PULLULAN

New specifications prepared at the 65th JECFA and published in FNP 52 Add 13 (2005). An ADI 'not specified' was established was established at the 65th JECFA (2005).

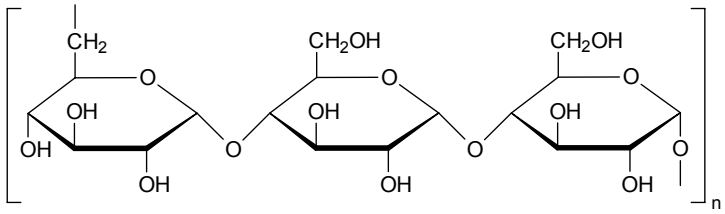
SYNONYMS INS No. 1204

DEFINITION Linear, neutral glucan consisting mainly of maltotriose units connected by α-1,6 glycosidic bonds. It is produced by fermentation from a food grade hydrolysed starch using a non-toxin producing strain of *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilized and pigments and other impurities are removed by adsorption and ion exchange chromatography.

C.A.S. number 9057-02-7

Chemical formula $(C_6H_{10}O_5)_x$

Structural formula



Assay Not less than 90% of glucan on the dried basis

DESCRIPTION White to off-white odourless powder

FUNCTIONAL USES Glazing agent, film-forming agent, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, practically insoluble in ethanol

pH (Vol. 4) 5.0 - 7.0 (10% solution)

Precipitation with polyethylene glycol 600 Add 2 ml of polyethylene glycol 600 to 10 ml of a 2% aqueous solution of pullulan. A white precipitate is formed.

Depolymerization with pullulanase Prepare two test tubes each with 10 ml of a 10% pullulan solution. Add 0.1 ml pullulanase solution having activity 10 units/g (refer to pullulanase activity, under Methods for enzyme preparations in Volume 4) to one test tube, and 0.1 ml water to the other. After incubation at about 25° for 20 minutes, the viscosity of the pullulanase-treated solution is visibly lower than that of the untreated solution.

PURITY

Loss on drying (Vol. 4) Not more than 6% (90°, pressure not more than 50 mm Hg, 6 h)

Mono-, di- and oligosaccharides	Not more than 10% (expressed as glucose) See description under TESTS
Viscosity	100-180 mm^2/s (10% w/w aqueous solution at 30°) See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, “Instrumental Methods”.
Microbiological criteria (Vol. 4)	Yeast and moulds: Not more than 100 CFU/g Coliforms: Negative in 25 g Salmonella: Negative in 25 g

TESTS

PURITY TESTS

Mono-, di- and oligosaccharides

Principle

The soluble mono-, di- and oligosaccharides of pullulan are measured using the anthrone-sulfuric acid method after pullulan has been precipitated with methanol and KCl.

Equipment

Spectrophotometer capable of measuring absorbance at 620 nm

Procedure

*Preparation of standard: W*eigh accurately 0.2 g glucose, dissolve in water and make up to 1 l.

Measurement of mono-, di- and oligosaccharides:
Weigh accurately 0.8 g sample and dissolve in water to make 100 ml (stock solution).
Place 1 ml of the stock solution in a centrifuge tube. Add 0.1 ml saturated potassium chloride solution. Add 3 ml methanol and mix vigorously for 20 sec. Centrifuge at 11000 rpm for 10 minutes. Add 0.2 ml of the supernatant to 5 ml modified anthrone solution (0.2 g anthrone in 100 g 75% (v/v) sulfuric acid, freshly prepared). Add 0.2 ml of glucose standard solution and 0.2 ml water (blank control) to separate 5 ml portions of modified anthrone solution. Mix rapidly. Place samples in a 90º water bath and incubate for 15 min. Measure absorbance of the test solution at 620 nm.

Calculate the percent of mono-, di- and oligosaccharides expressed as glucose, *C*, in the sample:

$$C(\%) = [(A_t - A_b)\times 0.41\times G\times 100]/(A_s - A_b)\times W$$

where

A_t is absorbance of the test solution
A_b is absorbance of the water blank
A_s is absorbance of the standard solution
G is weight of the glucose (g)
W is weight of the sample (g)

Viscosity

Dry the sample for 6 h at 90º under reduced pressure (50 mm Hg). Weigh 10.0 g of the sample and dissolve in water to yield 100 g of solution.

Use an Ubbelohde-type (falling-ball) viscometer. Charge the viscometer with sample in the manner dictated by the design of the instrument. Immerse the viscometer vertically in the thermostatic tank at 30 ± 0.1º and allow to stand for 20 min so that the sample equilibrates with the temperature in the tank. Adjust the meniscus of the column of liquid in the capillary tube to a position about 5 mm above of the first mark. With the sample flowing freely, measure, in seconds, the time required for the meniscus to pass from the first to the second mark. Calculate the viscosity, V:

$V\ (mm^2/s) = C \times t$

where
C = calibration constant of the viscometer (mm^2/s^2)
t = flow time (s)

METHOD OF ASSAY

Calculate the percentage of pullulan on dried basis, P, as the difference between 100% and the sum of the percentages of known impurities (mono-, di- and oligosaccharides and water).

$P(\%) = 100 - (L+C)$

where
L is loss on drying
C is taken from the calculation for mono-, di- and oligosaccharides