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Determination of β-glucan in cereal and its products

谷物及其制品中 β-葡萄糖含量的测定

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Determination of β-glucan in cereal and its products

1 Scope

This standard specifies the enzymatic-colorimetric determination method for β-glucan content in cereals and their products.

This standard applies to the determination of β-glucan content in oats, barley, highland barley, rye and other cereals and their processed products (flour, bran, oatmeal, beverages, etc.).

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB 5009.3 National food safety standard - Determination of moisture in foods

GB/T 5491 Inspection of grain and oilseeds - Methods for sampling and sample reduction

GB/T 6682 Water for analytical laboratory use - Specification and test methods

3 Principle

This method uses the enzymatic hydrolysis of (1→3)(1→4)-β-D-glucan (mixed β-D-glucan, or β-glucan for short) in the sample by lichenase (or glucanase) and β-glucosidase, uses lichenase to specifically hydrolyze β-glucan into oligosaccharides, while β-glucosidase hydrolyzes oligosaccharides into glucose; glucose generates gluconic acid and hydrogen peroxide under the action of glucose oxidase; hydrogen peroxide is oxidized and condensed with 4-aminoantipyrine under the action of peroxidase to generate red quinone compounds. This compound absorbs at 510 nm, its absorbance value is proportional to the glucose content.

4 Reagents

Unless otherwise specified, only confirmed analytical reagents are used in the analysis; the experimental water shall meet the requirements of grade 3 water in GB/T 6682.

4.6.1 200 mmol/L, pH 4.0 sodium acetate buffer solution: Take 7.6 mL of glacial acetic acid in 900 mL water; add 4.8 g of sodium acetate trihydrate; dissolve it; adjust pH to 4.0; dilute to 1000 mL.

4.6.2 50 mmol/L, pH 4.0 sodium acetate buffer solution: Take 250 mL of 200 mmol/L, pH 4.0 sodium acetate buffer solution; dilute to 1000 mL.

4.7 1.000 mg/mL glucose standard stock solution

Dry glucose powder (purity greater than 99%) at 100 °C under normal pressure for 2 h; cool in a desiccator and store in a sealed container at room temperature. Accurately weigh 0.1000 g of dry glucose; dissolve it in 50 mmol/L, pH 4.0 sodium acetate buffer (4.6.2); make the volume reach to 100 mL. The standard solution can be stored at 4 °C for one month.

Note: The enzyme preparations involved in 4.1, 4.2, 4.3 can all be provided by Megazyme mixed β-D-glucan assay kit. Megazyme is the trade name of the products of Megazyme Company of Ireland. This information is given for the convenience of users of this standard. If there are other products with the same effect, these equivalent products can be used.

5 Instruments and equipment

5.1 Cyclone mill or pulverizer: The sample can be pulverized to pass through a 0.5 mm or 35 mesh sieve.

5.2 Centrifuge: Can accommodate glass test tubes of 15 mm × 100 mm or 10 mm × 75 mm, centrifugal force 1000 × g.

5.3 Water bath: Temperature stability ± 0.2 °C.

5.4 Vortex mixer.

5.5 pH meter: Accuracy 0.01.

5.6 Analytical balance: Sensitivity 0.0001 g.

5.7 Drying oven: Can maintain 105 °C ± 1 °C.

5.8 Spectrophotometer: Detection wavelength 510 nm.

5.9 Cuvette.

5.10 Pipette: Range 10 μL ~ 100 μL, 20 μL ~ 200 μL, 1000 μL ~ 5 000 μL, equipped with disposable pipette tips.

5.11 Volumetric flask: Volume 100 mL, 1000 mL.

Accurately weigh an appropriate amount of sample into a test tube; add 10 mL of 50% ethanol solution (4.4); pre-extract at 80 °C for 10 min; centrifuge (1000×g, 10 min); discard the supernatant; repeat the alcohol wash of the precipitate once; centrifuge (1000×g, 10 min); retain the precipitate to prepare for use.

7.1.3 Liquid samples

Pipette 1 mL ~ 5 mL (accurate to 0.01 mL) of the sample to be tested; place it at the bottom of a glass test tube (known weight); add 3 times the volume of 95% (v/v) ethanol solution; shake vigorously on a vortex mixer; place at room temperature for 5 min; centrifuge (1000×g, 10 min); discard the supernatant; add 10 mL of 50% ethanol solution (4.4) to disperse the precipitate again; centrifuge (1000×g, 10 min); discard the supernatant; retain the precipitate to prepare for use

Note: In this step, the mass of the test tube containing the precipitate needs to be weighed; the mass of the test tube and the mass of the dry matter of the sample to be tested need to be subtracted to obtain the water absorption of the sample, which is recorded in the total volume of the sample reaction.

7.2 Pre-enzymatic hydrolysis

7.2.1 Add 0.2 mL of 50% ethanol solution (4.4) to the test tube of the sample to be tested; oscillate and disperse on a vortex mixer; add 4.0 mL of sodium phosphate buffer (4.5); oscillate thoroughly.

7.2.2 Place the test tube in a boiling water bath and keep it for 1 min; take out the test tube and oscillate vigorously on a vortex mixer for several seconds; continue to keep it in the boiling water bath for 2 min; oscillate as before.

7.3 Enzymatic hydrolysis

7.3.1 Keep the test tube in a 50 °C water bath for 5 min; add 0.2 mL of lichenase solution (4.1); oscillate vigorously for several seconds; cover the test tube with a stopper; continue to keep it in a 50 °C water bath for 60 min; during this period, take out the test tube; oscillate it 3 ~ 4 times.

7.3.2 Take out the test tube; add 5 mL of 200 mmol/L sodium acetate buffer (4.6.1) to it; mix well; cool at room temperature for 5 min ~ 10 min; centrifuge (1000×g, 10 min); take the supernatant; prepare for use.

7.3.3 Accurately pipette 0.1 mL of supernatant to the bottom of three test tubes; add 0.1 mL of β-glucosidase solution (4.2) to each of the two test tubes; add 0.1 mL of 50 mmol/L sodium acetate buffer (4.6.2) to the other test tube as a reaction blank; keep the above test tubes at 50 °C for 10 min.

7.4 Color reaction

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