NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 1886.322-2021

National food safety standard - Food additives - Soluble soybean polysaccharide

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1 Scope

This Standard applies to the food additive soluble soybean polysaccharide that is produced by degreasing, extraction, purification, sterilization, drying and other processes, with soybean or soybean meal as raw materials.

3.1 Sensory requirements

Sensory requirements shall be in accordance with Table 1.

Table 1 - Sensory requirements

Appendix A Inspection method

A.1 General provisions The reagents and water that are used in this Standard, when no other requirements are specified, refer to analytical reagents and grade-III water which is specified in GB/T 6682. The standard solution, the standard solutions, preparations and products for impurity determination, which are used in the test, are all prepared in accordance with the provisions of GB/T 601, GB/T 602, and

GB/T 603, when no other requirements are specified. The solution used in the test, if not indicated which solvent is used, refers to aqueous solution.

A.2 Identification test A.2.1 Reagents and materials A.2.1.1 Sodium hydroxide.

A.2.1.2 Disodium hydrogen phosphate.

A.2.1.3 Standard relative molecular mass reference substance: Pullulan, P-5 (MW6200Da), P-20 (MW23000Da), P-50 (MW48800Da), P-400 (MW348000Da), P-800 (MW805000Da). Or choose more than 5 standard products between pullulan P-5 ~ P-800 to develop a standard curve.

A.2.2 Preparation of reagents A.2.2.1 4 mol/L sodium hydroxide solution: Weigh 16 g of sodium hydroxide;

use water to dissolve it to 100 mL; mix well.

A.2.2.2 0.1 mol/L disodium hydrogen phosphate buffer solution: Weigh 15.6 g of disodium hydrogen phosphate (A.2.1.2); use water to dissolve it to 1 L; mix well; use 4 mol/L sodium hydroxide solution to adjust the pH to 6.8.

A.2.2.3 Standard relative molecular mass solution: Respectively weigh 10.0 mg of the standard relative molecular mass reference substance (A.2.1.3); add 0.1 mol/L disodium hydrogen phosphate buffer solution (A.2.2.2) to dissolve and make the volume to 10 mL.

A.2.3 Instruments and apparatuses A.2.3.1 High performance liquid chromatography, with differential refractive index detector.

A.3.2.2 2-(N-morpholino) ethanesulfonic acid (MES).

A.3.2.3 Trihydroxymethyl aminomethane (TRIS).

A.3.2.4 Thermally stable alpha-amylase solution: CAS 9000-85-5; the enzyme activity is 10 000 U/mL +/- 1 000 U/mL; it is stored in a refrigerator at 0 °C ~ 5 °C;

the enzyme activity determination and judgment standards shall meet the requirements of Appendix A of GB 5009. 88-2014.

A.3.2.5 Protease solution: CAS 9014-01-1; the enzyme activity is 300 U/mL ~ 400 U/mL; it is stored in a refrigerator at 0 °C ~ 5 °C; the enzyme activity determination and judgment standard shall meet the requirements of Appendix A of GB 5009.88-2014.

A.3.2.6 Amyloglucosidase solution: CAS 9032-08-0; the enzyme activity is 2 000 U/mL ~ 3 300 U/mL; it is stored in a refrigerator at 0 °C ~ 5 °C; the enzyme activity determination and judgment standard shall meet the requirements of

Appendix A of GB 5009.88-2014.

A.3.2.7 Hydrochloric acid.

A.3.2.8 Diatomite: CAS68855-54-9.

A.3.2.9 Potassium dichromate A.3.2.10 Sulfuric acid.

A.3.2.11 95% ethanol.

A.3.2.12 Acetone.

A.3.3 Preparation of reagents A.3.3.1 6 mol/L sodium hydroxide solution: Weigh 24 g of sodium hydroxide;

use water to dilute it to 100 mL; mix well.

A.3.3.2 0.05 mol/L MES-TRIS buffer: Weigh 19.52 g of 2-(N-morpholino)

ethanesulfonic acid (MES) and 12.2 g of trihydroxymethyl aminomethane (TRIS); use 1.7 L of water to dissolve; use 6 mol/L sodium hydroxide solution to adjust the pH according to

the room temperature; adjust the pH to 8.3 at 20 °C; adjust the pH to 8.2 at 24 °C; adjust the pH to 8.1 at 28 °C; use the interpolation method to correct pH at other room temperature between 20 °C and 28 °C. Add water to dilute to 2 L.

A.3.3.3 Protease solution: Use 0.05 mol/L MES-TRIS buffer to dilute the protease solution (A.3.2.5) to 50 mg/mL; prepare it before use and temporarily store it in the refrigerator at 0 °C ~ 5 °C.

A.3.4.6 Constant temperature water bath: the temperature control range is 25 °C ~ 100 °C; the temperature fluctuation is +/-1 °C.

A.3.4.7 Magnetic stirrer.

A.3.4.8 Analytical balance: the sensitivities are 0.1 mg and 1 mg.

A.3.4.9 Dryer: equipped with effective silicon dioxide or equivalent desiccant.

A.3.4.10 pH meter: with temperature compensation function; the accuracy is +/-0.1. Use standard buffers of pH 4.0, pH 7.0 and pH 10 to calibrate before use.

A.3.5 Operation steps A.3.5.1 Weighing: Accurately weigh about 1 g of the double sample (m)

(accurate to 0.1 mg); the difference in the mass of the double sample is <=0.005 g. Place the samples in the 500 mL tall beakers respectively; add 40 mL of 0.05 mol/L MES-TRSI buffer solution (A.3.3.2); stir magnetically until the sample is completely dispersed in the buffer (stir evenly to avoid sample formation, to prevent the sample from being not able to fully contact the enzyme during the enzymolysis process). Simultaneously prepare two blank sample solutions and sample solutions for simultaneous operation, which are used to correct the influence of reagents on the determination.

A.3.5.2 Thermally stable alpha-amylase enzymolysis: use a pipette to respectively add 50 uL of thermally stable alpha-amylase solution (A.3.2.4) to the sample solution; use aluminum foil to cover (or cover a watch glass); then, place in a 95 °C water bath; continuously stir; when the temperature of the water bath rises to 95 °C, start timing and continue to react for 30 minutes. Take out the beaker; cool it to 60 °C; open the aluminum foil cover; use a spatula to gently scrape off the jelly attached to the inner wall of the beaker; use 10 mL of water to rinse the wall of the beaker and the spatula.

A.3.5.3 Protease enzymolysis: Place the sample solution in a 60 °C water bath;

add 100 uL of protease solution (A.3.3.3) to each beaker; cover with aluminum foil (or cover with a watch glass); stir continuously; let it react for 30 min. Open the aluminum foil cover; add 5 mL 0.6 mol/L hydrochloric acid solution while stirring; use 1 mol/L hydrochloric acid solution to adjust the pH of the sample solution to 4.0 ~ 4.7 at 60 °C.

A.3.5.4 Amyloglucosidase enzymolysis: Add 300 uL of amyloglucosidase solution (A.3.2.6); cover with aluminum foil (or cover with a watch glass);

continue to stir in a 60 °C water bath; react for 30 minutes.

A.3.5.5 Suction filtration and washing: Take the filter crucible (A.3.4.4) that is treated with acetone; add 1 g of diatomite (A.3.3.5); use 3 mL of water to wet the diatomite in the filter crucible; use the suction filtration device to spread the m3 - the mass of protein in the sample residue, in milligrams (mg);

m5 - the mass of ash in the sample residue (m4 - m0), in milligrams (mg);

m2' - the average value of the residual mass of the blank test, in milligrams (mg);

m3' - the mass of protein in the residue of the blank test, in milligrams (mg);

m5' - the mass of ash in the residue of the blank test, in milligrams (mg);

m - the average of the masses of two samples, in milligrams (mg).

Keep three significant figures for the calculation result.

It is expressed by the arithmetic mean of two independent test results under repeatability, with three significant figures kept.

The absolute difference of two independent test results under repeatability cannot exceed 10% of the arithmetic mean value.

A.4 Determination methods of viscosity, gelation and pH of soluble soybean polysaccharide A.4.1 Instruments and apparatuses A.4.1.1 Low temperature constant temperature bath: the temperature control range is 0 °C ~ 90 °C; the temperature fluctuation +/-0.5 °C.

A.4.1.2 Rotational viscometer: the range is 10 mPa.s ~ 1x105 mPa.s; the accuracy is +/-1%.

A.4.1.3 pH meter: accuracy of 0.05.

A.4.2 Operating methods A.4.2.1 Viscosity determination: Weigh 20.00 g of sample; add it to 180.0 mL of water; stir evenly to make a 10% solution. Place the above solution in a low temperature constant temperature bath at 20 °C and keep it at 20 °C +/- 0.5 °C.

Use the rotational viscometer to measure the viscosity of the solution; record the viscosity value.

A.4.2.2 Gelation determination: Heat the sample solution after measuring the viscosity to 100 °C; slowly cool it to 4 °C; observe whether it is gelatinous (use a glass rod to gently stir and observe if necessary).