NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.303-2025

National food safety standard - Determination of yeast beta-glucan in food

食品安全国家标准 食品中酵母beta-葡聚糖的测定

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Standardization Administration of the PRC

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1 Scope

1 The document lays down the method for determining insoluble yeast beta-glucan in food.

1 It applies to the determination of insoluble yeast beta-glucan in milk and milk products, protein powder, confectionery and beverages.

2 Principle

2 Fat and protein are removed from the test portion by protease and/or lipase, and the yeast beta-glucan is then brought down by centrifugation.

2 The precipitated yeast beta-glucan is hydrolysed to glucose under the action of several enzymes. Glucose oxidase catalyses the oxidation of glucose under aerobic conditions, giving D-glucuronolactone and hydrogen peroxide; under peroxidase the hydrogen peroxide reacts with 4-aminoantipyrine and 4-hydroxybenzoic acid to form a red quinoneimine. This is the glucose oxidase-peroxidase, or GODPOD, reaction.

2 The absorbance of the quinoneimine is measured with a spectrophotometer at a wavelength of 510 nm, and the yeast beta-glucan content of the sample is calculated using the conversion factor of 0.9 between yeast beta-glucan and glucose.

3 Reagents and materials

3 Unless otherwise stated, all reagents used are of analytical grade and the water is grade three water as defined in GB/T 6682.

3.1 The reagents include alkaline, neutral and acid proteases and a lipase, each specified

by a minimum enzyme activity; a lyticase; a beta-(1,6)-glucanase; a mixed beta-(1,3)-glucanase and glucosidase preparation; glucose oxidase; peroxidase; tetrasodium ethylenediaminetetraacetate dihydrate; tris(hydroxymethyl)aminomethane; glacial acetic acid; anhydrous sodium acetate; sodium chloride; potassium hydroxide; sodium hydroxide; hydrochloric acid; 4-aminoantipyrine; potassium dihydrogen phosphate; 4-hydroxybenzoic acid; sodium azide; and a filter membrane of 0.45 micrometre pore size.

3.2 The clause gives the preparation of the protease mixture, of potassium hydroxide, sodium hydroxide and hydrochloric acid solutions, of two sodium acetate buffers A and B adjusted to pH 5.0 plus or minus 0.05 and pH 3.8 plus or minus 0.05, of buffer C adjusted to pH 7.5 plus or minus 0.05, of the lyticase, beta-(1,6)-glucanase and mixed enzyme solutions, and of the GODPOD buffer and GODPOD working solution, which is adjusted to pH 7.4 plus or minus 0.05 and stored at 4 °C protected from light. A note allows commercial reagents or kits to be used in place of those preparations.

3.3 The standards are an insoluble yeast beta-glucan reference material, CAS number 9012-72-0, of purity not less than 70%, and anhydrous glucose, CAS number 50-99-7, of purity not less than 99%, or standards nationally certified and issued with a reference material certificate.

3.4 A glucose stock standard solution of 2.00 g/L is prepared from anhydrous glucose dried for 2 h at 98 °C to 100 °C, and a series of glucose working standard solutions is prepared from it by dilution, all to be made up immediately before use.

4 Instruments and equipment

4 The equipment is a spectrophotometer able to measure absorbance at 510 nm and fitted with a 1 cm cell; a thermostatic water bath covering 40 °C to 80 °C with an accuracy of 1.0 °C; balances with graduations of 0.01 g and 0.1 mg; pipettes of 1.00 mL and 200 microlitre range; a pH meter accurate to 0.01; a vortex mixer; a centrifuge reaching 8000 r/min; a drying oven; and a round-hole sieve of 2.0 mm aperture.

5 Preparation of the test sample

5.1 For solid samples, 5.0 g to 50.0 g is taken, ground in a mortar and passed through the 2.0 mm round-hole sieve, then kept for analysis. A note adds that samples such as soft sweets that cannot be ground are to be cut up as finely as possible.

6 Analytical procedure

6.1 A matrix control sample is prepared by weighing 3 mg to 10 mg of the yeast beta-glucan reference material, to the nearest 0.0001 g and within 20% of the yeast beta-glucan content of the sample to be tested, into a conical centrifuge tube and mixing it thoroughly with 10.0 g of the corresponding blank sample.

6.2 For precipitation, liquid samples and the matrix control are weighed into conical centrifuge tubes, the protease mixture is added, or acid protease for acid samples such as yoghurt, and the tubes are incubated at 40 °C for 2 h, cooled, left to stand for 10 min and centrifuged at 8000 r/min for 5 min; the upper fat layer and the middle enzymatic digest are drawn off with a dropper and the precipitate kept. Solid samples treated according to 5.1 are handled in the same way after dissolution in water. A note deals with samples that give no precipitate, or that contain 10% or more fat, for which lipase is added after the protease step, or the excess fat is removed by reference to 6.1.3 of GB 5009.88-2023 before the protein is hydrolysed.

6.3 The precipitate is washed with water, left to stand, centrifuged and the supernatant removed, the washing being repeated at least three times until no glucose is detected in the supernatant by the method of 6.4; only then is the precipitate taken on to the enzymatic hydrolysis.

6.4 For the measurement of the supernatant, an aliquot is filtered through a 0.45 micrometre membrane, 100 microlitres are transferred to a centrifuge tube, 3 mL of GODPOD working solution is added, the tube is held in a water bath at 40 °C for 20 min and cooled, and the absorbance is read at 510 nm in a 1 cm cell against the GODPOD working solution as blank. The glucose concentration is taken from the calibration curve.

6.5 In the enzymatic hydrolysis the washed precipitate is dispersed in cold potassium hydroxide solution in an ice-water bath with repeated short vortexing, sodium acetate buffer B and lyticase solution are added and the total volume is recorded, the reaction mixture is incubated at 50 °C for 12 h to 18 h and cooled, an aliquot is taken and treated with beta-(1,6)-glucanase at 80 °C for 15 min, and the mixed enzyme solution is then added, the total volume recorded, and the mixture incubated at 40 °C for 1 h. An enzyme blank solution is carried through the same sequence without sample. Both solutions are filtered through a 0.45 micrometre membrane before measurement.

6.6 The calibration curve is drawn by treating the series of glucose working standard solutions with the GODPOD working solution in the same way and plotting absorbance against concentration. The sample solution, the matrix control solution and the enzyme blank solution are then measured under the same conditions, and dilution may be applied according to the particular sample.

7 Expression of results

7 The yeast beta-glucan content of the sample is calculated by formula (1), whose terms are the measured glucose concentration of the final enzymatic digest of the sample, the measured glucose concentration of the enzyme blank, the dilution factors of the sample digest and of the matrix control digest, the measured and the theoretical glucose concentrations of the matrix control digest, the factor 0.9 for converting glucose to yeast beta-glucan, the conversion factors between gram and kilogram and between millilitre and

litre, the sample mass, the volume of the digest after lyticase hydrolysis, the volume taken from that digest and the final volume after all the hydrolysis steps. The result is expressed in grams per kilogram.

7 The theoretical glucose content of the matrix control after hydrolysis is calculated by formula (2), whose terms are the mass of the yeast beta-glucan reference material in milligrams, its purity in grams per 100 grams, the corresponding volumes of the matrix control digest, the factor 0.9, the purity unit conversion factor 100 and the conversion factors between millilitre and litre and between milligram and gram.

7 When the sample and the reference material are carried through 6.5 in such a way that the corresponding volumes and dilution factors of the two are identical, formula (1) reduces to formula (3), which uses only the measured glucose concentrations of the sample digest, the enzyme blank and the matrix control digest, the mass and purity of the reference material, the sample mass and the conversion factors.

7 The result is reported to three significant figures.

8 Precision

8 The absolute difference between two independent results obtained under repeatability conditions is not to exceed 10% of their arithmetic mean.

9 Other

9 For a test portion of 10.0 g the detection limit is 0.0500 g/kg and the quantification limit is 0.150 g/kg.