# NATIONAL STANDARD OF THE

# PEOPLE'S REPUBLIC OF CHINA

# GB 1903.80-2025

## National Food Safety Standard - Food Nutritional Fortification Substance - Yeast Beta-Glucan

食品安全国家标准 食品营养强化剂 酵母beta-葡聚糖

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**Standardization Administration of the PRC**

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## 1 Scope

This standard applies to the food nutritional fortification substance yeast beta-glucan, whose main component is beta-1,3 and beta-1,6 glucan, obtained from brewing yeast (Saccharomyces cerevisiae) as raw material through cell wall disruption and extraction, acid and alkali treatment, separation and purification, drying and other operations.

Note: the species name Saccharomyces cerevisiae is rendered in Chinese as brewing yeast; the baker's yeast referred to in Ministry of Health Announcement No. 6 of 2012, in the wording made from baker's yeast (Saccharomyces cerevisiae) as raw material, is a common English name for that same yeast.

## 2 Molecular formula, structural formula and relative molecular mass

2.1 Molecular formula. (C6H10O5)n, where n is the degree of polymerisation, from 125 to 25000 inclusive.

2.2 Structural formula. Printed in the standard as a drawing, which the digitised text does not carry.

2.3 Relative molecular mass. From 20000 to 4000000, based on the 2022 international relative atomic masses.

## 3 Technical requirements

3.1 Sensory requirements. The sensory requirements shall comply with Table 1. Table 1 fixes the colour as pale yellow to yellowish brown, the state as powder, and the odour as the odour characteristic of the product. The test method given against all three is the same: take a suitable amount of the sample, place it in a clean, dry white porcelain dish, observe its colour and state under natural light, and smell it.

3.2 Physical and chemical indexes. The physical and chemical indexes shall comply with

Table 2. Table 2 fixes the following limits, each with the test method shown against it. Yeast beta-glucan content, mass fraction not less than 75 percent, by A.3 of Annex A. Protein, mass fraction not more than 3.5 percent, by the Kjeldahl method of GB 5009.5. Fat, mass fraction not more than 10.0 percent, by the acid hydrolysis method of GB 5009.6. Moisture, mass fraction not more than 8.0 percent, by the direct drying method of GB 5009.3. Ash, mass fraction not more than 3.0 percent, by the determination of total ash in foods of GB 5009.4. Lead, not more than 0.5 mg/kg, by GB 5009.12 or GB 5009.75. Total arsenic, expressed as As, not more than 0.5 mg/kg, by GB 5009.11 or GB 5009.76. Total mercury, expressed as Hg, not more than 0.05 mg/kg, by GB 5009.17. Cadmium, not more than 0.5 mg/kg, by GB 5009.15.

3.3 Microbiological limits. The microbiological limits shall comply with Table 3, which is written as a sampling plan with the number of sample units n, the maximum allowable number of sample units between m and M given as c, and the two limits m and M, expressed in CFU per gram unless otherwise stated. For aerobic plate count, n is 5, c is 2, m is 10000 and M is 50000, by GB 4789.2. For coliforms, n is 5, c is 2, m is 10 and M is 100, by the plate count method of GB 4789.3. For Staphylococcus aureus, n is 5, c is 0 and m is zero in 25 g, with no M given, by GB 4789.10. For Salmonella, n is 5, c is 0 and m is zero in 25 g, with no M given, by GB 4789.4. A footnote to the table states that the collection and handling of the samples follow GB 4789.1.



## A.1 General provisions

Unless other requirements are stated, the reagents and the water used in this standard are analytically pure reagents and grade three water as laid down in GB/T 6682.

Unless other requirements are stated, the standard solutions, the standard solutions for the determination of impurities, and the preparations and products used in the tests are prepared as laid down in GB/T 601, GB/T 602 and GB/T 603.

Where the solvent used to prepare a solution is not stated, an aqueous solution is meant.

## A.2 Identification test

Identification of yeast beta-glucan by its characteristic infrared spectrum is carried out by the potassium bromide pellet method following GB/T 6040. The infrared spectrum of the yeast beta-glucan shall match the features of the infrared spectrum of the yeast beta-glucan reference substance given in Annex B, with a broad, fairly strong absorption band near 3419 reciprocal centimetres, which is the O-H stretching absorption of sugars, and weaker absorption bands near 2923 reciprocal centimetres, which is the C-H stretching absorption of sugars, and near 889 reciprocal centimetres, which is the absorption characteristic of the beta configuration.

## A.3 Determination of yeast beta-glucan content

A.3.1 Acid hydrolysis method (first method). Dissolve the sample in water, filter off the insoluble matter, wash the filter residue with water so that it is completely separated from the bulk of the sample, dry it, and weigh the mass of the water insoluble impurities on the balance.

A.3.1.1 Principle of the acid hydrolysis method. After the yeast beta-glucan sample has been hydrolysed with acid, the glucose and the other components in the sample are separated on a high performance liquid chromatographic column, detected with a differential refractive index detector, and determined quantitatively by the external standard method. Note: during the hydrolysis of yeast beta-glucan the hydrolysis may be incomplete, and the glucose produced may partly undergo other side reactions at high temperature, so that the yeast beta-glucan content found is lower than the true value.

A.3.1.2 Apparatus and equipment. Electronic balance, readability 0.001 g. Constant temperature drying oven, 100 degrees Celsius plus or minus 2 degrees Celsius. Constant temperature water bath, 30 degrees Celsius plus or minus 1 degree Celsius. Autoclave. Vortex mixer. High performance liquid chromatograph with differential refractive index detector.

A.3.1.3 Reagents and materials. Water: grade one water of GB/T 6682. Hydrochloric acid: 37 percent. Anhydrous glucose (CAS number 50-99-7), purity analytically pure, not less than 99.5 percent. Glucose standard solution, 2.0 g/L: weigh 0.2 g, to the nearest 0.001 g, of glucose that has been dried at 98 degrees Celsius to 100 degrees Celsius for 2 h, dissolve it in water and make up to 100 mL, then shake well. Yeast beta-glucan reference substance: of known purity, the purity being not less than 75 percent. Sodium hydroxide solution, 300 g/L: weigh 300 g of sodium hydroxide to the nearest 0.01 g, make up to 1000 mL with water and shake well. Cellulose acetate membrane: pore size 0.22 micrometres.

A.3.1.4 Treatment of the sample. Weigh 0.4 g, to the nearest 0.001 g, of the sample or of the yeast beta-glucan reference substance into a 20 mL screw capped tube, add 6.0 mL of hydrochloric acid, cap tightly and shake to obtain a uniform suspension. Keep the tube in a water bath at 30 degrees Celsius for 45 min, mixing on the vortex mixer once every 15 min. Transfer the suspension from the water bath into a 200 mL heat resistant screw capped bottle, washing the tube several times with 100 mL to 120 mL of water and adding the washings to the bottle. Place the screw capped heat resistant bottle in the autoclave and sterilise at 121 degrees Celsius for 60 min. Take it out, cool it to room temperature, adjust the pH to between 6 and 7 with the sodium hydroxide solution, transfer to a 200 mL volumetric flask, make up to the mark with water and mix. Filter through the cellulose acetate membrane of 0.22 micrometre pore size before use.

A.3.1.5 Reference chromatographic conditions. Chromatographic column: a sugar column, 6.5 mm by 300 mm, or a separation column of equivalent analytical performance. Mobile

phase: pure water. Column temperature: 80 degrees Celsius. Flow rate: 0.5 mL/min. Injection volume: 20 microlitres.

A.3.1.6 Drawing of the calibration curve. Measure 2.0 mL, 4.0 mL, 6.0 mL, 8.0 mL and 10.0 mL of the glucose standard solution into 10 mL volumetric flasks, make up to the mark with water and shake well, giving a series of standard solutions with glucose mass concentrations of 400 mg/L, 800 mg/L, 1200 mg/L, 1600 mg/L and 2000 mg/L. Inject 20 microlitres under the chromatographic conditions of A.3.1.5 and draw the calibration curve from the chromatographic peak areas and the glucose concentrations.

A.3.1.7 Determination of the sample and of the reference substance. Under the same chromatographic conditions, inject the sample solution and the yeast beta-glucan reference substance solution prepared by the method of A.3.1.4 into the chromatograph and record the retention time and the peak area of each chromatographic peak. Identify by the retention time of the glucose standard solution peak and quantify by the peak area of the glucose standard solution peak.

A.3.1.8 Calculation of the result. The yeast beta-glucan content is calculated by formula (A.1). In the formula: X1 is the yeast beta-glucan content of the sample, in grams per 100 grams; c1 is the glucose content of the sample solution calculated from the peak area of the sample solution by means of the calibration curve, in milligrams per litre; 0.2 is the volume to which the sample or the reference substance was made up after treatment, in litres; 100 is the conversion factor for percentage content; m1 is the mass of sample taken, in grams; 1000 is the conversion factor between milligrams and grams; 0.9 is the factor for converting glucose to yeast beta-glucan; and F1 is the empirical compensation factor for the low result caused by destruction of glucose during acid hydrolysis of the sample. The value of F1 is calculated by formula (A.2), in which P1 is the purity of the yeast beta-glucan reference substance, taken from the test report supplied by its manufacturer, in grams per 100 grams; 100 is the conversion factor for percentage content; W is the moisture of the yeast beta-glucan reference substance, taken from the test report supplied by its manufacturer, in grams per 100 grams; m2 is the mass of yeast beta-glucan reference substance taken, in grams; 1000 is the conversion factor between milligrams and grams; c2 is the glucose content of the reference substance solution calculated from the peak area of that solution by means of the calibration curve, in milligrams per litre; 0.2 is the volume to which the sample or the reference substance was made up after treatment, in litres; and 0.9 is the conversion factor between glucose and yeast beta-glucan. The result is given as the arithmetic mean of two independent determinations obtained under repeatability conditions, kept to the nearest whole number.

A.3.1.9 Precision. The absolute difference between two independent results obtained under repeatability conditions shall not exceed 5 percent of their arithmetic mean.

A.3.2.1 Principle of the enzymatic hydrolysis method. The yeast beta-glucan is gelatinised with potassium hydroxide solution and then hydrolysed in several stages with lyticase,