CELLULASE from *PENICILLIUM FUNICULOSUM*

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). No ADI was allocated at the 31st JECFA (1987).

SOURCES Produced by the controlled fermentation of non-toxicogenic and non-pathogenic strains of *Penicillium funiculosum* and isolated from the growth medium.

Active principles
Cellulase (endo-1,4-beta-glucanase)
Endo-1,3(4)-beta-glucanase
Endo-1,4- beta-xylanase

Systematic names and numbers
1,4-(1,3; 1,4)- beta-D-Glucan-4-glucanohydrolase (EC 3.2.1.4)
1,3-(1,3; 1,4)- beta-D-Glucan-3(4)-glucanohydrolase (EC 3.2.1.6)
1,4- beta-D-xylan xylohydrolase (EC 3.2.1.8)

Reactions catalyzed
Hydrolyzes 1,4-beta-glucan linkages in polysaccharides such as cellulose, yielding beta-dextrins.

Secondary enzyme activities
alpha-N-Arabinofuranosidase
Cellulose 1,4- beta-cellobiosidase
beta-glucosidase
Xylan 1,4-beta-xylosidase

DESCRIPTION Typically off-white to tan amorphous powders, or liquids dispersed in food-grade carriers or diluents; soluble in water; practically insoluble in ethanol and ether.

FUNCTIONAL USES Enzyme preparation. Used in the preparation of fruit juices, wine, beer and vegetable oils

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

CHARACTERISTICS

IDENTIFICATION

Cellulase activity
The sample shows cellulase activity
See description under TESTS

Glucanase activity (Vol. 4)
The sample shows glucanase activity

Xylanase activity
The sample shows xylanase activity
See description under TESTS

TESTS

Cellulase activity
Principle

The assay is based on the ability of the enzyme to hydrolyze carboxymethyl cellulose (CMC) to reducing sugars. The reaction products are determined photometrically at 540 nm by measuring the resulting increase in reducing groups using 3,5-dinitrosalicylic acid. One cellulase unit is defined as the amount of enzyme that liberates reducing sugar at the rate of 1 µmol/min under the conditions of the assay.

Apparatus
Spectrophotometer set at 540 nm.
Water-bath set at 40.0 ± 0.1°

Reagents
1. CMC substrate solution (1.0%): Accurately weigh 0.500 g of CMC (SIGMA C5678-7 or equivalent) and sprinkle on to warm 40 ml of water in a beaker. Place beaker on a hot-plate equipped with a magnetic stirrer, apply heat and stir vigorously. When the liquid has started to boil, cover the beaker with a watch glass, turn off the hot plate and continue stirring until the solution is cool. Quantitatively transfer the solution into a 50 ml volumetric flask, add 5 ml acetate buffer, adjust the pH to 5.0 and make up to volume.

2. 3,5-Dinitrosalicylic acid (DNS) solution: Accurately weigh 10 g of DNS into a 2000-ml beaker. Add 16 g of sodium hydroxide pellets, 300 g of potassium sodium (+)-tartrate and 500 ml of water. Place the beaker on a heater/stirrer and warm gently, whilst stirring, to dissolve. Cool to ambient temperature and transfer the contents of the beaker into a 1000-ml volumetric flask. Rinse the beaker with water, add to volumetric flask and make up to volume with water. Store the solution at ambient temperature for up to 10 weeks. It is possible that DNS reagent get overheated during the preparation making the solution quite dark. The maximum absorbance at 540 nm for a blank (without glucose standard) measured against water shall not be more than 0.050 absorbance units.

3. DNS-lactose solution: Dissolve lactose monohydrate with water to obtain 0.120 g/l solution. Mix l50 ml of DNS solution and 50 ml of Lactose solution. Use freshly prepared mixture.

4. Samples preparation: Dissolve known quantity of sample in distilled water. Make serial dilutions to get a working solution in the absorbance range of 0.150 - 0.400

5. Glucose standard solution: Accurately weigh 0.5g of anhydrous glucose and make up to volume in a 100 ml volumetric flask. Dilute the solution with water to get 5, 10 and 15 µmoles/l of glucose.

Procedure
Measurement of enzyme activity
Add 1 ml of substrate solution (pre-warmed to 40.0± 0.1° for 5 min) to an equal volume of sample solution also pre-warmed to 40.0 ± 0.1°. Mix the resulting solution thoroughly and transfer to a water-bath maintained at 40.0 ± 0.1 °. After 10 minutes (reaction step) remove the test tube from the water bath, and add 4 ml of DNS-Lactose solution and mix to stop the enzymatic reaction. Cover tubes and place in a boiling water bath for 15 min. and then cooled to room temperature with a cooling water bath. Remove insoluble

substances by centrifugation (3000 rpm, 10 min). Determine the absorbance at 540 nm against water blank. Prepare a reaction blank in a similar manner but without a reaction step. Prepare a reagent blank omitting substrate and read absorbance against water.

Standard curve

Prepare the glucose standard curve by adding 1 ml glucose standard solution (5, 10 and 15 µmoles/l) instead of CMC substrate solution in the procedure described above. Draw the standard curve in a coordinate system using glucose concentration (µmol/l) as the abscissa and absorbance as the ordinate. The standard curve is a straight line passing through the origin and linear regression can therefore be applied.
Calculate the glucose concentration in the sample from the standard curve and calculate the enzyme activity as follows.

Calculation

Calculate the sample enzyme activity (U/g) by reading the equivalent glucose concentration on the standard curve for the sample and the reaction blank and inserting them in the following formula:

$$\text{Cellulase Activity, U/g} = \frac{(C_G - C_{RB}) \times D}{W \times 10 \times V}$$

Where
C_G : Reading from the standard curve for sample enzyme, µmol/l
C_{RB} : Reading from the standard curve for reagent blank, µmol/l
D : Dilution factor of the sample
W : Weight of sample taken, g
10 : Incubation time, min
V : Volume of sample solution taken, 1 ml

Xylanase activity

Principle

This assay is based on the enzymatic hydrolysis of sodium arabinoxylan. The resulting reducing sugar is allowed to react with 3,5-dinitrosalicylic acid and is determined photometrically at 540 nm. One xylanase unit is defined as that quantity of enzyme that liberates reducing sugar at a rate of 1 µmol /min under the conditions of the assay.

Apparatus

Spectrophotometer set at 540 nm.
Water bath set at 40.0 ± 0.1°

Reagents and solutions

1. Xylan substrate solution (1.0%): Accurately weigh 1.0 g xylan (dry base, from oat spelts; such as SIGMA X-0627), transfer to a beaker with 60 ml of 0.2 M acetate buffer (pH 4.5). Stir for 30 min and incubate at 60 ° for l hr with gradually stirring and check pH (4.50 ± 0.05). Transfer the solution into a 100 ml volumetric flask and make up to volume with water.

2. 3,5-Dinitrosalicylic acid (DNS) solution: Accurately weigh 10 g of DNS into a 2000-ml beaker. Add 16 g of sodium hydroxide pellets, 300 g of potassium sodium (+)-tartrate and 500 ml of water. Place the beaker on a heater/stirrer

and warm gently, whilst stirring, to dissolve. Cool to ambient temperature and transfer the contents of the beaker into a 1000-ml volumetric flask. Rinse the beaker with water, add rinsings to the volumetric flask and make up to volume with water. Store the solution at ambient temperature for up to 10 weeks. It is possible that DNS reagent get overheated during the preparation making the solution quite dark. The maximum absorbance at 540 nm for a blank (without xylose standard) measured against water shall not be more than 0.050 absorbance units.

3. DNS-lactose solution: Dissolve lactose monohydrate with water to obtain 0.120 g/l solution. Mix l50 ml of DNS solution and 50 ml of Lactose solution. Use freshly prepared mixture.

4. Samples preparation: Dissolve known quantity of sample in distilled water. Make serial dilutions to get a working solution in the absorbance range of 0.150 - 0.400

5. Xylose standard dilutions: Accurately weigh 0.5g of anhydrous xylose with distilled water and make up to 100 ml in a volumetric flask. Dilute with water to get working standard solutions containing 250, 500 and 750 µmoles/l of xylose.

Procedure

Measurement of enzyme activity

Add 0.1 ml of sample solution to 1.9 ml of substrate solution pre-warmed to 40.0 ± 0.1° for 5 min. Mix the resulting solution thoroughly and transfer to a water-bath maintained at 40 ± 0.1°. After 10 minutes (reaction step) remove the test tube from the water bath, and add 4 ml of DNS-Lactose solution and mix to stop the enzymatic reaction. Cover tubes and place in a boiling water bath for 15 min. and then cooled to room temperature with a cooling water bath. Remove insoluble substances by a centrifuge (3000 rpm, 10 min). Determine the absorbance at 540 nm against water blank. Prepare a reagent blank in a similar manner but without a reaction step.

Standard curve

Prepare the xylose standard curve by adding 0.1 ml xylose standard solution (250, 500 and 750 µmoles/l) instead off xylan substrate solution in the procedure described above. Draw the standard curve in a coordinate system using glucose concentration (µmol/l) as the abscissa and absorbance as the ordinate The standard curve is a straight line passing through the origin and linear regression can therefore be applied.

Calculate the xylose concentration in the sample from the standard curve and calculate the enzyme activity as follows.

Calculation

Calculate the sample enzyme activity (U/g) by reading the equivalent xylose concentration on the standard curve for the sample and the reaction blank and inserting them in the following formula:

$$\text{Xylanase Activity, U / g} = \frac{(C_X - C_{RB}) \times D}{W \times 10 \times V}$$

Where
C_x : Reading from the standard curve for sample enzyme, µmol/l
C_{RB}: Reading from the standard curve for reagent blank, µmol/l
D : Dilution factor of the sample
W : Weight of sample taken, g
10 : Incubation time, min
V : Volume of sample taken, 0.1 ml