NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 31614.1-2023

National food safety standard - Determination of sialic acid in foods

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1 Scope

This Standard specifies the methods for the determination of sialic acid in foods.

In this Standard, Method 1 "liquid chromatography - UV detection" is applicable to the determination of bound sialic acid in swiftlets nest and swiftlets nest products; Method 2 "liquid chromatography - fluorescence detection" and Method 3 "liquid chromatography - mass spectrometry / mass spectrometry" are applicable to the determination of sialic acid in liquid milk, milk powder, pastries and beverages.

Method 1 - Liquid Chromatography - UV Detection

2 Principle

The sample is heated and hydrolyzed in hydrochloric acid solution to release bound sialic acid.

The sample test solution is separated by a strong anion exchange chromatography column and detected by a UV detector or diode array detector. Adopt the external standard method for quantitative determination.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

3.1.4 Dialysis bag. capable of passing through compounds with a relative molecular mass of

less than 7,000.Before use, soak in water for 1 hour.

3.2 Preparation of Reagents

5 Analytical Procedures

5.1 Pre-treatment of Samples

5.1.1 Preparation of specimens

5.1.1.1 Swiftlets nest (cups, strips and chips, etc.) and solid swiftlets nest products Weigh-take 10 g of swiftlets nest and solid swiftlets nest products and dry them in an oven at 101 ?C ~ 105 ?C to a constant mass (the mass difference between the two times shall not exceed

2 mg). After cooling in a dryer, grind them and pass them all through a 100-mesh sieve (with a mesh size of 0.150 mm), then, put them into a clean container, place it in a dryer and seal for later use.

5.1.1.2 Liquid swiftlets nest products After evenly mixing liquid swiftlets nest products, take 200 g, homogenize it at a low speed first. Then, slowly raise the speed to 10,000 r/min, homogenize it for 3 minutes, let it stand, until the foam disappears. Put it into a clean container, seal it, keep it in the dark, and store it in a refrigerator.

5.1.2 Specimen extraction

5.1.2.1 Swiftlets nest (cups, strips and chips, etc.) and solid swiftlets nest products The determination of total sialic acid content. weigh-take 0.1 g (accurate to 0.0001 g) of sample into a 25 mL stoppered colorimetric tube, add 10 mL of hydrochloric acid solution, use a glass stopper to cover it, and conduct vortex mixing. Place it in 80 ?C water bath to conduct hydrolysis for 40 min. During the water bath, oscillate it every 5 min, then, take out the centrifuge tube and cool to room temperature. Transfer the hydrolysate to a 100 mL volumetric flask, use an appropriate amount of water to wash the centrifuge tube twice and transfer it to a volumetric flask. Use 0.1% phosphoric acid solution - acetonitrile (4 + 6) to reach a constant volume to the scale and evenly mix it. Transfer-take 2 mL into a centrifuge tube, at 15,000 r/min, centrifuge for 3 min. Take the supernatant, pass it through a 0.45 ?m microporous filter membrane, and reserve it for determination.

The determination of free sialic acid content. weigh-take 0.1 g (accurate to 0.0001 g) of sample into a 25 mL stoppered colorimetric tube, add 10 mL of water, and conduct vortex oscillation for 3 minutes. Transfer the specimen solution to a 100 mL volumetric flask, use an appropriate amount of water to wash the centrifuge tube twice and transfer it to a volumetric flask. Use 0.1% phosphoric acid solution - acetonitrile (4 + 6) to reach a constant volume to the scale and evenly mix it. Transfer-take 2 mL into a centrifuge tube, at 15,000 r/min, centrifuge for 3 min. Take the supernatant, pass it through a 0.45 ?m microporous filter membrane, and reserve it for determination.

5.1.2.2 Liquid swiftlets nest products The determination of bound sialic acid content. weigh-take 10 g (accurate to 0.01 g) of sample and place it in a dialysis bag (the specimen volume does not exceed 20% of the capacity of the dialysis bag). Tie both ends of the dialysis bag, place it in a 1,000 mL beaker and dialyze it under flowing tap water for 24 hours. After dialysis, transfer the test solution in the dialysis bag to a 25 mL stoppered colorimetric tube, add a small amount of water to rinse the dialysis bag, and combine it into the colorimetric tube. Add 104 ?L of concentrated hydrochloric acid, add water to reach a constant volume of 25 mL, and evenly mix it, until the mass concentration of hydrochloric acid in the hydrolysate is 0.05 mol/L. Use a glass stopper to cover it, place it in 80 ?C water bath to conduct hydrolysis for 40 min, then, take out the colorimetric tube and cool it to room temperature. Transfer the hydrolysate to a 100 mL volumetric flask, use an appropriate amount of water to wash the centrifuge tube twice and transfer it to a volumetric flask. Use 0.1% phosphoric acid solution - acetonitrile (4 + 6) to reach a constant volume to the scale and evenly mix it. Transfer-take 2 mL into a centrifuge tube, at 15,000 r/min, centrifuge for 3 min. Take the supernatant, pass it through a 0.45 ?m microporous filter

membrane, and reserve it for determination.

5.2 Reference Conditions of Liquid Chromatography The reference conditions of liquid chromatography are as follows.

a) Chromatographic column. SAX strong anion exchange column, 250 mm ? 4.6 mm (inner diameter), 5 ?m; or one with equivalent performance.

b) Column temperature. 30 ?C.

c) Detection wavelength. 205 nm.

d) Mobile phase. 0.1% phosphoric acid solution - acetonitrile (40 + 60, volume ratio).

e) Flow rate. 1.0 mL/min.

f) Injection volume. 10 ?L.

5.3 Drawing of Standard Curve Respectively inject the standard series of working solutions into the liquid chromatograph to determine the corresponding peak area. Take the mass concentration of sialic acid in the standard series of working solutions as the x-coordinate, and the peak area as the y-coordinate to draw a standard curve. For the chromatogram of sialic acid standard solution, see Figure A.1 in Appendix A.

5.4 Determination of Specimen Solution Inject the specimen solution into the liquid chromatograph to obtain the peak area. In accordance with the standard curve, obtain the mass concentration of sialic acid in the solution to be tested. The response values of sialic acid in the standard working solution and the specimen solution to be tested shall be within the linear response range of the instrument. If the of 1. 9.

10.2.3 O-phenylenediamine hydrochloride solution (50 mg/mL). weigh-take 2.5 g of o-phenylenediamine hydrochloride, add 50 mL of 0.05 mol/L hydrochloric acid solution to dissolve and evenly mix it. Prepare it right before use.

10.2.4 0.2% phosphoric acid solution. draw-take 2 mL of phosphoric acid, dissolve it in water and dilute to 1,000 mL.

10.3 Reference Material Sialic acid (N-acetylneuraminic acid, C11H19NO9, CAS No.. 131-48-6). purity ? 96%, or a standard substance certified by the state and awarded a reference material certificate.

10.4 Preparation of Standard Solutions

10.4.1 Sialic acid standard stock solution (1,000 mg/L). accurately weigh-take an appropriate amount of sialic acid reference material (converted in accordance with purity), use water to dissolve and prepare it into a standard stock solution with a mass concentration of 1,000 mg/L.

Store it in the dark at 4 ?C. It shall remain valid for 6 months.