XYLANASE (resistant to xylanase inhibitor) from *BACILLUS SUBTILIS* containing a MODIFIED XYLANASE GENE from *BACILLUS SUBTILIS*

New specifications prepared at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004). An ADI "not specified" was established at the 63rd JECFA (2004).

SYNONYMS

Beta-1,4-D-xylan xylanohydrolase; endo-1,4-beta-xylanase; beta-D-xylanase; beta-xylanase

SOURCES

Produced by pure culture fermentation of a nonpathogenic and nontoxigenic genetically modified strain of *Bacillus subtilis* containing the xylanase gene derived from *B. subtilis* and modified to encode xylanase that is resistant to the xylanase inhibitor present in flour. The enzyme is secreted to the fermentation broth which is subsequently separated from the bacterial cells and subjected to ultrafiltration to obtain the concentrated xylanase. The concentrated enzyme is either dried on a suitable carrier (e.g., starch and salt) or formulated as a liquid product using food grade compounds for stabilization and preservation. The dried product can be prepared either as a free-flowing microgranulate or tablets.

Active principles: Xylanase

Systematic names and numbers: 1,4-beta-D-xylan xylanohydrolase; EC 3.2.1.8; CAS No. 9025-57-4

Reactions catalyzed: Endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans and arabinoxylans

DESCRIPTION

Brownish liquid, off-white microgranulate or tablets.

FUNCTIONAL USES

Enzyme preparation.
Used in baked goods and pasta to increase dough stability.

GENERAL SPECIFICATIONS

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

CHARACTERISTICS

IDENTIFICATION

Xylanase activity: The sample shows endo-xylanase activity
See description under TESTS

TESTS

Xylanase activity

Principle

Xylanase samples are incubated with azurine-crosslinked wheat arabinoxylan substrate. Xylanase hydrolyses the substrate to water-soluble fragments with the concomitant change in colour. The reaction is terminated after a designated time and the optical density (OD) of the reaction mixture is measured at 590 nm (OD_{590}). Xylanase activity is calculated based on the rate of release of the azurine dye. One xylanase unit (XU) is defined as the amount of enzyme that increases the OD_{590} at a rate of one OD per 10 minutes under standard conditions (pH=5.00; 40^{o}).

Apparatus

Spectrophotometer
Magnetic stirrer
Thermostatic water bath
Whatman No. 1 filter paper
Test tubes (15 ml)

Reagents

Citric acid monohydrate
Disodium hydrogen phosphate dihydrate
TRIS (tris (hydroxyl methyl) amino methane)
Sodium hydroxide
Substrate (azurine-crosslinked wheat arabinoxylan: Xylazyme tablets from Megazyme, Ireland)

Note: a new batch of the substrate should be compared with a previous batch by analyzing the same enzyme preparation using both substrates. If a difference in enzymatic activity is noted, an appropriate correction factor should be calculated and applied to the results obtained with the new batch of the substrate.

Reagent solutions

Reaction buffer (McIlvaine buffer, pH 5.00): Dissolve 10.19 g of citric acid monohydrate and 18.33 g disodium hydrogen phosphate dihydrate in 850 ml distilled water in a 1000 ml volumetric flask. Adjust the pH to 5.00 using either 0.1M citric acid monohydrate or 0.2M disodium hydrogen phosphate dihydrate. Add water to 1000 ml. The buffer can be stored for up to 6 months at 2-5^{o}.

Stop solution (2% w/v TRIS, pH 12.0): Dissolve 20 g of TRIS in 850 ml distilled water in a 1000 ml volumetric flask. Adjust the pH to 12.0 with 5M NaOH. The solution can be stored for up to six months at 2-5^{o}.

Test sample solutions

Accurately weigh a quantity of the enzyme preparation that would give an OD increase within the range of 0.3 – 1.2 in a 100 ml volumetric flask. Add 60 ml of the reaction buffer. Stir the solution using a magnetic stirrer for 10 minutes. Remove the magnet and add the reaction buffer to volume. Transfer the enzyme solution to a glass beaker and let it stand for 5 minutes or until the precipitate settles. Use clear solution for analysis.

Blank

Pre-heat 1.0 ml reaction buffer at 40.0^{o} for 5 minutes. Add one Xylazyme tablet. After exactly 10 minutes at 40.0^{o}, add 10.0 ml stop solution and filter the sample through Whatman No.1 filter.

Procedure

1) Prepare 3 test tubes for each test sample. Pipette 1 ml of the reaction buffer to each tube and add 50, 75, and 100 microliters of the test sample solution.
2) Pre-heat all test sample solutions at 40.0^{o} for 5 minutes.
3) Add one Xylazyme tablet to each tube. Do not stir.
4) After 10 minutes (±1 sec.), terminate the reaction by adding 10

ml stop solution.

5) Filter all solutions through Whatman No. 1 filter paper.
6) Measure OD of each test sample solution against the blank at 590 nm.

Calculations

Perform linear regression on OD_{590} as a function of test sample volumes (in milliliters) used in the analysis. Calculate the activity of the enzyme preparation in xylanase units (XU) per gram (g) using the following equation:

$$\frac{XU}{g} = S\frac{V}{W}$$

Where:

S = Slope obtained from linear regression of the OD_{590} as a function of sample volume in ml
V = Volume of the volumetric flask used to prepare the test sample solution in ml (multiplied by further dilutions, if applicable)
W = Weight of the enzyme preparation in grams