GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.303-2025

National food safety standard - Determination of yeast *β*-glucan in food

食品安全国家标准 食品中酵母 *β*-葡聚糖的测定

Issued on: March 16, 2025 **Implemented on: September 16, 2025**

Issued by: National Health Commission of the People's Republic of China; State Administration for Market Regulation.

Table of Contents

National food safety standard - Determination of yeast *β*-glucan in food

1 Scope

This Standard specifies the method for the determination of insoluble yeast *β*-glucan in food.

This Standard applies to the determination of insoluble yeast *β*-glucan in milk and dairy products, protein powder, candy and beverages.

2 Principle

After the sample is freed of fat and protein by protease and/or lipase, yeast *β*-glucan is precipitated by centrifugation. The precipitated yeast *β*-glucan is hydrolyzed into glucose under the action of multiple enzymes. Glucose oxidase (GOD) is used to catalyze the oxidation of glucose under aerobic conditions to generate D-glucuronic acid-*d*-lactone and hydrogen peroxide, hydrogen peroxide is catalyzed by peroxidase (POD) to react with 4-aminoantipyrine and p-hydroxybenzoic acid to generate red quinone imine (GODPOD method). The absorbance of quinone imine is measured at a wavelength of 510 nm using a spectrophotometer. Finally, the content of yeast *β*-glucan in the sample is calculated by the conversion coefficient of yeast *β*-glucan and glucose (0.9).

3 Reagents and materials

Unless otherwise specified, all reagents used in this method are analytically pure, and the water is grade 3 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Alkaline protease, liquid, enzyme activity ≥ 240000 U/mL.

3.1.2 Neutral protease, liquid, enzyme activity ≥ 80000 U/mL.

3.1.3 Acidic protease, liquid, enzyme activity ≥ 50000 U/mL.

3.1.4 Lipase, liquid, enzyme activity ≥ 20000 U/mL.

3.1.5 Tetrasodium ethylenediaminetetraacetic acid dihydrate ($C_{10}H_{12}N_2Na_4O_8 \cdot 2H_2O$).

3.1.6 Tris(hydroxymethyl)aminomethane (tris) ($C_4H_{11}NO_3$).

3.2.5 Sodium acetate buffer solution A (0.2 mol/L): respectively weigh 5.25 g of anhydrous sodium acetate and 2.16 g of glacial acetic acid into 400 mL of water, adjust the pH to 5.0 ± 0.05 with sodium hydroxide solution or glacial acetic acid, add water to make up to 500 mL, and mix well.

3.2.6 Sodium acetate buffer solution B (1.2 mol/L): respectively weigh 4.96 g of anhydrous sodium acetate and 32.32 g of glacial acetic acid into 400 mL of water, adjust the pH to 3.8 ± 0.05 with sodium hydroxide solution or glacial acetic acid, add water to make up to 500 mL, and mix well.

3.2.7 Buffer solution C (pH 7.5): dissolve 1.212 g of tris(hydroxymethyl)aminomethane, 1.169 g of sodium chloride, and 0.416 g of tetrasodium ethylenediaminetetraacetic acid dihydrate in 90 mL of water. Adjust the pH to 7.5 ± 0.05 with hydrochloric acid solution or sodium hydroxide solution, add water to make up to 100 mL, and mix well. Before use, transfer an appropriate amount and dilute 10 times with water. Prepare before use.

3.2.8 Lytic enzyme solution (5 U/μL): weigh an appropriate amount of lytic enzyme into buffer solution C to make the final concentration of lytic enzyme 5 U/μL. Prepare before use.

3.2.9 β-(1,6)-glucanase solution: dissolve an appropriate amount of β-(1,6)-glucanase in sodium acetate buffer solution A to make a suspension with the final concentration of 1 U/300 μL. Prepare before use.

3.2.10 Mixed enzyme solution: pipette an appropriate amount of β-(1,3)-glucanase and β-glucosidase mixed enzyme, add an appropriate amount of sodium acetate buffer solution A to dilute and mix well, so that the final concentrations are 20 U/mL and 4 U/mL respectively. Prepare before use. It shall be stored in an ice water bath during use.

3.2.11 Glucose oxidase-peroxidase (GODPOD) buffer: add about 160 mL of water to a 200 mL volumetric flask, then add 27.2 g of potassium dihydrogen phosphate, 8.4 g of sodium hydroxide, and 6.0 g of p-hydroxybenzoic acid, stir to dissolve completely, and adjust the pH to 7.4 ± 0.05. Finally, add 0.8 g of sodium azide, add water to make up to the mark after dissolution, and prepare before use. It shall be diluted before use. Measure 48 mL of GODPOD buffer into a 1000 mL volumetric flask, add water to make up to the mark, and mix thoroughly. Prepare before use.

3.2.12 GODPOD working solution: weigh 400 U of glucose oxidase, 1000 U peroxidase, and 31.3 mg of 4-aminoantipyrine into a 1000 mL volumetric flask, add 20 mL diluted GODPOD buffer, shake gently to fully dissolve, then make up to the mark with GODPOD buffer, mix well and store at 4 °C in the dark. Prepare before use.

NOTE: 3.2.8 ~ 3.2.12 can also use commercial reagents or kits.

3.3 Standards

NOTE: Samples that cannot be crushed, such as soft candy, shall be cut into pieces as much as possible.

6 Analysis steps

6.1 Preparation of matrix control sample

Weigh 3 mg ~ 10 mg of yeast β-glucan reference substance (accurate to 0.0001 g, and the deviation from the yeast β-glucan content in the sample to be tested is not more than 20 %) in a conical centrifuge tube, add 10.0 g (accurate to 0.01 g) of the corresponding blank sample, and mix thoroughly.

6.2 Precipitation

6.2.1 Liquid sample

Respectively weigh 10.0 g (accurate to 0.01 g, or a sample mass equivalent to 3 mg ~ 10 mg yeast β-glucan) of the mixed liquid sample and the corresponding matrix control sample in 15 mL conical centrifuge tubes, each add 200 μL of protease mixed solution (add 200 μL of acidic protease for acidic samples such as yogurt), and incubate at 40 ℃ for 2 h and cool to room temperature. After standing for 10 min, centrifuge at 8000 r/min for 5 min, slowly remove the upper fat and intermediate enzymatic hydrolysate with a dropper, and retain the precipitate.

6.2.2 Solid sample

Respectively weigh 10.0 g (accurate to 0.01 g, or a sample mass equivalent to 3 mg ~ 10 mg of yeast β-glucan) of the solid sample treated in 5.1 and the corresponding matrix control sample in 50 mL centrifuge tubes, and each add 20 mL of water to fully dissolve. Add 500 μL of protease mixed solution and mix well. Incubate at 40 ℃ for 2 h and then cool to room temperature. After standing for 10 min, centrifuge at 8000 r/min for 5 min, slowly remove the upper fat and the intermediate enzymatic hydrolysate with a dropper, and retain the precipitate.

NOTE: For samples with no yeast β-glucan precipitation after centrifugation or samples with a fat content of ≥ 10 %, add 500 μL of lipase after protease incubation for 2 h, and incubate at 40 ℃ for 2 h. Or weigh 10.0 g of sample, refer to 6.1.3 of GB 5009.88-2023 to remove excess fat in the sample, and then use protease to hydrolyze the protein.

6.3 Precipitation cleaning

Add 5 mL of water to the precipitate treated in 6.2, fully disperse it, let stand for 10 min, centrifuge at 8000 r/min for 5 min, and slowly remove the supernatant with a dropper. The precipitation cleaning needs to be repeated more than 3 times until no glucose is detected in the supernatant according to the method in 6.4. The bottom precipitate can be subjected to the next step of enzymatic hydrolysis.

6.4 Determination of supernatant

Take an appropriate amount of the supernatant treated in step 6.3 and filter it with a 0.45 μm filter membrane, transfer 100 μL to a 5 mL centrifuge tube, each add 3 mL of GODPOD working solution, and place in a 40 ℃ water bath for 20 min. Remove from the water bath, cool to room temperature, transfer to a 1 cm cuvette, use the GODPOD working solution as blank, measure the absorbance at 510 nm with a spectrophotometer, and record. Calculate the concentration of glucose in the sample solution using the standard curve.

6.5 Enzymatic hydrolysis

6.5.1 Sample and matrix control sample

Add 400 μL of cold potassium hydroxide solution to the sample and matrix control sample treated in 6.3, place in an ice-water bath for 20 min. During the ice-water bath, vortex briefly several times until all the precipitates are dispersed and no lumps are visible, add 1.6 mL of sodium acetate buffer solution B, then add 500 μL of lytic enzyme solution, and record the total volumes as V_1 and V'_1 at this moment. Incubate the reaction solutions in a 50 ℃ constant temperature water bath for 12 h ~ 18 h, and then cool to room temperature. Respectively transfer 150 μL (V_2 and V'_2) of the enzymatic hydrolysate to 2 mL centrifuge tubes, add 300 μL of β-(1,6)-glucanase solution, incubate at 80 ℃ for 15 min, and cool to room temperature. Add 300 μL of mixed enzyme solution, record the total volumes as V_3 and V'_3, incubate at 40 ℃ for 1 h, and then cool to room temperature. Pipette an appropriate amount of solution, filter through a 0.45 μm filter membrane, and then test.

6.5.2 Enzyme blank solution

Pipette 200 μL of potassium hydroxide solution into a 5 mL centrifuge tube, add 0.8 mL of sodium acetate buffer solution B, mix well and then pipette 120 μL into a 2 mL centrifuge tube, add 30 μL of lytic enzyme solution, and mix well. Incubate at 50 ℃ for 12 h ~ 18 h, cool to room temperature. Add 300 μL of β-(1,6)-glucanase solution, incubate at 80 ℃ for 15 min, and cool to room temperature. Finally, add 300 μL of mixed enzyme solution, incubate at 40 ℃ for 1 h, and cool to room temperature. Pipette an appropriate amount of solution, filter through a 0.45 μm filter membrane, and then test.

6.6 Determination

6.6.1 Plotting of standard curve

Respectively pipette 100 μL of glucose series standard solutions into 5 mL centrifuge tubes, each add 3 mL of GODPOD working solution, and incubate in a 40 ℃ water bath for 20 min. Remove from the water bath, cool to room temperature, pipette an appropriate amount of solution, filter through a 0.45 μm filter membrane, and transfer to 1 cm cuvettes, use GODPOD working solution as blank, measure the absorbance at

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

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