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DD ISO/TS 17193:2011

BSI Standards Publication

Milk — Determination of the lactoperoxidase activity — Photometric method (Reference method)

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It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis, so that information and experience of its practical application can be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the international organization responsible for its conversion to an international standard. A review of this publication will be initiated not later than 3 years after its publication by the international organization so that a decision can be taken on its status. Notification of the start of the review period will be made in an announcement in the appropriate issue of *Update Standards.*

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into an international Standard, to extend the life of the Technical Specification or to withdraw it. Comments should be sent to the Secretary of the responsible BSI Technical Committee at British Standards House, 389 Chiswick High Road, London W4 4AL.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Chemical analysis of milk and milk products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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TECHNICAL SPECIFICATION

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IDF/RM 208

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Milk — Determination of the lactoperoxidase activity — Photometric method (Reference method)

Lait — Détermination de l'activité de la lactoperoxydase — Méthode photométrique (Méthode de référence)

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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/TS 17193|IDF/RM 208 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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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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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/TS 17193|IDF/RM 208 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 Project Group on *Photometric lactoperoxidase activity* of the Standing committee on *Analytical methods for processing aids and indicators* under the aegis of its project leaders, Mr D. Tanzer (DE) and Mrs M. Nicolas (FR).

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TECHNICAL SPECIFICATION

ISO/TS 17193:2011(E)
IDF/RM 208:2011(E)

Milk — Determination of the lactoperoxidase activity — Photometric method (Reference method)

1 Scope

This Technical Specification specifies a photometric method for the determination of the lactoperoxidase activity in milk in amounts exceeding 50 U/l.

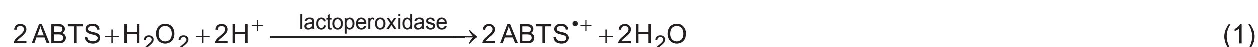
2 Terms and definitions

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

2.1
unit of lactoperoxidase activity
amount of lactoperoxidase enzyme that catalyses the transformation of 1 µmol of substrate per minute

3 Principle

In the presence of any lactoperoxidase enzyme derived from the sample, ABTS [2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid)] is catalytically converted to its radical cation ($ABTS^{\bullet +}$) at pH 6,0 and 25 °C. The amount of ($ABTS^{\bullet +}$) liberated per minute is proportional to the lactoperoxidase activity and measured photometrically at 420 nm. The liberation of the $ABTS^{\bullet +}$ is based on the following chemical reaction:



$$2\,ABTS + H_2O_2 + 2H^+ \xrightarrow{\text{lactoperoxidase}} 2\,ABTS^{\bullet +} + 2H_2O \qquad (1)$$

4 Reagents

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

4.1 Buffer solution, pH 6,0. Dissolve 0,72 g of disodium hydrogenphosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$) and 3,99 g of potassium dihydrogenphosphate (KH_2PO_4) in a beaker in a volume of about 450 ml of water. Adjust the pH of the solution, if necessary, to 6,0 ± 0,03 with a dilute sodium hydroxide, potassium hydroxide or phosphoric acid solution. Transfer the solution to a 500 ml volumetric flask (5.6). Make up to the mark with water and mix.

NOTE The buffer solution contains 8,09 mmol/l of disodium hydrogenphosphate dihydrate and 58,6 mmol/l of potassium dihydrogenphosphate.

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4.2 Hydrogen peroxide solution.

WARNING — Hydrogen peroxide is corrosive and should not be brought in contact with metals or readily flammable organic substances to prevent explosive mixtures forming.

Pipette 0,1 ml of 30 % mass fraction H_2O_2 into a 100 ml one-mark volumetric flask (5.6). Make up to the mark with water and mix.

Prepare the hydrogen peroxide solution immediately before use (see 4.3).

4.3 ABTS reagent solution, 2 mmol/l solution of the diammonium salt of 2,2-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) $[C_{18}H_{16}N_4O_6S_4(NH_4)_2]$.

Weigh 55 mg of the di-ammonium salt of ABTS into a 50 ml volumetric flask (5.6). Add about 30 ml of buffer solution (4.1) to dissolve the salt. Then add 1,0 ml of hydrogen peroxide solution (4.2). Make up to the 50 ml mark with the buffer solution and mix.

Prepare a fresh reagent solution every day.

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 pH-meter, capable of measuring 0,1 unit of pH, with a readability of 0,01 unit.

5.3 Spectrophotometer, capable of measuring absorbance at 420 nm, with a temperature-controlled cuvette holder capable of maintaining a temperature of 25 °C ± 0,5 °C.

5.4 Plastic cuvette, of pathlength 1,0 cm, provided with a lid.

NOTE The use of glass or quartz cuvettes can give results that are too low due to adsorption losses.

5.5 Air-displacement pipettes or **piston-operated pipettes**, ISO 8655-2[5], of nominal capacities 0,05 ml, 0,1 ml, 1,0 ml, and 2,0 ml.

5.6 One-mark volumetric flasks, of nominal capacities 5 ml, 10 ml, 25 ml, 50 ml, 100 ml and 500 ml, ISO 1042[2], class A.

5.7 Water bath or **dry bath**, capable of maintaining a temperature of 25 °C ± 0,5 °C.

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

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7 Procedure

7.1 Preparation of test sample dilution

Warm refrigerated test samples to room temperature and mix carefully. It is usually not necessary to reheat the test sample for proper mixing. In no case shall the temperature exceed 35 °C.

Depending on the lactoperoxidase content of the sample, mix the test sample with water in a suitable ratio by volume of at least 1→5.

To calculate the lactoperoxidase activity, the differential absorbance, ΔA, found has to be in the range 0,02 min^{-1} to 0,5 min^{-1}. Depending on the measurement range, the dilutions with water specified in Table 1 are applicable.

Table 1 — Test sample dilution

Measurement range U/l	**Dilution**
50 to 1 200	1→5
100 to 2 500	1→10
250 to 6 000	1→25

For dilution of UHT milk samples, a ratio of 1→5 (by volume) shall be used.

7.2 Determination

Prior to the photometric determination, adjust the photometer (5.3) to its zero level at a wavelength of 420 nm with air as reference (without a cuvette in the beam path).

Pipette 2 ml of ABTS reagent solution (4.3) into a cuvette (5.4). Cover the cuvette and place it in the water bath or the dry bath (5.7) set at 25 °C for the time necessary to attain this temperature into the cuvette.

Then add 0,05 ml of the test sample diluted as specified in 7.1. Immediately cover the cuvette again and mix its contents by carefully inverting. Place the cuvette quickly in the cuvette holder of the photometer (5.3) preheated at 25 °C.

Within 15 s after adding the diluted test sample to the reagent solution in the cuvette, measure the absorbance A_1 at a wavelength of 420 nm using air as reference. Record the absorbance A_2 after precisely 2 min.

IMPORTANT — For a reliable measurement, make sure that the interval between adding the diluted test sample to the reagent and reading A_1 is ≤15 s.

If using a 0,05 ml piston-operated pipette, ensure that the tip is rinsed beforehand with the sample dilution.

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8 Calculation and expression of results

8.1 Calculation

NOTE In the chemical reaction underlying this determination, there is a linear relation between the change in absorbance per minute and the lactoperoxidase activity. The activity of an enzyme in a dilute solution determined by absorption photometry conforms to the Lambert-Beer formula.

Calculate the lactoperoxidase activity, a_l, expressed as units per litre (U/l) of the sample using Equation (2):

$$a_l = \frac{V_1 \, \Delta A \, f \times 1000}{\varepsilon \, d \, V_2 \times 2} \tag{2}$$

where

V_1 is the total volume, in millilitres, of the test solution (7.2) ($V_1 = 2{,}05$ ml);

V_2 is the volume, in millilitres, of the sample dilution (7.1) ($V_2 = 20{,}05$ ml);

ΔA is the differential absorbance, $(A_2 - A_1)$/min;

ε is the absorptivity of the oxidized ABTS at 420 nm ($\varepsilon = 243{,}2$ µmol^{-1} cm^{-1});

d is the pathlength, in centimetres, of the cuvette ($d = 21{,}0$ cm);

f is the dilution factor ($f = 25$ for UHT milk);

1 000 is the factor for conversion to units per litre [U/l];

2 is the stoichiometric coefficient.

8.2 Expression of results

Express the results to the nearest whole number.

9 Precision

At the time of publication, no interlaboratory study fulfilling the requirements of ISO 5725-1[3] and ISO 5725-2[4] could be organized. Consequently this method is published as a Technical Specification and not as an International Standard.

10 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 Technical Specification (ISO/TS 17193 | IDF/RM 208:2012);

d) all operating details not specified in this Technical Specification, 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.

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[1] ISO 707 | IDF 50, *Milk and milk products — Guidance on sampling*

[2] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

[3] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[4] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[5] ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

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