XYLANASE from *THERMOMYCES LANUGINOSUS* expressed in *FUSARIUM VENENATUM*

New specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). An ADI "not specified" was established.

SYNONYMS Endo-1,4-beta-xylanase, endo-xylanase, beta-D-xylanase, beta-xylanase, pentosanase

SOURCES Produced by submerged fermentation of a non-pathogenic and non-toxigenic strain of *Fusarium venenatum* carrying the gene from *Thermomyces lanuginosus* inserted by r-DNA techniques encoding a xylanase. The purification process involves primary separation, concentration, pre and germ filtration and then preservation and stabilization. The cell mass and other solids are separated from the broth by vacuum drum filtration or centrifugation. Ultrafiltration and/or evaporation are applied for concentration and further purification followed by germ filtration.

Active principles: Xylanase

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

Reactions catalysed: Hydrolyses xylosidic linkages in arabinoxylans into smaller oligosaccharides.

DESCRIPTION Light brown powder.
The liquid concentrate produced as above is mixed with granulation aids such as dextrin and/or sorbitol syrup and stabilised by the addition of sodium chloride. The product is standardized to the declared enzyme activity by the addition of wheat flour.

FUNCTIONAL USES Enzyme preparation.
Used in baking applications to increase the elasticity of the gluten network, whereby handling and stability of the dough is improved. It can be used in the pH range 4-6 and at temperatures up to 75°. The enzyme is denatured and inactivated in the baking process.

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

CHARACTERISTICS

IDENTIFICATION

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

IDENTIFICATION TEST

Xylanase activity: Principle (Determination of endoxylanase, pH 6.0):

Xylanase samples are incubated with a remazol-stained wheat arabinoxylan substrate. Unconverted substrate is precipitated with ethanol. The intensity of the blue colouring of the supernatant due to unprecipitated remazol-stained substrate degradation products is proportional to the endoxylanase activity. Endoxylanase activity in FXU (Farvet Xylan Units) is measured relative to an enzyme standard. Different types of xylanases some colour, but the colour profile may vary from enzyme to enzyme. This can result in major dilution effects.

Apparatus:
- Spectrophotometer
- Thermostatic water bath
- Centrifuge
- 10 ml plastic test tubes
- Stopwatch

Reagents and substrates:
Phosphate buffer stock solution, 1.0 M: Dissolve 1210 g sodium dihydrogen phosphate monohydrate and 218.9 g disodium hydrogen phosphate dihydrate in demineralised water. Add 40 ml 4 N NaOH and make up to 10 l with water.
Phosphate buffer, 0.1 M, pH 6.00 ± 0,05: Take 1000 ml phosphate buffer stock solution and make up to 10 l with demineralised water. Adjust the pH to 6.0 $\pm$ 0.05 using either 4 N NaOH (reagent 6.2.4) or 2 N HCl
HCl, 2 M
NaOH, 4 M
Azo-wheat arabinoxylan substrate, 0.5% w/v pH 6.00 ± 0,05: Weigh 0.500 g Azo-Wheat arabinoxylan into a 150 ml beaker. Add approx. 90 ml 0.1 M phosphate buffer, heated to approx. 50°C, while stirring. Continue stirring at 50° for a further 20 minutes. Cool the substrate solution and adjust to pH 6.00 $\pm$ 0.05 before transferring to a 100 ml graduated flask. Fill to the mark with phosphate buffer.
Stop reagent: Pipette 6.65 ml 2 N HCl into a 100 ml graduated flask. Fill up to the mark with 99.9% ethanol.

Samples and standards:
Reference enzyme stock solution for standard curve: Calculate and accurately weigh approximately 1g FXU standard into a suitable graduated flask, dissolve in 0.1 M phosphate buffer by stirring for approximately 15 min and prepare at least six FXU standard solutions to give a range of activities between 0.2 and 1.4 FXU/ml. A sample of known activity is included at the beginning and the end of each analysis series or at least every 20 samples.
Samples: Samples are diluted on the basis of their anticipated activity so that the activity of the final dilution is between. 0.4-1.4 FXU/ml. Results outside the working range may be used to assess the activity of the sample for the next run. Weigh dry or liquid samples directly into the flask. Granulated products may take considerable time to dissolve.

Procedure:

Pipette 0.100 ml standard or sample solution into separate 10 ml test tubes, add 0.900 ml of substrate and mix. Incubate the tubes in a 50° water bath for 30 min. Add 5 ml stop reagent and mix for 10-20 sec. Leave the tubes to stand at room temperature, 15-60 min, centrifuge at 4000 rpm for 15 min and measure the absorbance of the supernatant at 585 nm within 20 minutes.

Calculation:
Use the measurements for the enzyme standards to plot a standard curve. The data may be fitted to a third order polynomial. Determine the corresponding enzyme activity values from the standard curve for the samples. The activity of each sample is then calculated as follows:

$$\text{Sample activity (in FXU)/g)} = \frac{\text{C x F x D}}{\text{W}}$$

Where:
C = enzyme activity read from the standard curve (FXU/ml)
F = volume of sample
D = further dilution of sample (e.g. second or third dilution)
W = weight of sample