

Translated English of Chinese Standard: GB5009.255-2016

www.ChineseStandard.net → Buy True-PDF → Auto-delivery.

Sales@ChineseStandard.net

GB

NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.255-2016

**National food safety standard -
Determination of fructan in food**

食品安全国家标准

食品中果聚糖的测定

Issued on: August 31, 2008

Implemented on: March 01, 2017

Issued by: National Health and Family Planning Commission of PRC

Table of Contents

1 Scope.....	3
2 Principle	3
3 Reagents and materials	3
4 Apparatus.....	5
5 Analysis steps	6
6 Expression of analysis results.....	8
7 Precision	9
8 Other.....	10
Annex A Fructose standard solution, milk powder sample chromatogram	11
Annex B Method for determination of enzyme activity	14

National food safety standard - Determination of fructan in food

1 Scope

This Standard specifies the method for determination of fructan content in food by ion chromatography.

This Standard is applicable to the determination of individually added oligofructose, polyfructose or inulin content in milk and dairy products, infant formula, infant cereal supplements, solid beverages, blended wine.

2 Principle

The specimen is soaked and extracted by hot water. Sucrose in the sample solution is hydrolyzed by sucrase into glucose and fructose. Glucose and fructose are reduced to the corresponding sugar alcohol by sodium borohydride. Excess sodium borohydride is neutralized by acetic acid. The fructan in the sample solution is hydrolyzed by inulinase into fructose and glucose. Use ion chromatography-pulse amperometric detector to determine the fructose content. The content of fructan is obtained by conversion factor.

3 Reagents and materials

3.1 Reagents

Unless otherwise stated, the reagents used in this method are analytically pure and the water is grade one water specified in GB/T 6682.

3.1.1 Sodium hydroxide (NaOH).

3.1.2 Maleic acid (C₄H₄O₄).

3.1.3 Sucrase: from yeast, enzyme activity $\geq 300\text{U}$.

3.1.4 Sodium borohydride (NaBH₄).

3.1.5 Glacial acetic acid (CH₃COOH).

3.1.6 Sodium acetate trihydrate (CH₃COONa·3H₂O).

3.1.7 Inulinase: derived from *Aspergillus niger*, enzyme activity $\geq 100000\text{U}$.

3.1.8 50% sodium hydroxide solution (NaOH): chromatographically pure.

3.1.9 Anhydrous sodium acetate (CH_3COONa): purity $\geq 99.0\%$.

3.1.10 Nitrogen (N_2): purity ≥ 99.9 .

3.2 Reagent preparation

3.2.1 Sodium hydroxide solution (1 mol/L): Weigh 40 g of sodium hydroxide (to the nearest of 0.01 g). Dissolve in water and dilute to 1000 mL. It may be stored at room temperature for 2 months.

3.2.2 Sodium maleate buffer solution (100 mmol/L, pH 6.5): Weigh 1.16 g of maleic acid (to the nearest of 0.01 g) in a 150mL beaker. Add about 70 mL of water to dissolve. Use 1 mol/L sodium hydroxide solution to adjust pH 6.5. Use water to dilute to 100 mL. Store at 4°C. It may be stored for 3 months.

3.2.3 Sucrase solution (4.5 U/mL): Dissolve sucrase (activity is 300 U) in 66 mL of sodium maleate buffer solution. Dispense into 2 mL centrifuge tubes. Store at -20°C. It may be stored for 6 months. Before use, it needs to determine enzyme activity.

3.2.4 Sodium hydroxide solution (50 mmol/L): Weigh 2 g of sodium hydroxide (to the nearest of 0.01 g). Dissolve in water and dilute to 1000 mL. It may be stored at room temperature for 2 months.

3.2.5 Sodium borohydride solution (10 mg/mL): Accurately weigh an appropriate amount of sodium borohydride (to the nearest of 0.001 g) in polypropylene centrifuge tube. Use 50 mmol/L sodium hydroxide solution to dissolve. The final mass concentration is 10 mg/mL. Prepare when required.

3.2.6 Acetic acid solution (200 mmol/L): Pipette 0.6 mL of glacial acetic acid. Use water to dilute to 50 mL. Store at 4°C. It may be stored for 2 months.

3.2.7 Sodium acetate solution (200 mmol/L): Weigh 1.36 g of sodium acetate trihydrate (to the nearest of 0.01 g). Dissolve in water and dilute to 50 mL. Store at 4°C. It may be stored for 2 months.

3.2.8 Sodium acetate buffer solution (pH 4.5): Pipette 14 mL of acetic acid solution and 11 mL of sodium acetate solution to mix. Use water to dilute to 50 mL. Prepare when required.

3.2.9 Inulinase solution (455 U/mL): Dissolve inulinase (activity is 10000 U) in 22 mL of sodium acetate buffer solution. Dispense into 2mL centrifuge tubes. Store at -20°C. It may be stored for 6 months. Before use, it needs to determine enzyme activity.

4.4 Vortex oscillator.

4.5 Constant temperature water bath shaker: temperature control accuracy of $\pm 1^{\circ}\text{C}$.

4.6 Centrifuge: rotating speed ≥ 3000 r/min.

5 Analysis steps

5.1 Specimen preparation

5.1.1 Specimen pretreatment

5.1.1.1 Solid sample: Use "quadruple method" to divide to about 200 g of sample. Crush by a mincer. Mix well, for use.

5.1.1.2 Liquid sample: Load the specimen into a container with lid that is twice specimen volume. Shake well, for use.

5.1.2 Extraction

Accurately weigh 1g~5g (to the nearest of 0.001 g, at least containing 5 mg of fructan) of specimen into a 150mL Erlenmeyer. Add into about 50 mL of $80^{\circ}\text{C} \pm 1^{\circ}\text{C}$ hot water. Place in the $80^{\circ}\text{C} \pm 1^{\circ}\text{C}$ constant temperature water bath shaker. Shake at 150 r/min for 15 min. Take out. Cool to room temperature. Transfer to a 100mL volumetric flask. Use water to rinse the Erlenmeyer three times. Set volume. Shake well. The solution is filtered or centrifuged through a filter paper. The filtrate or supernatant determines the dilution factor based on the linear range of the standard curve, for use.

Take 200 μL of the above alternate sample solution in a 10mL stoppered glass test tube. Estimate the possible sucrose content in the sample solution. Add 300 μL of sucrase solution per milligram of sucrose. Vortex and mix well. Place in the $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ constant temperature water bath shaker. Shake at 150 r/min for 60 min. Add 300 μL of sodium borohydride solution. Vortex and mix well. Place in the $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ constant temperature water bath shaker. Shake at 150 r/min for 30 min. Take out. Cool to room temperature. Add into 750 μL of acetic acid solution. Place still for 10 min. Estimate the possible fructan content in the sample solution. Add 1.2 mL of inulinase solution per milligram of fructan. Vortex and mix well. Place in the $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ constant temperature water bath shaker. Shake at 150 r/min for 30 min. Take out. Cool to room temperature. Transfer to a 10mL volumetric flask. Use water to rinse the glass tube three times. Set volume. Shake well.

Activate the purification column. The sample solution passes through a $0.22\mu\text{m}$ aqueous phase filter membrane and a purification column in sequence. Discard

Annex B

Method for determination of enzyme activity

B.1 Method for determination of sucrase activity

B.1.1 Principle

Sucrase (EC 3.2.1.26), is also known as invertase. It can cut the fructose glycosidic bond of sucrose from the end of fructose so as to make hydrolysis of sucrose to produce glucose and fructose. Glucose and fructose are reducing sugars. The content can be measured by 3,5-dinitrosalicylic acid colorimetric method so as to measure the size of enzyme activity. Since the sucrase is easily inactivated under alkaline conditions, the alkali can be used to terminate the reaction. 3,5-dinitrosalicylic acid and reducing sugars form a brown-red amino compound. Measure at a maximum absorption wavelength of 540 nm. The absorbance value is linear with the reducing sugar content within a certain concentration range, so it can be used for the determination of reducing sugar content to determine the activity of sucrase.

Sucrase activity is defined as: The amount of enzyme (activity) required to hydrolyze 1 μmol of glucose per minute at 37°C and pH 6.5 is defined as one enzyme activity unit.

B.1.2 Reagents

Unless otherwise stated, the reagents used in this method are analytically pure and the water is grade one water specified in GB/T 6682.

B.1.2.1 Glucose standard substance ($\text{C}_6\text{H}_{12}\text{O}_6$): purity $\geq 99.0\%$.

B.1.2.2 Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$): purity $\geq 99.0\%$.

B.1.2.3 Sodium hydroxide (NaOH).

B.1.2.4 Maleic acid ($\text{C}_4\text{H}_4\text{O}_4$).

B.1.2.5 Dinitrosalicylic acid ($\text{C}_7\text{H}_4\text{N}_2\text{O}_7$).

B.1.2.6 Sodium potassium tartrate ($\text{C}_4\text{H}_4\text{KNaO}_6$).

B.1.2.7 Phenol ($\text{C}_6\text{H}_5\text{OH}$).

B.1.2.8 Sodium sulfite (Na_2SO_3).

B.1.3 Reagent preparation

B.1.3.1 2.5 μmol/mL glucose standard solution: Accurately weigh 0.045 g (to the nearest of 0.1 mg) of glucose standard substance that has been dried to constant weight at 80°C in a 50 mL beaker. Add into about 10 mL of water. After the glucose is dissolved, cool to room temperature. Use water to dilute to a 100 mL volumetric flask. Shake well. Store at 4°C. It may be stored for 1 month.

B.1.3.2 Sodium hydroxide solution (1 mol/L): Weigh 40 g of sodium hydroxide (to the nearest of 0.01 g). Dissolve in water and dilute to 1000 mL. It may be stored for 2 months at room temperature.

B.1.3.3 Sodium maleate buffer solution (0.1 mol/L, pH 6.5): Weigh 1.16 g of maleic acid (to the nearest of 0.01 g) in a 150 mL beaker. Add into about 70 mL of water to dissolve. Use 1 mol/L sodium hydroxide solution to adjust pH to 6.5. Use water to dilute to 1000 mL. Store at 4°C. It may be stored for 3 months.

B.1.3.4 1.0 mol/L sucrose solution: Accurately weigh 34.2 g (to the nearest of 0.01 g) of sucrose. Use about 50 mL of water to dissolve. Use sodium maleate buffer solution to dilute to 100 mL. Shake well. Prepare when required.

B.1.3.5 Sodium hydroxide solution (2 mol/L): Weigh 80 g of sodium hydroxide (to the nearest of 0.01 g). Use water to dissolve and dilute to 1000 mL. It may be stored for 2 months at room temperature.

B.1.3.6 3,5-dinitrosalicylic acid solution: Add 6.3 g of dinitrosalicylic acid and 262 mL of 2 mol/L NaOH solution into 500 mL of hot water solution that contains 185 g of potassium sodium tartrate. Then add 5 g of phenol and 5 g of sodium sulfite. Stirring to dissolve. After cooling, use water to dilute to 1000 mL. Store in a brown bottle, for use.

B.1.4 Apparatus

B.1.4.1 Constant temperature water bath: temperature control accuracy of ± 1°C.

B.1.4.2 Spectrophotometer: with 1 cm glass cuvette.

B.1.4.3 Micropipette: 100 μL, 1000 μL.

B.1.5 Analysis steps

B.1.5.1 Preparation of enzyme to be tested

Accurately weigh 0.1 g of testing enzyme sample (to the nearest of 0.001 g). Use 0.1 mol/L sodium maleate buffer solution to dissolve and dilute. Control the enzyme activity concentration in the range of 1 U/mL~5 U/mL.

B.1.5.2 Determination

c - Molar concentration of glucose in the testing enzyme solution, in micromoles per milliliter ($\mu\text{mol/mL}$);

c_0 - Molar concentration of glucose in the reagent blank testing solution, in micromoles per milliliter ($\mu\text{mol/mL}$);

V - Final set volume of enzyme hydrolysate (5 mL), in milliliters (mL);

V_x - The amount of enzyme to be tested, in milliliters (mL);

30 - Reaction time, in minutes (min).

B.2 Method for determination of inulinase activity

B.2.1 Principle

Inulinase (EC 3.2.1.80), also known as inulase, is a type of hydrolase capable of hydrolyzing β -2,1-D-fructo polyglycoside linkages. It hydrolyzes inulin to produce glucose and fructose. Glucose and fructose are reducing sugars. The content can be measured by 3,5-dinitrosalicylic acid colorimetric method so as to measure the size of enzyme activity. 3,5-dinitrosalicylic acid and reducing sugars form a brown-red amino compound. Measure at a maximum absorption wavelength of 540 nm. The absorbance value is linear with the reducing sugar content in a certain concentration range, so it can be used for the determination of reducing sugar content, thereby measuring the viability of inulinase.

The inulinase activity is defined as the amount of enzyme (activity) required to hydrolyze 1 μmol of fructose per minute at 55°C and pH 4.5 as one enzyme activity unit.

B.2.2 Reagents

B.2.2.1 Fructose standard substance ($\text{C}_6\text{H}_{12}\text{O}_6$): purity $\geq 99.0\%$.

B.2.2.2 Inulin: purity $\geq 90.0\%$.

B.2.2.3 Glacial acetic acid (CH_3COOH).

B.2.2.4 Sodium acetate trihydrate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$).

B.2.2.5 Dinitrosalicylic acid ($\text{C}_7\text{H}_4\text{N}_2\text{O}_7$).

B.2.2.6 Sodium potassium tartrate ($\text{C}_4\text{H}_4\text{KNaO}_6$).

B.2.2.7 Phenol ($\text{C}_6\text{H}_5\text{OH}$).

B.2.2.8 Sodium sulfite (Na_2SO_3).

B.2.3 Reagent preparation

B.2.3.1 2.5 $\mu\text{mol/mL}$ fructose standard solution: Accurately weigh 0.045 g (to the nearest of 0.1 mg) of fructose standard substance that has been dried to constant weight at 80°C into a 50mL beaker. Add about 10 mL of hot water. After the fructose is dissolved, cool to room temperature. Use water to dilute to a 100mL volumetric flask. Shake well. Store at 4°C. It may be stored for 1 month.

B.2.3.2 Acetic acid solution (200 mmol/L): Pipette 0.6 mL of glacial acetic acid. Use water to dilute to 50mL. Store at 4°C. It may be stored for 2 months.

B.2.3.3 Sodium acetate solution (200 mmol/L): Weigh 1.36 g of sodium acetate trihydrate (to the nearest of 0.01 g). Dissolve in water and dilute to 50 mL. Store at 4°C. It may be stored for 2 months.

B.2.3.4 Sodium acetate buffer solution (pH 4.5): Pipette 14 mL of acetic acid solution and 11 mL of sodium acetate solution to mix. Use water to dilute to 50mL. Prepare when required.

B.2.3.5 10% inulin solution: Accurately weigh 10 g (to the nearest of 0.01 g) of inulin. Use about 50 mL of hot water to dissolve. Cool to room temperature. Use sodium acetate buffer solution to dilute to a 100mL volumetric flask. Shake well. Store at 4°C. It may be stored for 1 month.

B.2.3.6 Sodium hydroxide solution (2 mol/L): Weigh 80 g of sodium hydroxide (to the nearest of 0.01 g). Dissolve in water and dilute to 1000 mL. It may be stored for 2 months at room temperature.

B.2.3.7 3,5-dinitrosalicylic acid solution: Add 6.3 g of dinitrosalicylic acid and 262 mL of 2 mol/L NaOH solution into 500 mL of hot water solution that contains 185 g of potassium sodium tartrate. Then add 5 g of phenol and 5 g of sodium sulfite. Stirring to dissolve. After cooling, use water to dilute to 1000 mL. Store in a brown bottle for use.

B.2.4 Apparatus

B.2.4.1 Constant temperature water bath: temperature control accuracy of $\pm 1^\circ\text{C}$.

B.2.4.2 Spectrophotometer: with 1cm glass cuvette.

B.2.4.3 Micropipette: 100 μL , 1000 μL .

B.2.5 Analysis steps

B.2.5.1 Preparation of enzyme to be tested

Use sodium acetate buffer solution to dilute the testing enzyme sample. Control enzyme activity concentration in the range of 1U/mL~5U/mL.

B.2.5.2 Determination

This is an excerpt of the PDF (Some pages are marked off intentionally)

Full-copy PDF can be purchased from 1 of 2 websites:

1. <https://www.ChineseStandard.us>

- SEARCH the standard ID, such as GB 4943.1-2022.
- Select your country (currency), for example: USA (USD); Germany (Euro).
- Full-copy of PDF (text-editable, true-PDF) can be downloaded in 9 seconds.
- Tax invoice can be downloaded in 9 seconds.
- Receiving emails in 9 seconds (with download links).

2. <https://www.ChineseStandard.net>

- SEARCH the standard ID, such as GB 4943.1-2022.
- Add to cart. Only accept USD (other currencies - <https://www.ChineseStandard.us>).
- Full-copy of PDF (text-editable, true-PDF) can be downloaded in 9 seconds.
- Receiving emails in 9 seconds (with PDFs attached, invoice and download links).

Translated by: Field Test Asia Pte. Ltd. (Incorporated & taxed in Singapore. Tax ID: 201302277C)

About Us (Goodwill, Policies, Fair Trading...): <https://www.chinesestandard.net/AboutUs.aspx>

Contact: Wayne Zheng, Sales@ChineseStandard.net

Linkin: <https://www.linkedin.com/in/waynezhengwenrui/>

----- The End -----