MALTOGENIC AMYLASE from *BACILLUS STEAROTHERMOPHILUS* expressed in *BACILLUS SUBTILIS*

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 5, Add 6 (1998). ADI "not specified" established at the 51st JECFA in 1998.

SOURCES

Prepared by submerged fermentation of *Bacillus subtilis*, which through recombinant DNA techniques, contains the gene coding for maltogenic amylase. The strain of *Bacillus subtilis* is non-pathogenic and non-toxicogenic e. g. strain DN 252 (a derivative of *Bacillus subtilis* 168, strain QB 1133, BGSC1A289) The gene is imported from *Bacillus stearothermophilus.*

Active principles: Exo-acting alpha-amylase

Systematic names and numbers: Glucan 1,4-alpha-maltohydrolase, E.C. 3.2.1.133

Reactions catalyzed: Exohydrolysis of 1,4-alpha-glucosidic linkages in amylose, amylopectin and related glucose polymers. Maltose units are successively removed from the non-reducing end of the polymer chain until the molecule is completely degraded or, in the case of amylopectin, a branch point is reached.

DESCRIPTION

Liquids, granulates or powders

FUNCTIONAL USES

Enzyme preparation
Used in the retardation of staling in baked goods; preparation of high maltose glucose syrup

GENERAL SPECIFICATIONS

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

CHARACTERISTICS

IDENTIFICATION

Maltogenic amylase activity: The sample shows maltogenic amylase activity
See description under TESTS

TESTS

IDENTIFICATION TESTS

Maltogenic amylase activity

Principle

The method is based on the ability of the enzyme to hydrolyze maltotriose to maltose and glucose. The reaction is stopped by raising the pH of the reaction medium to about 11. The formed glucose is converted to gluconolactone by glucose dehydrogenase in the presence of NAD^+ , which is converted to NADH. The change in the concentration of NAD^+ is measured photometrically at 340 nm. One enzyme unit (Maltogenic

Amylase Unit, MAU) is defined as the amount of enzyme, which, under standard assay conditions, cleaves 1 µmol of maltotriose per minute. Assay conditions being as follows: Substrate concentration 10 mg/ml; temperature 37°; pH 5.0; incubation time 30 min.

Preparation of 20 mg/ml-maltotriose substrate
Dissolve 1000 mg maltotriose (e.g. Sigma M8378) in 0.1 M citrate buffer, pH 5.0, making a total volume of 50 ml (to be prepared each day of analysis).

Preparation of glucose dehydrogenase (GluDH) reagent
Add buffer solution (Merck No. 14051 or equivalent) to the enzyme mixture (Merck No. 14055, bottles "1" and "2", or equivalent). Allow the mixture to stand for 15 min. Transfer the solution to a 500-ml volumetric flask, containing 200 ml of 0.1 M citrate buffer, pH 5.0. Fill the flask to the mark with the same buffer. This solution can be stored for two weeks at room temperature (not in a refrigerator).

Preparation of standard enzyme solutions
Dilute a maltogenic amylase standard with known activity in 10 mM NaCl to give the following enzyme units per ml: 0.015, 0.030, 0.045, 0.060 and 0.075.

Measurement of enzyme activity
Add 500 µl of substrate solution (20 mg/ml maltotriose, prepared in 0.1 M citrate buffer, pH 5.0, pre-warmed to 37°) to an equal volume of enzyme solution also pre-warmed to 37°. Mix the resulting solution thoroughly and transfer to a water bath maintained at 37°. After 30 min remove the test tube from the water bath, and add 1000 µl of the stop reagent (0.06N NaOH) and shake vigorously. Add 3 ml of GluDH reagent and mix again. Leave the test tube at room temperature for exactly 30 min and measure optical density (OD) at 340 nm. Prepare a blank in a similar manner except that the stop reagent is added before the substrate.

Calculation of enzyme activity
Draw the enzyme standard curve in a coordinate system using enzyme activity (MAU/ml) as the abscissa and ΔOD (sample - blank) as the ordinate. Calculate the activity of the sample by reading ΔOD of sample dilutions on the standard curve and then multiplying by the corresponding dilution factors. The standard curve is a straight line passing through the origin and linear regression can therefore be applied.

Basic standardisation
Glucose solutions:

a) Stock solution: Accurately weigh 1.60 g of glucose, analytical grade, in a 1000-ml volumetric flask and make up to volume with demineralised water.
b) standard solutions: Dilute 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of stock solution to volume with deionised water in 100-ml volumetric flask to give standard solutions of 88.8, 178, 266, 355, 444 and 533 µmol/l.

Standard curve

Prepare the glucose standard curve by mixing 2.0 ml glucose solution with 3.0 ml GluDH reagent and incubating at room temperature for 30 min. Read OD at 340 nm. Prepare a common blank using demineralised water instead of glucose solution. Plot glucose concentration (µmol/l) versus ΔOD.

Calculation

Calculate the enzyme activity by reading the equivalent glucose concentration on the standard curve and inserting it in the following formula:

$$\text{MAU/g} = \frac{A \times 4 \times F}{30 \times 1000}$$

where:
A = reading on the standard curve, µmol/l
F = dilution factor of the enzyme solution, ml/g
4 = the ratio between amount of enzyme dilution in sample and amount of glucose solution in standard
30 = incubation time, 30 min
1000 = conversion from litre to ml