PECTINASE from *ASPERGILLUS NIGER*, var.

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 31st JECFA (1987) and published in FNP 38 (1988) and in FNP 52 (1992). An ADI "Not specified" was established at the 35th JECFA (1989).

SOURCES

Produced by the controlled fermentation of non-pathogenic and non-toxicogenic strains of *Aspergillus niger* and isolated from the growth medium.

Active principles

1. Pectinesterase (synonym: pectin methylesterase)
2. Polygalacturonase
3. Pectin lyase (synonym: pectin depolymerase)

Systematic names and numbers

1. Pectin pectylhydrolase (EC 3.1.1.11; C.A.S. No. 9025-98-3)
2. Poly (1,4-alpha-D-galacturonide)glycanohydrolase (EC 3.2.1.15; C.A.S. No. 9032-75-1)
3. Poly (methoxy-L-galacturonide) lyase (EC 4.2.2.10; C.A.S. No. 9033-35-6)

Reactions catalyzed

1. Demethylation of pectin
2. Hydrolysis of 1,4-alpha-galacturonide linkages in pectin
3. Eliminative cleavage of pectin to give oligosaccharides

Secondary enzyme activities

alpha-Amylase (1,4-alpha-D-glucan-glucanohydrolase, EC 3.2.1.1; C.A.S. No. 9000-90-2)
beta-Glucanase (EC 3.2.1.6; C.A.S. No. 9074-99-1)
beta-Glucosidase (EC 3.2.1.21; C.A.S. No. 9001-22-3)
Cellulase ((1,4-[1,3;1,4]-beta-D-Glucan 4-glucano.-hydrolase; EC 3.2.1.4; C.A.S. No. 9012-54-8)
Xylanase (EC 3.2.1.32)

DESCRIPTION

Typically off-white to tan amorphous powders or tan to dark-brown liquids. The unformulated product is usually diluted and standardised with food grade glycerol, water and potassium chloride to obtain commercial products. Soluble in water and practically insoluble in ethanol and ether.

FUNCTIONAL USES

Enzyme preparation.
Used mostly to reduce viscosity, to improve filtration to clarify products, to avoid particle sedimentation and to prevent pectin gel formation in the manufacture of fruit juice and wine.

GENERAL SPECIFICATIONS

Must conform to the General Specifications for Enzyme Preparations used in Food Processing (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Pectinase activity

The sample shows Pectinase activity
See description under TESTS

TESTS

Pectinase activity

Principle
This assay method is based on the enzymatic hydrolysis of pectin, the resulting galacturonic acid being determined spectrophotometrically at 235 nm. One unit of pectinase activity (PTA) causes an increase of 0.010 of absorbance per minute under the conditions of the assay (Pectin 0.5 %, pH 5.8 and 30°)

Apparatus
Spectrophotometer set at 235 nm (if possible double beam type, preferably with reference automation and cuvette changer).
Water bath set at 30.0 ± 0.1°

Reagents and solutions
Citrate buffer pH 5.8
35.6 g Disodium hydrogen phosphate ($Na_2HP0_4.2H_20$) are dissolved in 1000 ml of water (A). 21 g of Citric acid monohydrate are dissolved in 1000 ml of water (B). 57 ml of solution A and 43 ml of solution B are mix to obtain working buffer solution. pH can be adjusted to 5.8 by adding one of the aforesaid solutions.

Pectin substrate solution
0.5 g of pectin (pectin, such as Copenhagen Pectin X, 2955, or Sigma, P2157) are mashed with 2 ml ethanol in a beaker and stirred to homogeneity with a magnetic stirrer. Slowly (allow 2 minutes for this step) add 80 ml of 0.1 M citrate buffer and continue stirring, avoiding foam formation. Measure pH and if necessary adjust to pH= 5.8 with solutions A or B respectively. Transfer solution to a volumetric flask and add 0.1 M citrate buffer up to 100 ml. Keep substrate solution cool over night (refrigerator). Next day centrifuge solution 10 minutes at 12000 x g.

Samples preparation
Weigh 0.5 g to 2 g of sample and dissolve with buffer solution into a volumetric flask. Fill up to 100 ml and transfer 3 ml of this solution to a next volumetric flask and fill up with buffer to 25 ml. Sample solutions have to be prepared immediately before analysis.

Standard enzyme solution
Dilute standard enzyme (such as Sigma P 9179, Rohapect D5L or equivalent) with known activity in buffer solution to give 12 PTA enzyme units per ml.

Procedure
Measurement of enzyme activity
Add 0.1 ml of sample or standard solution to 3.0 ml of substrate solution pre-warmed to 30 ± 0.1° for 5 min. After short mixing, the absorbance at 235 nm is registered each minute over 8 minutes using distilled water as blank. Determination is done in triplicate.
Calculation
Plot absorbance (sample-water blank) data as the abscissa and time of reaction as the ordinate. The slope of the extinction, ($\Delta A/\Delta t$), is determined in the linear section of the function. The linear part should encompass at

least 5 minutes (6 data points). Delays of the enzyme reaction before the linear part (lag-phase) are not taken into account. Pectinase activity units is calculated as:

$$\text{Pectinase Activity, U / mg} = \frac{\Delta A_{235} / \Delta t}{0.01 \times C \times V}$$

Where:
V = final reaction volume, ml (0.1 ml of sample or standard solution plus 3.0 ml of substrate solution)
C = final sample or standard concentration, mg/ml
The change in absorbance should not exceed 0.03 per min. Best values are within a range of 0.02 to 0.03.