

GB

Nation Standard of the People's Republic
of China

GB21703-2010

食品安全国家标准

乳与乳制品中苯甲酸和山梨酸的测定

National food safety standard

Determination of benzoic and sorbic acid in milk and milk
products

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Forward

This standard adopts standard of IDF139:1987 Milk, Dried Milk, Yogurt and other Fermented Milks-Determination of Benzoic and Sorbic Acid.

Appendix A is reference appendix.

Replaced previous published standards:

——GB 21703-2008。

National food safety standard

Determination of benzoic and sorbic acid in milk and milk products

1 Scope

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

The standard is applicable to the determination of the benzoic and sorbic in milk and milk products.

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.

3 Principle

Fats and proteins are removed. Following dilution of the resultant solution with methanol, the supernatant liquid is filtered. The benzoic acid and sorbic acid are determined by Reversed Phase Liquid Chromatography.

4 Reagents and Materials

Unless otherwise specified, use only reagents of recognized analytical grade. Water is the first grade water according to GB/T6682.

4.1 Methanol (CH₃OH): Chromatographic pure

4.2 Potassium hexacyanoferrate(II) solution(92g/L): dissolve 106 g of potassium hexacyanoferrate(II) trihydrate (K₄[Fe(CN)₆].3H₂O) in water in a 1000 ml volumetric flask, dilute to the mark with water and mix.

4.3 Zinc acetate solution(183g/L): dissolve 219 g of zinc acetate dihydrate [(CH₃COO)₂Zn.2H₂O] and 32 ml of acetic acid (CH₃COOH) in water in a 1000 ml volumetric flask, dilute to the mark with water and mix.

4.4 Phosphate buffer solution(pH 6.7): dissolve 2.5 g of potassium dihydrogen phosphate (KH₂PO₄) and 2.5 g of potassium hydrogen phosphate trihydrate (K₂HPO₄.3H₂O) in a 1000 ml volumetric flask, dilute to the mark with water and mix, filter the solution thus obtained through the filtration membrane (4.9).

4.5 Sodium hydroxide solution(0.1 mol/L): dissolve 4 g of Sodium hydroxide (NaOH) in water in a 1000 ml volumetric flask, and dilute to the mark with water and mix.

4.6 Sulfuric acid solution(0.5 mol/L): Pour slowly 30 ml of concentrated sulfuric acid

(H₂SO₄) into 500 ml of water with agitation. Transfer the solution into a 1000 ml volumetric flask after cooling down to room temperature and dilute to the mark with water and mix.

4.7 Aqueous-methanol solution: the volume fraction is 50 %.

4.8 Standard solution

4.8.1 Stock standard solution of sorbic acid and benzoic acid: contains 500 µg/ml of both the sorbic and benzoic acid.

Dissolve 50 mg of standard sorbic acid and 50 mg of standard benzoic acid in methanol (4.1) in a 100 ml volumetric flask, dilute with methanol to the mark and mix. The stock standard solution's shelf-life is 2 months if stored in a refrigerator.

4.8.2 Sorbic acid and benzoic acid working standard solutions: contains 10 µg/ml of both the sorbic and benzoic acid.

Pipette 5 ml of Sorbic acid and benzoic acid stock standard solution (4.8.1) respectively into a 250 ml volumetric flask, dilute with the 50 % aqueous-methanol solution (4.7) to the mark and mix. The resulting working standard solution contains 10 µg/ml of both the sorbic and benzoic acid. The working standard solution's shelf-life is 5 days if stored in a refrigerator.

4.9 Filtration membrane: 0.45µm

5 Apparatus

5.1 High-performance liquid chromatography: equipped with UV detector.

5.2 Analytical balance: with a sensibility of 0.1mg,0.01g.

6 Procedure

6.1 Preparation of test sample

6.1.1 Liquid Sample

Take out the milk and dairy products beforehand if stored in a refrigerator and warm it gently to room temperature. Weigh 20 g (to the nearest 0,01 g) of sample into a 100ml volumetric flask.

6.1.2 Solid Sample

Weigh 3 g(to the nearest 0,01 g) of sample into a 100ml volumetric flask, disperse the test sample completely in 10 ml water added while stirring with a glass rod.

6.2 Extraction and Clarification

Add 25 ml of sodium hydroxide solution (4.5) to the test sample (6.1) and mix. Either place the flask and its contents in an ultrasonic bath for 15 min or place the flask and its contents in a water bath maintained at 70 °C and heat for 15 min. After cooling, adjust the pH to 8(use pH meter or pH paper) by adding sulfuric acid solution (4.6). Then add 2 ml of potassium hexacyanoferrate (II) solution (4.2) and 2 ml of zinc acetate solution (4.3). Shake vigorously, then stand for 15 min and cool to room temperature. Dilute with methanol (4.1) to the mark and mix. Allow the mixture to stand for another 15 min. Filter the supernatant liquid using the sample filtration membrane (4.9). Collect the filtrate as the test sample for the HPLC (5.1) determination.

6.3 Chromatographic conditions

HPLC column: C₁₈, 250 mm×4.6 mm, 5 µm.

Mobile phase: methanol (4.1)- Phosphate buffer solution (4.4)= 1+9.

Flow rate: 1.2 mL/min.

Wavelength Detection: 227 nm.

Column Temperature: room temperature.

Sample Size: 10 µL.

6.4 Determination

Precisely pipette 10 µl of test sample solution (6.2) and sorbic acid and benzoic acid working standard solutions (4.8.2), duplicate. Calculate the content by the peak area of chromatogram. Under above chromatographic condition, benzoic acid and sorbic acid will appear in sequence. The liquid chromatogram figure of the standard solutions refers to figure A.1.

7 Expression of results

Calculate the sorbic acid content, using the following equation(1):

$$X = \frac{A \times C_s \times V}{A_s \times m} \dots\dots\dots(1)$$

Where,

X —the sorbic acid ,the benzoic acid content, both expressed in milligrams per kilogram(mg/kg);

A —the peak area of the sorbic acid, the benzoic acid in sample;

A_s—the peak area of the sorbic acid, the benzoic acid in standard solution;

C_s—the concentration, in micrograms per milliliter (µg/ml), of the working standard solution;

V—the volume, in milliliters (ml), of test sample after diluted;

m—the mass, in grams, of the test sample.

The result should be expressed by arithmetic mean of two results of duplicate determination. Express the result to three decimal places.

8 Precision

The absolute difference between results of duplicate determination should not exceed 10% of the arithmetic mean.

9 Other

The detecton limit of the benzoic and sorbic acid in this method is 1mg/kg.

Appendix A

(Appendix of Reference)

Typical chromatogram of the sorbic acid and the benzoic acid

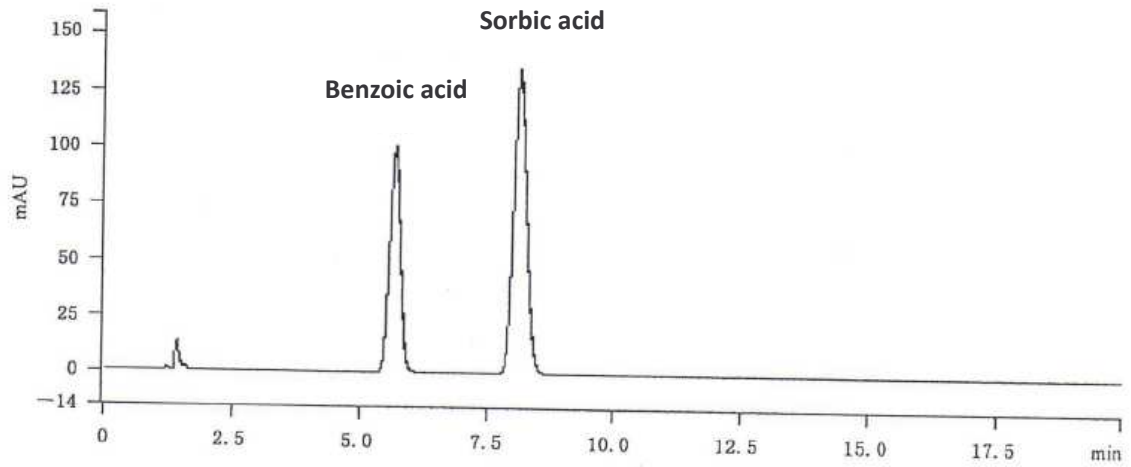


Figure A.1 Typical chromatogram of the sorbic acid and the benzoic acid