GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.245-2016

National Food Safety Standard – Determination of Polydextrose in Food

Issued on: July 31, 2016 **Implemented on: March 1, 2017**

Issued by: National Health and Family Planning Commission of the People's Republic of China

Table of Contents

1 Application Scope....3
2 Terms and Definitions....3
3 Principle....3
4 Reagents and Materials....3
5 Apparatus....5
6 Operating Procedure....6
7 Accuracy....7
8 Others....7
Annex A Enzyme Activity Determination....9
Annex B Reference Conditions for Chromatography....17

National Food Safety Standard – Determination of Polydextrose in Food

1 Application Scope

This Standard specifies the method for determination of polydextrose in food.

This Standard applies to the determination of polydextrose added in food.

2 Terms and Definitions

For the purposes of this document, the following term and definition apply.

2.1 polydextrose [$(C_6H_{10}O_5)_n$]

A polymer which is made by mixing glucose, sorbitol and citric acid (or phosphoric acid) in accordance with a certain proportion and heating polymerization, refining and dying at high temperature, with an average polymerization degree 12. It is a soluble dietary fiber.

3 Principle

Carry out hot water extraction and ultrafiltration centrifugation of polydextrose in food; remove interfering substances in the filtrate such as starch and fructan; then use an ion chromatography-pulsed amperometric detector for the quantitative determination of polydextrose content.

4 Reagents and Materials

Unless specified otherwise, all reagents used for this method are guaranteed reagents and the water is water grade II specified in GB/T 6682.

4.1 Reagents

4.1.1 Sodium hydroxide solution (50%): chromatographically pure, exclusive use for ion chromatography.

4.1.2 Glacial acetic acid (CH_3COOH).

4.1.3 Sodium acetate trihydrate ($CH_3COONa·3H_2O$).

4.1.4 Sodium acetate anhydrous (CH_3COONa).

4.1.5 Fructanase: ≥ 2 000 U/mL.

4.1.6 Amyloglucosidase: ≥ 3 260 U/mL (soluble starch); ≥ 200 U/mL (p-NP-β maltoside).

4.1.7 Isoamylase: ≥ 1 000 U/mL.

4.2 Preparation of reagents

4.2.1 Acetic acid solution (0.2 mol/L): accurately absorb 1.2 mL of glacial acetic acid; use water to dilute to 100 mL.

4.2.2 Sodium acetate solution (0.2 mol/L): accurately weigh 2.72 g of sodium acetate trihydrate; use water to dilute to 100 mL.

4.2.3 Acetate buffer solution (of pH 4.5): mix 28 mL of acetic acid solution (0.2 mol/L) and 22 mL of sodium acetate solution (0.2 mol/L); use water to dilute to 100 mL.

4.2.4 Mixed enzyme solution: absorb and mix 680 μL of fructanase, 84 μL of amyloglucosidase, 17 μL of isoamylase apiece; add acetate buffer solution to dilute to 20 mL. Prepare mixed enzyme solution immediately prior to use.

4.3 Mobile phase

4.3.1 Mobile phase A (containing 0.15 mol/L sodium hydroxide): accurately absorb 15.7 mL of sodium hydroxide solution (50%); use pre-degassed water to dilute to 2 L; use an inert gas for protection.

4.3.2 Mobile B (containing 0.15 mol/L sodium hydroxide, 0.50 mol/L sodium acetate): accurately weigh 41 g of sodium acetate anhydrous (or 68 g of sodium acetate trihydrate); use mobile phase A to dissolve before adding dropwise to 1 L; mix up; pass through 0.45 μm membrane; conduct degassing.

NOTE When preparing mobile phase, deionized water shall be pre-degassed for 0.5 h; mobile phase shall be inverted for 5 ~ 6 times for mixing up before adding dropwise to a constant volume; it shall not be shaken violently. Prepared mobile phase is poured in slowly along bottle wall; it shall be protected with an inert gas after 0.5 h of degassing.

4.4 Reference substance

Polydextrose [$(C_6H_{10}O_5)_n$]: purity greater than 90%; CAS 68424-04-4.

NOTE In order to ensure the accuracy of results, reference substance from the same source as polydextrose added in food shall be selected and achieve grade FCC, if the source of polydextrose added can be determined.

4.5 Preparation of reference substance

4.5.1 Reference substance stock solution (2.00 mg/g): accurately weigh 0.20 g of polydextrose reference substance (accurate to 0.000 1 g) to place into a pre-weighed

Annex A

Enzyme Activity Determination

A.1 Fructanase enzyme activity determination method

A.1.1 Principle

Under the standard conditions of pH 4.5, temperature 40°C and substrate (kestose) concentration 10 mmol/L, the amount of enzyme required for release of 1 μmol of fructose for 1 min is 1 U.

A.1.2 Reagents and solvents

A.1.2.1 Acetic acid (0.2 mol/L): accurately absorb 12 mL of glacial acetic acid; use water to dilute to 1 000 mL.

A.1.2.2 Sodium acetate solution (0.2 mol/L): accurately weigh 27.2 g of sodium acetate trihydrate; use water to dissolve and dilute to 1 000 mL.

A.1.2.3 Acetate buffer solution (of pH value 4.5): mix 280 mL of acetic acid solution (0.2 mol/L) and 220 mL of sodium acetate solution (0.2 mol/L); use water to dilute to 1 000 mL.

A.1.2.4 Sodium hydroxide solution (2 mol/L): accurately weigh 80 g of sodium hydroxide; use water to dissolve and dilute to 1 000 mL.

A.1.2.5 3,5-dinitrosalicylic acid reagent (DNS): add 6.3 g of 3,5-dinitrosalicylic acid reagent and 262 mL of 2 mol/L NaOH solution to 500 mL of hydrothermal solution containing 185 g of seignette salt; then add 5 g of phenol and 5 g of sodium sulfite; stir to dissolve; add distilled water dropwise to 1 000 mL after cooling; store in brown bottle as standby.

A.1.2.6 Fructose standard solution (1 mg/mL): accurately weigh 100 mg of analytical pure fructose dried to a constant weight at 80°C and place into small beaker; transfer to 100 mL volumetric flask; use acetate buffer solution to add dropwise to 100 mL; mix up evenly; store in refrigerator at 4°C as standby.

A.1.2.7 Kestose solution (10 mmol/L): accurately weigh 5.04 g of kestose; use acetate buffer solution to dissolve and dilute to 1 000 mL.

A.1.3 Apparatus

A.1.3.1 Analytical balance.

A.1.3.2 Electric heating constant temperature water bath.

A.2.2.5 Oyster glycogen (10 mg/mL): accurately weigh 1.0 g of oyster glycogen; use acetate buffer solution to dissolve and dilute to 100 mL.

A.2.3 Apparatus

A.2.3.1 Analytical balance.

A.2.3.2 Electric heating constant temperature water bath.

A.2.3.3 Spectrophotometer.

A.2.3.4 Timer.

A.2.4 Analytical procedure

A.2.4.1 Plotting of glucose standard curve

Absorb 4.0 mL of acetate buffer solution to pour into 25 mL graduated test tube; add 5.0 mL of DNS reagent; maintain in boiling water bath for 5 min; cool to room temperature; use water to add dropwise to 25 mL; then obtain standard blank sample.

Absorb respectively 1.00 mL, 2.00 mL, 3.00 mL, 4.00 mL, 5.00 mL, 6.00 mL, 7.00 mL of glucose standard solution to pour into 100 mL volumetric flask; use acetate buffer solution to add dropwise to 100 mL; obtain glucose standard working solution of concentrations 0.10 mg/mL, 0.20 mg/mL, 0.30 mg/mL, 0.40 mg/mL, 0.50 mg/mL, 0.60 mg/mL, 0.70 mg/mL; absorb 2.0 mL of glucose standard working solutions apiece, add respectively to 25 mL graduated test tube, then add respectively 2.0 mL of acetate buffer solution and 5.0 mL of DNS reagent. Shake up each test tube; heat accurately for 5 min in boiling water bath; take out; cool to room temperature; add water dropwise to 25 mL; mix up evenly by inverting after putting on stopper; store for 30 min; use standard blank solution as contrast for zero setting; measure absorbance value at 540 nm. Plot standard curve with absorbance value as ordinate and glucose content (in mg) abscissa.

A.2.4.2 Preparation of enzyme solution to be tested

Weigh enzyme sample to be tested; use acetate buffer solution to dilute and add dropwise to a volume (the activity of fructanase in enzyme solution after dilution is 0.04 U/mL ~ 0.08 U/mL).

A.2.4.3 Determination procedure

Absorb 10.0 mL of oyster glycogen solution; balance at 40°C for 20 min.

Absorb 10.0 mL of enzyme solution to be tested; balance at 40°C for 20 min.

Absorb 2.0 mL of enzyme solution to be tested balanced; add to 25 mL graduated test tube; add 5.0 mL of DNS reagent; mix up evenly; add 2.0 mL of oyster glycogen solution balanced; balance at 40°C for 30 min; heat in boiling water bath for 5 min; cool

Under the standard conditions of pH 4.5, temperature 40°C, substrate (paranitrophenol-*β*-maltoside) concentration 10 mmol/L and existence of excessive *β*-glucosidase, the amount of enzyme required for release of 1 μmol of paranitrophenol from paranitrophenol-*β*-maltoside for 1 min is 1 U.

A.3.2.2 Reagents and solvents

A.3.2.2.1 Acetic acid (0.2 mol/L): accurately absorb 12 mL of glacial acetic acid; use water to dilute to 1 000 mL.

A.3.2.2.2 Sodium acetate solution (0.2 mol/L): accurately weigh 27.2 g of sodium acetate trihydrate; use water to dissolve and dilute to 1 000 mL.

A.3.2.2.3 Acetate buffer solution (of pH 4.5): mix 280 mL of acetic acid solution (0.2 mol/L) and 220 mL of sodium acetate solution (0.2 mol/L); use water to dilute to 1 000 mL.

A.3.2.2.4 Sodium carbonate solution (1 mol/L): accurately weigh 105.9 g of sodium carbonate; use water to dissolve and dilute to 1 000 mL.

A.3.2.2.5 Paranitrophenol standard solution (1 mg/mL): weigh 100 mg of paranitrophenol; add acetate buffer solution to dissolve; add dropwise to 100 mL.

A.3.2.2.6 *β*-fructanase solution: use acetate buffer solution to dilute and add dropwise (the activity of *β*-fructanase in enzyme solution diluted is 2 U/mL ~ 4 U/mL).

A.3.2.2.7 Paranitrophenol-*β*-maltoside solution (10 mmol/mL): accurately weigh 4.63 g of paranitrophenol-*β*-maltoside; use *β*-fructanase solution to dissolve and dilute to 1 000 mL.

A.3.2.3 Apparatus

A.3.2.3.1 Analytical balance.

A.3.2.3.2 Electric heating constant temperature water bath.

A.3.2.3.3 Spectrophotometer.

A.3.2.3.4 Timer.

A.3.2.4 Analytical procedure

A.3.2.4.1 Plotting of paranitrophenol standard curve

Absorb 2.0 mL of acetate buffer solution; add 1.0 mL of sodium carbonate solution; mix up evenly; then obtain standard blank sample.

Absorb respectively 1.00 mL, 2.00 mL, 3.00 mL, 4.00 mL, 5.00 mL, 6.00 mL, 7.00 mL of paranitrophenol standard solution to pour into 100 mL volumetric flask; use acetate buffer solution to add dropwise to 100 mL; obtain paranitrophenol standard working

Annex B

Reference Conditions for Chromatography

B.1 Testing conditions for four-potential ion chromatography

B.1.1 Ion chromatography conditions

Analytical column: CarboPa PA1 (grain diameter 10 μm, 250 mm × 2 mm); guard column: CarboPa PA1 (grain diameter 10 μm, 50 mm × 2 mm), or equivalent column.

Sample volume: 20 μL;

Column temperature: 30°C.

NOTE The analytical column information given is for the convenience of use by the users of this Standard, which is not an approval of the product; if other equivalent products have the same functions, then these equivalent products can be used.

B.1.2 Mobile phase

NOTE Mobile phase prepared needs to be degassed and protected with inert gas.

Solution A: containing 0.15 mol/L sodium hydroxide;

Solution B: containing 0.15 mol/L sodium hydroxide and 0.50 mol/L sodium acetate;

Flow velocity: 0.5 mL.

B.1.3 Gradient elution procedure

See Table B.1 for gradient elution procedure.

Table B.1 – Gradient Elution Procedure

Time/min	Mobile phase A/%	Mobile phase B/%
0.0	70	30
10.0	0	100
15.0	0	100
25.0	70	30

B.1.4 Detector

Use four-potential waveform. See Table B.2.

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

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