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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 28402-2012

National food safety standards of food additives -- Pullulan

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1 Scope

This standard is applicable to the fermentation of carbohydrates by *Aureobasidium pullulans*.

Made of food additives made of pullulan polysaccharide.

2.1 Molecular formula

(C₆H₁₀O₅)_n

3.1 sensory requirements. should be consistent with the provisions of Table 1.

Table 1 sensory requirements

The project requires a test method Color close to white State powder Take appropriate sample in a clean, dry glass container In the natural light to observe its color and state

3.2 Physical and chemical indicators. should be consistent with the provisions of Table 2.

Table 2 Physical and chemical indicators

Item Index Test Method Viscosity (10% solution, 30 ° C)/(mm²/s) 15 to 180 Appendix A A.3 Monosaccharides, disaccharides and oligosaccharides (based on glucose), w /% ≤ 10 Appendix A, A.4 Total nitrogen, w /% ≤ 0.05 GB/T 609

Table 2 (continued)

Item Index Test Method Dry reduction, w /% ≤ 10 GB 5009.3 direct drying method a Lead

(Pb)/(mg/kg) $\leq$ 2.0 GB 5009.12 Burning residue, w /% $\leq$ 8 GB 5009.4 pH 6.0 to 8.0
Appendix A, A.5 a Drying temperature and time are 105 degrees C and 2.5h respectively.

3.3 Microbiological indicators. should be consistent with the provisions of Table 3.

Table 3 Microbial limits

Item	Index	Test Method	Total number of colonies/(CFU/g) $\leq$ 10000	GB 4789.2	Escherichia coli/(MPN/g) $<$ 3.0	GB 4789.3

Appendix A Testing method

A.1 General provisions The reagents and water used in the standard, when not specified in other requirements, refer to the analytical reagent and the third class specified in GB/T 6682-2008 water. Standard titration solution used in the test, the standard solution for the determination of impurities, preparations and products, without any other requirements, GB/T 601, GB/T 602, GB/T 603. The solution used in the test refers to water when it is not specified with the formulation of the solvent Solution.

A.2 Identification test A.2.1 Weigh 10 g of the sample, dissolve in 100 mL of water, and add the sample while stirring to form a viscous solution.

A.2.2 To a 10 mL sample solution (A.2.1), add 0.1 mL of aqueous solution of pullulanase (10 units/mL), mix, So that no viscous solution is obtained.

A.2.3 Weigh 1 g of the sample and dissolve in 50 mL of water. Take 10 mL of this solution and add 2 mL of polyvinyl alcohol 600 to form white precipitation.

A.3 Determination of viscosity A.3.1 Instruments and equipment A.3.1.1 Inner diameter 2mm flat glass capillary viscometer.

A.3.1.2 Agitator (800r/min).

A.3.2 Analysis steps A.3.2.1 Preparation of sample solution Measure 100mL of water in the mixing cup, placed on the stirrer, open the stirring. Accurately weighed 10.0g dried at 105 degrees C for 2.5h Product, slowly added to the mixing cup, stirring at 800r/min to full dissolution, and then put it aside for 1h.

A.3.2.2 Determination With 10mL suction tube to absorb 10mL sample solution, into the viscometer, the viscometer vertical into the 25 degrees C $\pm$ 0.1 degrees C incubator, Insulation 20min, with the suction ear to the viscometer liquid to the ball between the first tick on the line 5mm, so that the sample free flow, to be liquid Flow to the first tick line, press the stopwatch timing, to reach the second tick line, the timer stops, the downstream time to s, calculate the viscosity.

A.3.3 Calculation of results Sample viscosity w_0 calculated according to formula (A.1).

$$w_0 = C \times \tau \quad (\text{A.1})$$

Where.

w_0 --- sample viscosity, in units of centis (mm^2/s);

C - constant value of viscometer in centis per second (mm^2/s^2);

τ --- flow time in seconds (s).

The experimental results are based on the arithmetic mean of the parallel measurement results. The absolute difference between the two independent determinations obtained under repeatability conditions The value is not greater than 2% of the arithmetic mean.

A.4 Determination of monosaccharides, disaccharides and oligosaccharides A.4.1

Methodological Summary After the samples were precipitated with methanol and potassium chloride, the contents of monosaccharides, disaccharides and oligosaccharides in the samples were determined by anthrone-sulfuric acid method.

A.4.2 Reagents and materials A.4.2.1 Glucose.

A.4.2.2 Methanol.

A.4.2.3 anthrone solution. 0.2g anthrone dissolved in 100g 75% (volume fraction) of sulfuric acid solution, is now available.

A.4.2.4 saturated potassium chloride solution. the amount of water to take appropriate, placed in a beaker, add potassium chloride crystals, while adding side of the stirring, added to the added crystal When it is no longer dissolved, then take the supernatant.

A.4.3 Instruments and equipment Spectrophotometer, detection wavelength of 620nm.

A.4.4 Analysis steps A.4.4.1 Preparation of standard solutions Weigh 0.2g glucose, accurate to 0.001g, dissolved in water, diluted volume to 1L, from the amount of 0.2mL, add to 5mL anthracene Ketone solution, mixed evenly, this is the standard solution.

A.4.4.2 Preparation of blank solution Measure 0.2mL of water, add to 5mL anthrone solution, mixed evenly, this is a blank solution.

A.4.4.3 Preparation of sample solution Weigh 0.8g sample, accurate to 0.001g, dissolved in water, diluted volume to 100mL, this is the sample stock solution. Measure 1mL The sample stock solution was placed in a centrifuge tube and 0.1 mL of saturated potassium chloride solution and 3 mL of methanol were added and vigorously mixed for 20 s.

11000r/min under the conditions of centrifugation 10min. The amount of 0.2mL centrifugal supernatant, added to 5mL anthrone solution, mixed evenly, This is the sample solution.

A.4.4.4 Determination The sample solution, standard solution and blank solution in 90

degrees C water bath for 15min, with a spectrophotometer, measured at 620nm Determine the absorbance of these solutions.

A.4.5 Calculation of results The content of monosaccharides, disaccharides and oligosaccharides is expressed in terms of mass fraction w_1 of glucose, expressed in%, calculated according to formula (A.2)

$$w_1 = (A_t - A_b) \times 0.41 \times m_1 / (A_s - A_b) \times m_0 \times 100\% \quad (A.2)$$

Where.

A_t - absorbance of the measured sample solution;

A_b - measured absorbance value of the blank solution;

0.41 --- conversion factor;

A_s - the measured absorbance of the standard solution;

m_1 --- the value of glucose quality, in grams (g);

m_0 --- the value of the sample quality, in grams (g).

The experimental results are based on the arithmetic mean of the parallel measurement results. The absolute difference between the two independent determinations obtained under repeatability conditions The value is not greater than 2% of the arithmetic mean.

A.5 Determination of pH A.5.1 Instruments and equipment A.5.1.1 Acidity meter. Accuracy 0.01pH units.

A.5.1.2 Analytical balance. Accuracy 0.001g.

A.5.1.3 Agitator. 800r/min.

A.5.2 Analysis steps A.5.2.1 measure 270mL water in the mixing cup, placed on the stirrer, open the stirring.

A.5.2.2 Accurately weigh 30.0 g of the sample, slowly add to the stirring cup and stir at 800r/min for 30min.

A.5.2.3 The pH of the solution (accurate to 0.01pH units) was measured with an acidity at 25 ° C +/- 1 ° C.

The experimental results are based on the arithmetic mean of the parallel measurement results. The absolute difference between the two independent determinations obtained under repeatability conditions The value is not greater than 2% of the arithmetic mean.

No. 1 amendment This modification was approved by the National Health and Family Planning Commission of the People's Republic of China on April 29, 2014, No. 7, The date of approval.