NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 1886.321-2021

National food safety standard - Food additives - Thaumatin

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1 Scope

This Standard is applicable to the food additive thaumatin obtained from the arils of mature fruit of African thaumatococcus daniellii using water extraction method.

2 Relative molecular mass

Thaumatin I: 22209 (according to the international relative atomic mass in 2018)

Thaumatin II: 22293 (according to the international relative atomic mass in 2018)

3.1 Sensory requirements

The sensory requirements shall meet the requirements of Table 1.

Table 1 - Sensory requirements

3.2 Physical and chemical indicators

The physical and chemical indicators shall meet the requirements of Table 2.

Annex A Inspection methods

A.1 General When other requirements are not specified, the reagents and water used in this Standard refer to analytically-pure reagents and grade 3 water specified in GB/T 6682. All standard solutions used in the test, standard solutions for impurity determination, preparations and products are prepared in accordance with the provisions of GB/T 601, GB/T 602, and GB/T 603 when other requirements are not indicated. The solution used in the test refers to an aqueous solution when it is not specified which solvent is used to prepare it.

A.2 Identification test A.2.1 Solubility identification It is easily soluble in water but insoluble in acetone.

A.2.2 Ninhydrin test In 5mL of 0.1% sample solution, add 1mL of freshly prepared ninhydrin solution (200mg of ninhydrin is dissolved in water, and the volume is set to 100mL). Blue shall appear.

A.2.3 Liquid chromatography test On the liquid chromatogram of content determination, the retention time of the main peak of the specimen solution and the main peak of the reference solution shall be consistent.

A.3 Determination of content (on a dry basis)

A.3.1 Principle of determination Thaumatin is a protein compound. It has a characteristic absorption peak of ultraviolet absorption spectrum at 279nm. The specimen is dissolved by water.

Use liquid chromatography to separate. Use UV detector to test. Use external standard method to quantify.

A.3.2 Reagents and materials A.3.2.1 Water: Grade 1 water.

A.3.2.2 Ammonium acetate.

Under reference chromatographic conditions, respectively inject a series of standard solutions and specimen solutions to determine. According to the external standard method, use a series of standard solutions as a calibration table. Refer to Annex B for the reference retention time and chromatogram of each component.

A.3.6 Result calculation The mass fraction w1 of thaumatin content is calculated according to formula (A.1).

Where, c - The concentration of thaumatin in the test solution, in micrograms per milliliter (ug/mL);

V - The volume of specimen solution, in milliliters (mL);

m - The specimen mass, in milliliters (mL);

1000 - The conversion factor;

f - The dilution times.

The test results are based on the arithmetic mean of the parallel determination results. The absolute difference between the two independent determination results obtained under repeatability conditions is not more than 5% of the arithmetic mean.

A.4 Determination of specific absorption rate A.4.1 Principle of determination Perform spectrophotometric detection at the maximum wavelength (typical wavelength is 279nm).

A.4.2 Reagents and materials A.4.2.1 Hydrochloric acid.

A.4.2.2 Water (equivalent to grade two water in GB/T 6682).

A.4.3 Instruments and equipment A.4.3.1 Dual-beam UV/Visible spectrophotometry.

C - 0.05% thaumatin absorption rate;

100 - The conversion factor;

W - The moisture content of thaumatin (%);

WCF - The weight correction factor;

WT - The mass of the thaumatin specimen actually used, in grams (g);

0.5 - The mass of the theoretically used thaumatin specimen, in grams (g).

The test results are based on the arithmetic mean of the parallel determination results. The absolute difference between two independent determination results obtained under repeatability conditions is not more than 0.2% of the arithmetic mean.

A.5 Determination of absorbance A.5.1 Reagents and materials A.5.1.1 Hydrochloric acid.

A.5.1.2 Water (equivalent to grade two water in GB/T 6682).

A.5.2 Instruments and equipment A.5.2.1 Dual beam UV/Visible spectrophotometer.

A.5.2.2 Quartz cuvette with a light path of 10mm.

A.5.2.3 50mL volumetric flask.

A.5.2.4 Analytical balance: Resolution is 0.1 mg.

A.5.2.5 Pasteur pipette.

A.5.2.6 pH meter.

A.5.3 Analysis steps A.5.3.1 Preparation of specimen solution Accurately weight about 0.5g of specimen in a 50mL volumetric flask. Use the right amount of pH2.5 blank solution to dissolve. Set volume to 50mL.

A.5.3.2 Determination A.6.2.3 75mmx10mm disposable test tube.

A.6.2.4 Vortex mixer.

A.6.2.5 Electric heating constant temperature water bath.

A.6.2.6 1mL adjustable pipette.

A.6.2.7 Analytical balance: Resolution is 0.1mg.

A.6.2.8 Pasteur pipette.

A.6.2.9 pH meter.

A.6.3 Analysis steps A.6.3.1 Preparation of specimen solution Accurately weigh about 0.2g of specimen in a 100mL volumetric flask. Use the right amount of pH2.5 blank solution to

dissolve. Set volume to 100mL. Take 0.2mL of specimen solution in a tube. Prepare a set of thaumatin specimens: 4 in total. Take 0.2mL of pH 2.5 blank solution to prepare a set of blank solution.

Use straws to respectively add 1.2mL of cysteine-sulfuric acid reagent to the blank and the specimen. Use a vortex mixer to thoroughly mix well. Place in ice for 2min. Move to place at room temperature for 3min. Then immerse in the boiling water for 3min. The blank solution and the specimen solution are cooled in ice for 5min.

A.6.3.2 Determination Adjust the wavelength to 412nm. Warm up the spectrophotometer for 10min.

Place the blank solution in the sample cuvette and reference cuvette to zero the spectrophotometer. Take out the sample cuvette containing the blank solution. Place the cuvette that contains the specimen solution. Read the absorbance value (E412). Repeat the test for each set of specimens and take the average value.

A.6.3.3 Preparation of standard curve Use 0.2mLof 10mg/mL~100mg/mL glucose solution to test according to the above methods. Prepare the standard curve.

A.6.4 Result calculation According to the standard curve, calculate the carbohydrate content w3 (as glucose) according to formula (A.5).