolve 0,1 g of disodium phenylphosphate dihydrate (4.3.1) in 100 ml of diluted barium borate-hydroxide buffer solution (4.1).

4.4 Zinc-copper precipitant solution

Dissolve 3,0 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and 0,6 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water in a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix.

4.5 2,6-Dibromoquinonechloroimide (BQC) solution, Gibb's reagent.

Dissolve 40 mg ± 1 mg of BQC ($C_6H_2Br_2ClNO$) in 10 ml of ethanol 96 % volume fraction.

Store the solution in a dark coloured bottle at 4 °C ± 2 °C. Reject if discoloured or more than 1 month old.

4.6 Copper sulfate solution

Dissolve 0,05 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water in a 100 ml one-mark volumetric flask (5.8). Make up to the 100 ml mark with water and mix.

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4.7 Sodium hydroxide solution, c(NaOH) = 0,5 mol/l.

4.8 Phenol standard solutions

4.8.1 Phenol standard stock solution

Transfer a weighed amount of 200 mg ± 2 mg of anhydrous phenol of purity higher than 99,5 % mass fraction into a 100 ml one-mark volumetric flask (5.8). Dissolve the phenol in water. Make up to the mark with water and mix.

The phenol standard stock solution remains stable at 4 °C ± 2 °C for 6 weeks.

4.8.2 Phenol standard working solutions

Pipette 10 ml of phenol standard stock solution (4.8.1) into a 100 ml one-mark volumetric flask (5.8). Make up to the mark with water and mix (1 ml contains 200 µg of phenol).

Use the diluted standard solution to prepare the appropriate phenol standard working solutions, containing 2 µg, 5 µg, 10 µg and 20 µg of phenol per millilitre respectively.

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.

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5.2 Photometer, suitable for measuring at a wavelength of 610 nm.

5.3 Water bath, capable of being maintained at 37 °C ± 1 °C under thermostatic control.

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5.4 Boiling water bath.

5.5 Vortex mixer.

5.6 Pipettes, capacities 0,1 ml, 1 ml, 5 ml and 10 ml, ISO 648[1], class A.

5.7 Glass test tubes, of appropriate volumes, with closures made from phenolic-free liners.

5.8 One-mark volumetric flasks, capacities 100 ml, 500 ml and 1000 ml, ISO 1042[3], class A.

5.9 Glass funnels, diameters about 60 mm and about 100 mm.

5.10 Filter paper, fast grade, diameters about 110 mm and about 185 mm.

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[2].

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

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7 Preparation of test sample

7.1 Preparation

Carefully mix the test sample before use. Usually, it is not necessary to pre-warm the test sample for proper mixing. If pre-warming is necessary, however, the temperature shall under no circumstances exceed 35 °C.

7.2 Milk powder, buttermilk powder and whey powder

Dissolve 10 g of test sample in 90 ml of water by heating, if necessary. The temperature, however, applied in dissolving the sample completely, shall under no circumstances exceed 35 °C.

7.3 Neutralization of acid test samples

If the test sample is acidic (pH < 7,0), adjust to neutral pH with sodium hydroxide solution (4.7)

8 Procedure

8.1 Preparation of the standard curve

8.1.1 Prepare a range of standard matching solutions in five glass test tubes (5.7) by pipetting 1 ml of water for the control or blank test tube and 1 ml of each of the four phenol standard working solutions (4.8.2) into each of the remaining four test tubes respectively. The standard test tubes contain 0 µg (control or blank), 2 µg, 5 µg, 10 µg and 20 µg of phenol respectively.

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8.1.2 Add to each test tube (8.1.1) 1 ml of copper sulfate solution (4.6), 5 ml of colour buffer solution II (4.2.2), 3 ml of water and 0,1 ml of BQC solution (4.5) and mix. Allow the colour to develop at room temperature for 30 min.

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8.1.3 Measure the optical density of the standard solutions against that of the control or blank solution at a wavelength of 610 nm.

8.1.4 Plot the optical density against the quantities of phenol in micrograms (8.1.1). Calculate the equation of the standard curve.

8.2 Determination

8.2.1 Avoid exposure to direct sunlight during the determination. Contamination with traces of saliva or perspiration can give false positive results and should be avoided.

8.2.2 Pipette into each of two test tubes (5.7) 1 ml of test sample or reconstituted sample. Use one of the tubes as control or blank test.

8.2.3 Stopper the blank test tube. Place the tube in a beaker with boiling water. Cover the beaker with aluminium foil. Heat the tube in the boiling water for 2 min while keeping the tube and the beaker covered with the foil to ensure the entire tube is heated. Then cool the tube to room temperature in cold water.

From this point on, treat the blank test tube and the tube with the test sample in the same way.

8.2.4 Add 10 ml of buffer substrate solution (4.3) to both the test sample tube (8.2.2) and the blank test tube (8.2.2) and mix.

8.2.5 Immediately heat both tubes in the water bath (5.3) at 37 °C for 60 min while mixing the contents occasionally.

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8.2.6 Place both tubes in a beaker with boiling water. Cover the beaker with aluminium foil. Heat both tubes in the boiling water for 2 min and cool them to room temperature in cold water.

8.2.7 Add 1 ml of zinc-copper precipitant solution (4.4) to each tube and mix thoroughly.

8.2.8 Filter the content of each tube through filter paper (5.10), discarding the first few millilitres. Pipette 5 ml of each filtrate into another glass test tube (5.7).

8.2.9 Add to each test tube (8.2.8) 5 ml of colour buffer solution I (4.2.1) and mix.

8.2.10 Add 0,1 ml of BQC solution (4.5) to each tube and mix. Allow the colour of both solutions to develop at room temperature for 30 min.

8.2.11 Measure the optical density of the test portion solution against that of the blank test solution at a wavelength of 610 nm.

8.2.12 If the optical density of the test sample, measured in 8.2.11, exceeds that of the phenol standard working solution containing 20 µg of phenol per millilitre as measured in 8.1.3, repeat the determination with an appropriate dilution of the test sample or the reconstituted test sample as follows.

Mix 1 volume of test sample or reconstituted test sample with an appropriate volume of the same test sample or reconstituted sample which has been carefully heated to boiling in order to inactivate the phosphatase. Continue as in 8.2.2.

9 Calculation and expression of results

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9.1 Calculation

9.1.1 Convert the optical density determined in 8.2.11 to micrograms of phenol by referring to the standard curve (8.1.4).

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9.1.2 Calculate the phosphatase activity, a_p, expressed in micrograms of phenol per millilitre of milk, by using the equation:

$$a_p = 2,4 \times m \times f_d$$

where

m is the mass, in micrograms, of phenol obtained in 9.1.1;

f_d is the dilution factor for the test sample or reconstituted sample (8.2.12), if needed (if not f_d = 1).

9.2 Expression of test results

Express the test results to one decimal place.

10 Precision

10.1 Interlaboratory test

The values for repeatability and reproducibility limits were derived from the results of interlaboratory tests carried out in accordance with ISO 5725-1[4] and ISO 5725-2[5]. The values are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

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