

SRI LANKA STANDARD 735 : PART 17:2017
(ISO 9231 : 2008)
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METHODS OF
TEST FOR MILK AND MILK PRODUCTS
PART 17: DETERMINATION OF THE BENZONIC AND
SORBIC ACID CONTENTS

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard
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PART 17: DETERMINATION OF THE BENZONIC AND SORBIC ACID CONTENTS

SLS 735 : Part 17:2017
(ISO 9231 : 2008)

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SRI LANKA STANDARDS INSTITUTION
17, Victoria Place
Elvitigala Mawatha
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Sri Lanka Standard
METHOD OF TESTS FOR MILK AND MILK PRODUCTS
PART 17: DETERMINATION OF THE BENZONIC AND SORBIC ACID
CONTENTS

NATIONAL FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the council of the Sri Lanka Standards Institution on 2017-05-04.

The Standard prescribes the test methods for determining whether the material conforms to the requirements of the relevant individual Standards and thus form a necessary adjunct to series of Sri Lanka Standard Methods of tests of milk and milk products. However, keeping in view the experience gained during the years and various International Standards brought out by the International Organization for Standardization (ISO) on the subject of testing of milk and milk products, it was decided to publish it with a view of updating the existing methods of test and by incorporating those not covered earlier.

In order to accommodate large number of test methods within the scope of one standard, this Standard is published in several parts.

This part of the Standard is identical with **ISO 9231 : 2008 (IDF 139)** Milk and milk products - Determination of the benzoic and sorbic acid content published by International Organization for Standardization (ISO).

TERMINOLOGY AND CONVENTIONS

The text of the International Standard has been accepted as a suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

- a) Wherever the words “International Standard” appear referring to this standard, they should be interpreted as “Sri Lanka Standard”.
- b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards it is the current practice to use the full point at the base line as the decimal marker.
- c) Wherever page numbers are quoted, they are ISO page numbers.

Cross Reference

International Standards

Corresponding Sri Lanka Standard

ISO 1042 Laboratory glassware- one mark No corresponding Sri Lanka Standard
volumetric flask

INTERNATIONAL
STANDARD

SLS 735-17:2017

ISO
9231

IDF
139

First edition
2008-07-15

**Milk and milk products — Determination
of the benzoic and sorbic acid contents**

*Lait et produits laitiers — Détermination de la teneur en acide
benzoïque et en acide sorbique*



Reference numbers
ISO 9231:2008(E)
IDF 139:2008(E)

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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

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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 9231|IDF 139 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.

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.

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 9231|IDF 139 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 *Food additives and vitamins* of the Standing Committee on *Analytical methods for additives and contaminants* under the aegis of its project leader, Dr. M. Carl (DE).

ISO 9231|IDF 139:2008 cancels and replaces IDF 139:1987, which has been technically revised.

Milk and milk products — Determination of the benzoic and sorbic acid contents

1 Scope

This International Standard specifies a method for the determination of the benzoic and sorbic acid contents in milk and milk products.

The method is applicable to milk, dried milk, yogurt and other fermented milks, and cheese and processed cheese, and is suitable for measuring the contents of both compounds at levels of more than 5 mg/kg.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

3 Terms and definitions

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

3.1

benzoic and sorbic acid contents

mass fractions of benzoic acid and sorbic acid determined by the procedure specified in this International Standard

NOTE The benzoic acid and sorbic acid contents are expressed in milligrams per kilogram of product.

4 Principle

Fats and proteins are removed from a slightly alkaline solution of the product by Carrez precipitation. Following dilution of the resultant solution with methanol, the supernatant liquid is filtered. The benzoic acid and sorbic acid are separated by high-performance liquid chromatography (HPLC) on a reversed-phase C₁₈ column, measuring the absorbance at 227 nm and 250 nm.

5 Reagents

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

5.1 Methanol (CH₃OH).

5.2 Precipitating reagents, as follows:

5.2.1 Potassium hexacyanoferrate(II) solution

Dissolve 10,6 g of potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6] \cdot 3H_2O$) in water in a 100 ml one-mark volumetric flask (6.3). Dilute to the mark with water and mix.

NOTE The 100 ml solution is sufficient for 40 runs.

5.2.2 Zinc acetate solution

Dissolve 21,9 g of zinc acetate dihydrate [$(CH_3COO)_2Zn \cdot 2H_2O$] and 32 ml of acetic acid (CH_3COOH) in water in a 100 ml one-mark volumetric flask (6.3). Dilute to the mark with water and mix.

If the zinc acetate dihydrate does not dissolve completely, heat the 100 ml flask and its contents in a water bath (6.2) maintained at 70 °C while swirling. When the zinc acetate dihydrate has dissolved completely, cool the solution thus obtained back to room temperature. Dilute to the mark with water and mix again.

NOTE The 100 ml solution is sufficient for 40 runs.

5.3 Phosphate buffer solution, pH 6,7.

Dissolve 2,5 g of potassium dihydrogen phosphate (KH_2PO_4) and 2,5 g of potassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$) in 1 l of water and mix. Filter the solution thus obtained through the solvent filtration system (6.8).

5.4 Mobile phase, for HPLC.

Mix 10 volumes of methanol (5.1) with 90 volumes of phosphate buffer solution (5.3). Remove any dissolved gas by applying a slight vacuum.

5.5 Sodium hydroxide solution, $c(NaOH) = 0,1$ mol/l.

Dissolve 4,0 g of sodium hydroxide pellets in water in a 1 000 ml one-mark volumetric flask (6.3). Dilute to the mark with water and mix.

5.6 Sulfuric acid, $c(H_2SO_4) = 0,5$ mol/l.

Pour cautiously 15 ml of concentrated sulfuric acid, with a mass fraction of at least 95 % to 98 %, into 250 ml of water in a 500 ml one-mark volumetric flask (6.3) and allow to cool. Dilute to the mark with water and mix.

5.7 Sorbic acid and benzoic acid standard solutions, as follows:

5.7.1 Stock standard solution

Dissolve 50 mg of sorbic acid and 50 mg of benzoic acid in methanol (5.1) in a 100 ml one-mark volumetric flask (6.3). Dilute with methanol (5.1) to the mark and mix.

The stock standard solution is stable for at least three weeks if stored in a refrigerator at between 4 °C and 7 °C.

5.7.2 Working standard solution

Mix 500 ml of methanol (5.1) with 500 ml of water to obtain an aqueous-methanol solution with a volume fraction of 50 %.

On the day of use, pipette 5 ml of stock standard solution (5.7.1) into a 250 ml one-mark volumetric flask (6.3). Dilute with the 50 % aqueous-methanol solution to the mark and mix. The resulting working standard solution contains 10 µg/ml of both the sorbic and benzoic acid.

6 Apparatus

Usual laboratory equipment and, in particular, the following:

- 6.1 Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.
- 6.2 Water bath**, capable of maintaining a temperature of $70\text{ °C} \pm 2\text{ °C}$.
- 6.3 One-mark volumetric flasks**, of capacities 100 ml, 250 ml, 500 ml and 1 000 ml, meeting the requirements for class A as specified in ISO 1042.
- 6.4 Liquid chromatograph**, equipped with a pump capable of generating pressures of up to 4,37 MPa (6 000 psi), an injector, a dual-wavelength or diode-array UV detector, and a recorder or integrator.
- The dual-wavelength detector shall have a 1 cm light path flow-through optical cell and shall be capable of measuring absorbance at 227 nm (for benzoic acid) and 250 nm (for sorbic acid).
- 6.5 HPLC column**, made of stainless steel, of length 250 mm, of internal diameter 4 mm, containing a reversed-phase, octadecyl (ODC) treated silica adsorbent, i.e. Micro-Bondapak C₁₈¹⁾ or similar.
- 6.6 Syringe for HPLC**.
- 6.7 Sample clarification kit**, for membrane filtration of sample extracts, with filters of pore size 0,45 µm for aqueous solutions.
- 6.8 Solvent filtration system**, for membrane filtration of solvents, with filters of pore size 0,45 µm for aqueous solutions.
- 6.9 Ultrasonic bath**.
- 6.10 pH-meter**.

7 Sampling

A representative sample should have 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 [1].

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

8 Preparation of test sample

8.1 Yogurt and other fermented milks

Prior to starting the procedure, homogenize the sample by warming it gently to 40 °C while stirring. Weigh, to the nearest 0,1 g, 20 g of the homogenized sample into a 100 ml one-mark volumetric flask (6.3).

1) Micro-Bondapak C₁₈[®] is the name of a product available commercially. This information is given for the convenience of the users of this International Standard but does not constitute an endorsement by either ISO or IDF of the product named.

8.2 Other milk products

Weigh, to the nearest 0,1 g, 3 g of sample into a 20 ml glass beaker. Disperse the test sample completely in 10 ml water added in small portions while stirring with a glass rod.

Transfer the solution quantitatively to a 100 ml one-mark volumetric flask (6.3), rinsing the beaker twice with 5 ml of water.

9 Procedure

9.1 Precipitation of fats and proteins and clarification

Add 25 ml of sodium hydroxide solution (5.5) to the test sample (see 8.1 or 8.2) and mix. Either place the flask and its contents in an ultrasonic bath (6.9) for 15 min or place the flask and its contents in a water bath (6.2) maintained at 70 °C and heat for 15 min, subsequently allowing the solution to cool to room temperature.

Adjust the pH to 8 ± 1 by adding sulfuric acid solution (5.6) while mixing. Then add 2 ml of potassium hexacyanoferrate(II) solution (5.2.1) and 2 ml of zinc acetate solution (5.2.2) to precipitate the fats and proteins. Shake vigorously and allow the suspension thus obtained to stand for 15 min. Subsequently, add about 40 ml of methanol (5.1) and mix. Cool to room temperature.

Dilute with methanol (5.1) to the mark and mix again. Allow the mixture to stand for another 15 min. Filter the supernatant liquid using the sample clarification kit (6.7).

9.2 High-performance liquid chromatography (HPLC)

Perform the HPLC analysis at room temperature, using the mobile phase (5.4) at a flow rate of about 1,2 ml/min. Allow the system to equilibrate for at least 30 min before injecting any solutions.

Then inject 5 µl to 20 µl of the clarified solution prepared in 9.1 and an equal volume of working standard solution (5.7.2). Monitor column effluent by UV detection at 227 nm and at 250 nm, using consecutive detection at the two wavelength settings or simultaneous dual-wavelength detection.

NOTE Provided that the calibration curve has been found to be linear, single-point calibration is sufficient.

The amount of methanol in the standard and the test sample solutions is much higher than that in the mobile phase, and this may influence peak shape and peak separation in certain cases. In such cases, check the peak shape and peak separation by comparing them with those obtained with injections of stock standard solution (5.7.1) in which the methanol (5.1) has been replaced by mobile phase (5.4), using equivalent volumes.

The approximate retention times for benzoic acid and sorbic acid under these conditions are 5,5 min and 7 min, respectively. If the peaks produced by either compound go off scale, prepare a suitable dilution in 50 % aqueous methanol and inject 5 µl to 20 µl of the diluted solution to obtain suitable peak heights.

To detect whether any interfering compound is co-eluting with the sorbic acid, check the ratio of the UV signals, both at 250 nm and 227 nm.

When analysing milk or dried milk, a third peak, eluting after about 8 min, is observed. This peak is produced by hippuric acid, a natural constituent. The hippuric acid peak may partly overlap that of sorbic acid. The column resolution between sorbic and hippuric acid should therefore preferably be >1 .

10 Calculation and expression of results

10.1 Calculation

Calculate the sorbic acid content, w_s , and/or the benzoic acid content, w_b , both expressed in milligrams per kilogram, using the following equation:

$$w_s \text{ (or } w_b) = \frac{H_{ts} \times m_s \times V_1}{H_{st} \times m \times V_2} \times 1000$$

where

H_{ts} is the peak height or area, in appropriate units, given by the test solution (see 9.2);

H_{st} is the peak height or area, in the same units, given by the working standard (see 9.2);

m_s is the mass, in micrograms, of working standard injected (5.7.2);

m is the mass, in grams, of the test sample (see 8.1 or 8.2);

V_1 is the volume, in millilitres, of the extract prepared in 9.1 (= 100 ml);

V_2 is the volume, in microlitres, of test solution injected (see 9.2).

10.2 Expression of results

Express the results to the nearest whole number.

11 Precision

11.1 Interlaboratory testing

The values derived from interlaboratory tests may not be applicable to content ranges and matrices other than those given.

The values of repeatability and reproducibility have been derived from the results of two interlaboratory trials carried out in 1984 and 2004 (see Annex A). The tests were carried out on samples with benzoic acid and sorbic acid contents ranging from 6 mg/kg to 920 mg/kg.

11.2 Repeatability

The absolute difference between two individual 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 be greater than $2,235 + 0,031 \bar{w}_s$ (or \bar{w}_b) mg/kg in not more than 5 % of cases.

11.3 Reproducibility

The absolute difference between two individual test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will be greater than $8,987 + 0,130 \bar{w}_s$ (or \bar{w}_b) mg/kg in not more than 5 % of cases.

12 Test report

The test report shall specify:

- a) all information necessary for complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this International Standard;
- d) all operational details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the result(s);
- e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Annex A (informative)

Interlaboratory trials

The values for the repeatability and reproducibility have been derived from the results of two international interlaboratory trials carried out in 1984^[4] and 2004^[6], respectively.

The results obtained in the two trials were subjected to statistical analysis in accordance with ISO 5725:1981 and ISO 5725-2:1994, respectively, to give the precision data shown in Table A.1.

Table A.1 — Results of interlaboratory trials

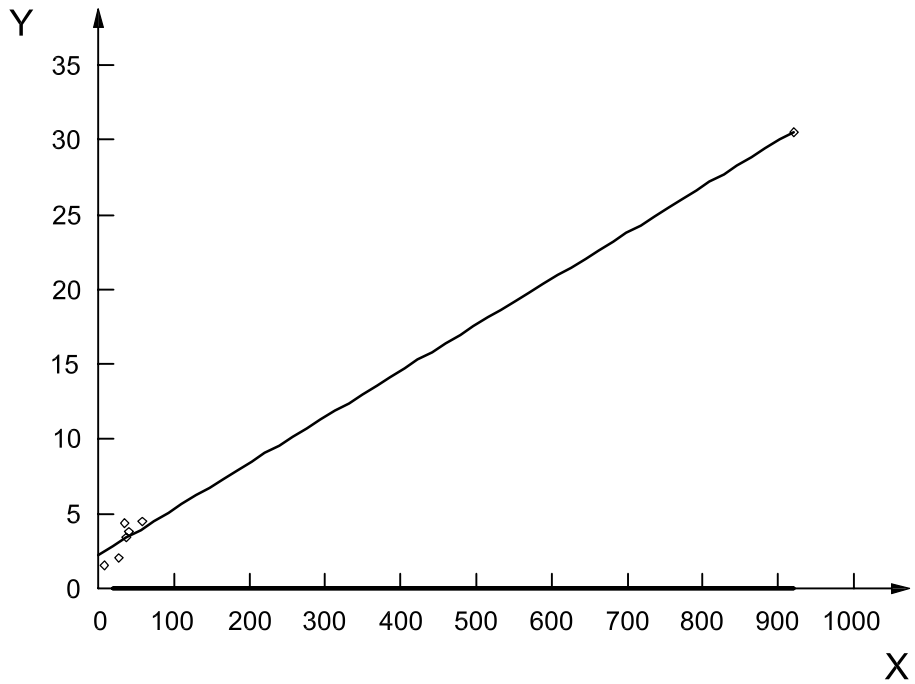
Parameter	Benzoic acid				Sorbic acid		
	1984 ^[4]	1984 ^[4]	2004 ^[6]	2004 ^[6]	1984 ^[4]	2004 ^[6]	2004 ^[6]
Samples	A ^a	B ^a	C ^b	D ^b	B+ ^c	C ^b	D ^b
Total number of laboratories	14	14	9	14	14	14	14
Outliers	2	2	1	1	3	1	0
Number of laboratories remaining	12	12	8	13	11	13	14
Mean value, mg/kg	26,4	40,6	7,61	35,0	36,1	920	57,0
Repeatability, r , mg/kg	2,0	3,8	1,6	4,4	3,4	30,5	4,5
Repeatability relative to mean, r_{rel} , %	7,7	9,4	20,5	12,7	9,4	3,3	7,8
Repeatability standard deviation, s_r , %	0,7	1,3	0,6	1,6	1,2	10,8	1,6
RSD _{r} , %	2,7	3,3	7,2	4,5	3,3	1,2	2,8
Reproducibility, R , mg/kg	13,5	15,3	11,0	9,9	12,1	128,9	18,6
Reproducibility relative to mean, R_{rel} , %	51,1	37,7	144	28,4	33,5	14,0	32,6
Reproducibility standard deviation, s_R , mg/kg	4,8	5,4	3,9	3,5	4,3	45,6	6,6
RSD _{R} , %	18,1	13,3	50,9	10,0	11,9	5,0	11,5
^a Samples A and B: Flavoured yogurt. ^b Samples C and D: Processed cheese (see also additional information in Reference [6]). ^c Spiked with 40 mg/kg of sorbic acid during preparation.							

Using linear regression analysis, the following regression line, $x = b + ay$, is obtained, with a coefficient of correlation of 0,997 348 6, for $n = 7$, $s = 0,824 9$ and $t_{0,95} = 2,57$ (see Figure A.1):

$$\text{for sorbic acid, } r = 2,235 408 8 + 0,030 778 7 \bar{w}_s \text{ and, for benzoic acid, } r = 2,235 408 8 + 0,030 778 7 \bar{w}_b.$$

Using linear regression analysis, the following regression line, $x = b + ay$, is obtained, with a coefficient of correlation of 0,998 965 3, for $n = 7$, $s = 2,181 5$ and $t_{0,95} = 2,57$ for the reproducibility (see Figure A.2):

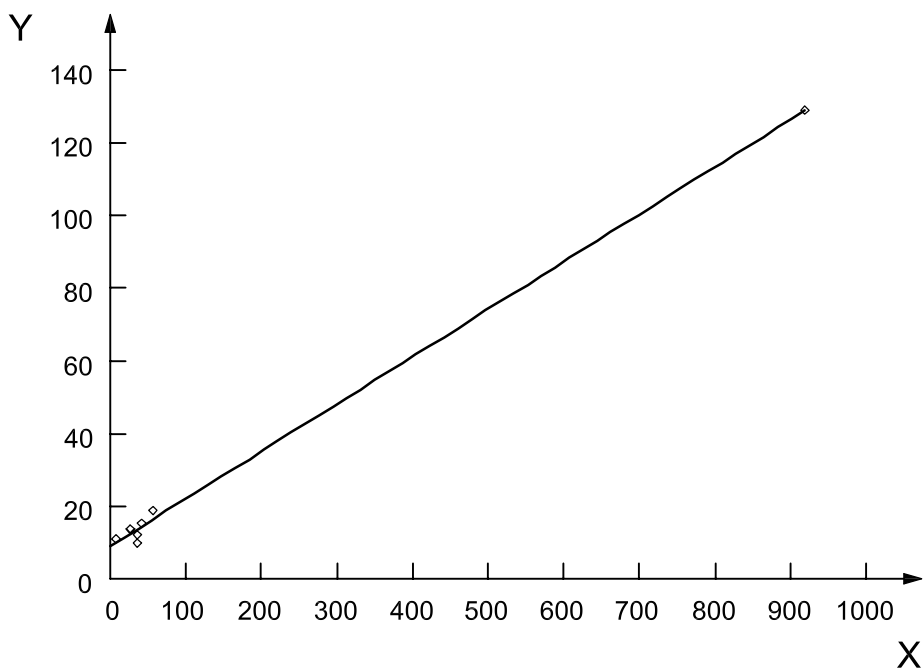
$$\text{for sorbic acid, } R = 8,987 496 5 + 0,130 466 3 \bar{w}_s \text{ and, for benzoic acid, } R = 8,987 496 5 + 0,130 466 3 \bar{w}_b.$$



Key

- X mean value (mg/kg)
- Y repeatability, r (mg/kg)

Figure A.1 — Repeatability as a function of mean value



Key

- X mean value (mg/kg)
- Y reproducibility, R (mg/kg)

Figure A.2 — Reproducibility as a function of mean value

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