INTERNATIONAL STANDARD

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)

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

Published in Switzerland

Contents

Page

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 C_{18} 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 (CH_3OH).

5.2 Precipitating reagents, as follows:

5.2.1 Potassium hexacyanoferrate(II) solution

Dissolve 10,6 g of potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6].3H_2O$) 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 [$(CH_3COO)_2Zn.2H_2O$] 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 ($K_2HPO_4.3H_2O$) 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 °C ± 2 °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 C_{18}[1)] 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 C_{18}® 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.