ISOAMYLASE FROM *PSEUDOMONAS AMYLODERAMOSA*

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS Debranching enzyme; α-1,6-glucan hydrolase

SOURCES Isoamylase is produced by submerged fed-batch pure culture fermentation of *Pseudomonas amyloderamosa*. The enzyme is isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration. The final product is formulated using food-grade stabilizing and preserving agents.

Active principles: Isoamylase

Systematic names and numbers: Glycogen α-1,6-glucanohydrolase; EC 3.2.1.68; CAS No. 9067-73-6

Reactions catalysed: Hydrolysis of α-1,6-D-glucosidic linkages in glycogen, amylopectin and their β-limit dextrins.

Secondary enzyme activities: Low levels of cellulase, lipase, and protease.

DESCRIPTION Yellow to brownish liquid

FUNCTIONAL USES Enzyme preparation.
Used in the production of food ingredients from starch.

GENERAL SPECIFICATIONS Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

CHARACTERISTICS

IDENTIFICATION

Isoamylase activity: The sample shows isoamylase activity.
See description under TESTS.

TESTS

Isoamylase activity

Principle

Isoamylase activity is determined by incubating the enzyme with soluble waxy corn starch as a substrate in the presence of iodine for 30 min under standard conditions (pH=3.5; 40.0±0.1°) and measuring absorbance of the reaction mixture at 610 nm. The change in

absorbance represents the degree of hydrolysis of the substrate. Isoamylase activity is calculated in isoamylase activity units (IAU) per gram of the enzyme preparation. One IAU is defined as the amount of isoamylase that increases absorbance of the reaction mixture by 0.008 in 30 min under the conditions of the assay.

Apparatus
Spectrophotometer (UV/VIS).
Thermostated water bath with agitator
Test tubes (18x180 mm)
Vortex mixer
Digital timer or stopwatch.

Reagents and solutions
Substrate solution: Accurately weigh 4.17 g (dried basis) of soluble waxy corn starch (Hayashibara Biochemical Laboratories, Inc., Cat. No. CS 101, or equivalent) in a 50-ml beaker. Add approximately 30 ml of water and stir to produce a suspension. Heat to boiling approximately 300 ml of water in a 500-ml beaker with stirring. Slowly add the starch slurry to the boiling water. Rinse the empty beaker with a small amount of water and add the rinse to the boiling water. Boil the starch suspension for 5 min. Quantitatively transfer the starch solution to a 500-ml volumetric flask and cool to room temperature under running water. Stir the solution continuously during cooling. Add 50 ml of 1 M acetate buffer solution (pH 3.5) and dilute with water to volume. The solution should be freshly prepared.

Acetate buffer solution (1 M; pH 3.5): Mix 1 M acetic acid and 1 M sodium acetate to obtain pH 3.5. The solution is stable for up to 3 months at room temperature.

Acetate buffer stock solution (1M; pH 4.5): Mix 1 M acetic acid and 1 M sodium acetate to obtain pH 4.5. The solution is stable for up to 3 months at room temperature.

Acetate buffer working solution (0.01M; pH 4.5): Transfer 1 ml of the acetate buffer stock solution (1M, pH 4.5) to a 100-ml volumetric flask and add water to volume. The solution should be freshly prepared.

Iodine solution (0.01 N): Transfer 10 ml of 0.1 N iodine solution to a 100-ml volumetric flask and add water to volume. The solution should be freshly prepared.

Sample solution: Accurately weigh (to four decimal places) approximately 3g of the sample into a 1000-ml volumetric flask and add the acetate buffer working solution (0.01 M, pH 4.5) to volume. Repeat the dilution with the acetate buffer working solution as necessary to obtain the activity of approximately 25-50 IAU/ml. The solution should be freshly prepared.

Procedure

Blank solution: Place 3.0 ml of freshly prepared substrate solution in a test tube and incubate in a water bath at 40.0±0.1^{o} for 10 min. Add 0.5 ml of the sample solution and mix rapidly. After 30 sec, transfer 0.5 ml of the reaction mixture to a test tube containing 15 ml of 0.02 N sulfuric acid and mix rapidly.

Test solution: Incubate the remaining reaction mixture in a water bath at 40.0±0.1^{o} for 30 min and 30 sec. Transfer 0.5 ml of the reaction mixture to a test tube containing 15 ml of 0.02 N sulfuric acid and mix rapidly.

Let the blank and test solutions stand at 25±1^{o} for at least 15 min. Then add 0.5 ml of 0.01 N iodine solution to both solutions and let the solutions stand at 25±1^{o} for another 15 min. Read the absorbance of each solution at 610 nm against water in a 10-mm cell.

Calculations

Calculate the activity of the sample in IAU/g according to the following equation:

$$\text{Activity(IAU/g)} = \frac{(E_{30} - E_0) \times V \times D \times F}{0.004 \times W}$$

where:

E_0 is the absorbance of the blank solution,
E_{30} is the absorbance of the test solution,
V is the volume of the volumetric flask in which the sample was initially dissolved (ml),
D is the dilution of the sample solution,
W is the sample weight (g),
0.004 is obtained by multiplying the absorbance corresponding to one IAU (0.008) by the volume of the sample solution used in the experiment (0.5 ml), and
F is the correction factor that accounts for discrepancies between various batches of the substrate. This factor is determined for each new batch of the substrate by measuring the isoamylase activity of the same sample of the enzyme preparation using the old and new batch of the substrate. F is calculated by dividing the result obtained with the old batch by that obtained with the new batch of the substrate.