MIXED β-GLUCANASE AND XYLANASE FROM *DISPOROTRICHUM DIMORPHOSPORUM* (TENTATIVE)

New tentative specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

Information required:

- *method to determine the identity for β-glucanase, including data from a minimum of five batches, using the method described;*
- *a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories and data from a minimum of five batches, using the method described.*

SOURCES

The mixed β-glucanase and xylanase enzyme preparation is produced by submerged fermentation of a non-pathogenic and non-toxigenic strain of *Disporotrichum dimorphosporum*. The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass by vacuum drum filtration or centrifugation. The enzyme is concentrated by ultrafiltration and/or evaporation. 40-45% glycerol is added to the polished liquid enzyme concentrate, to standardise to desired activity. Sodium benzoate is added as a stabilising agent, and the liquid enzyme preparation is filtered again prior to packaging.

Active principles: β-glucanase and xylanase

Systematic names and numbers:

β-glucanase:	3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase
EC No.:	3.2.1.6
CAS No.:	62213-14-3
Xylanase:	1,4-beta-D-xylan xylanohydrolase
EC No.:	3.2.1.8
CAS No.:	9025-57-4

Reactions catalyzed: β-glucanase: endohydrolysis of (1→3) or (1→4) linkages in β-D-glucans
Xylanase: endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans

DESCRIPTION

Light brown to dark brown liquid.

FUNCTIONAL USES

Enzyme preparation.
The mixed β-glucanase and xylanase-enzyme preparation is used as a processing aid in brewing and in potable alcohol production to degrade beta-D-glucans and xylans to reduce viscosity and improve grist stability. The enzyme preparation is also used in grain processing to degrade cell wall components, in order to improve mechanical treatments.

GENERAL SPECIFICATIONS

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

CHARACTERISTICS

IDENTIFICATION

β-Glucanase activity — The sample shows β-glucanase activity.
Method information required.

Xylanase activity — The sample shows xylanase activity.
Method information required.

TESTS

β-Glucanase activity

Principle

Enzyme activity is measured by a method based on the reduction of viscosity of a beta-glucan substrate at pH 5.60 and 45°, in the presence of β-glucanase. This is measured using a calibrated Ubbelhode viscosimeter. Activity is expressed in Beta-Glucanase Fungique (BGF) units. One BGF unit is the amount of enzyme per milliliter (ml) of reaction mixture (15 ml substrate and 2 ml enzyme solution) that causes a change in viscosity of the substrate with a speed giving a slope of 0.147 per min under the conditions of the test, when the reaction time is plotted against the inverse of the falling time.

Apparatus

Ubbelhode No. 1C Viscosimeter, with a circulation flow constant of approximately 0.03, or equivalent
Analytical balance, accuracy to within 0.001 g
Waterbath

Description of Ubbelhode Viscosimeter:

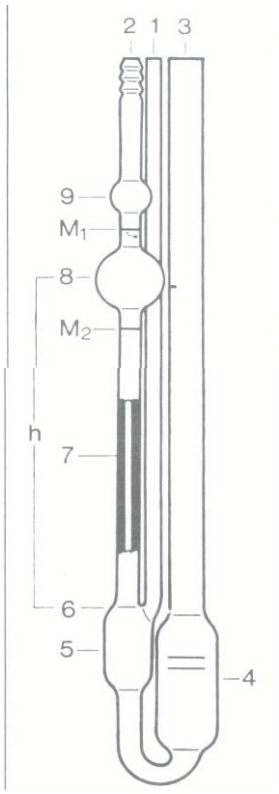

Figure legend:

1. Ventilating tube
2. Capillary tube
3. Filling tube
4. Reservoir
5. Reference level vessel
6. Dome-shaped top part
7. Capillary
8. Measuring sphere
9. Pre-run sphere

M1. Upper timing mark
M2. Lower timing mark

Reagents and solutions

Potassium dihydrogen phosphate (KH_2PO_4)
Phosphoric acid, 85%, P-BGBM beta-glucan from barleyv viscosity 20–30 cst. Megazyme
Sodium Hydroxide, 1 mol/l
Ultrahigh quality water, resistance >18.2 mega Ohm/cm, TOC < 500 µg/l.

Phosphoric acid 1.0 mol/l:
Slowly and while stirring continuously add 67 ml phosphoric acid 85% to approximately 500 ml water in a 1 l volumetric flask. Make up to volume and mix. This solution may be kept for 2 months at room temperature.

Phosphate Buffer, 1.0 mol/l, pH 5.00:
Dissolve 13.6 g potassium dihydrogen phosphate in approximately 80 ml water in a 100 ml volumetric flask. If necessary heat while dissolving and allow cooling to ambient temperature. Adjust the pH to 5.00 by adding phosphoric acid solution (1 mol/l) or sodium hydroxide solution (1 mol/l). Make up to volume with water and mix. This solution may be kept for 2 months.

Beta-glucan substrate solution:
Use a reserved batch of beta-glucan, with a known substrate batch factor. (This is the factor used to bridge new substrates to the existing substrate used for calibration).

Dissolve 1.0 g beta-glucan in approximately 30 ml water in a 100 ml Erlen Meyer vial. Stir for approximately 1 h. Next place the solution in the bath with boiling water for 5 min and allow cooling to ambient temperature. Add
10.0 ml phosphate buffer 1 mol/l, pH 5.00 and quantitatively transfer to a 100 ml volumetric flask with water. Make up to volume with water and mix.
Check the pH of the solution. It must be 5.60 +/- 0.05. Prepare a new solution when pH is out of range. Only use a freshly prepared solution.

Control: A β-glucanase preparation of known activity is used as assay control.

Samples: Allow the sample to attain room temperature. Weigh, accurately to within 0.0001 gram, and in duplicate an amount of sample corresponding to 10 000 BGF in a 100 ml volumetric flask. Dissolve in water by stirring on a magnetic stirrer. Make up to volume with water and

mix. Dilute 0.25 ml of these solutions with 4.75 ml water in a centrifuge tube and mix. The activity of the sample should fall between 3.4 and 6.8 BGF/ml.

Procedure

Allow the Ubbelhode viscosimeter to equilibrate at 45° for at least 20 min.

1. Place a reagent tube containing 15.0 ml of beta-glucan substrate in the 45° water bath and allow it to equilibrate for at least 20 min.
2. Start a stopwatch (Stopwatch 1) set to T= 0 min, and leave this running throughout the assay to record incubation time. Add 2.0 ml of sample solution to the equilibrated substrate at T = 0 min. Mix on a tube shaker.
3. Fill the reservoir of the viscosimeter through Filling Tube to a level between the two marks on the reservoir (refer to the Description of Ubbelhode Viscosimeter, if necessary).
4. Measure the viscosity of this solution at every three min for a 15 min period, by following step 5 - 9. This will result in 5 measurements in total.
5. At T = ~ 2.5 min close Ventilating Tube (with finger) and execute suction on Capillary Tube to fill the Capillary Tube up to the Pre-run sphere (refer to the Description of Ubbelhode Viscosimeter, if necessary).
6. Stop suction, remove finger from Ventilating Tube and start Stopwatch 2 when the liquid reaches the Upper Timing mark (M1).
7. Read the time on Stopwatch 1 (do not stop stopwatch 1!). This will be Rt 1 for the calculations.
8. Allow the liquid to run down to the Lower Timing mark, M2. Stop Stopwatch 2 when the Lower Timing mark, M2 is reached. This will be Vt 1 in the calculation.
9. Repeat this procedure every three min. Record Rt 2 to Rt 5 and Vt 2 to Vt 5.
10. Repeat Steps 1-9 using a mixture of 15.0 ml substrate and 2.0 ml water as the substrate blank. Record the viscosity as Vtb.
11. Repeat Steps 1-9 using a mixture of 17.0 ml water as the water blank. Record the viscosity as Vtw.
12. Repeat Steps 1-9 if the enzyme activity is outside the linear range of the assay, with appropriate dilutions.

Calculations:

Calculate the Reaction Time (T1-T5), in sec, accurately to 0.01 sec using the equations:

$$T1 = \{Rt1 + (½\ Vt1)\}$$
$$T2 = \{Rt2 + (½\ Vt2)\}$$
$$T3 = \{Rt3 + (½\ Vt3)\}$$
$$T4 = \{Rt4 + (½\ Vt4)\}$$
$$T5 = \{Rt5 + (½\ Vt5)\}$$

1. Calculate the average time for drop in viscosity, for the five measuring points of the substrate blank in sec, using the equation:
   Vtbl = (Sum of Vt 1 to 5) / 5
2. Calculate the average time for drop in viscosity, for the five measuring points of the water blank in sec, using the equation:
   Vtw = (Sum of Vt 1 to 5) / 5
3. For each of the five measuring points of sample, calculate X using the equation:
   X = Vtbl / (Vt - Vtw).
4. For each of the measuring points, plot the T values (obtained in

Step 1, x axis) against the corresponding X (obtained in Step 4, y-axis). Calculate the slope according to the linear equation
$y = Px + b$
From this line calculate the slope, P

5. Calculate the enzyme concentration in the incubation mixture as follows:

$C = (W / 100) \times (0.25 / 5.00)$

Where
C is Sample Concentration in g/ml
W is Sample weight, g
0.25/5.00 is Dilution Factor
100 is Volume of Dilution, ml

6. Calculate the enzyme activity of the sample as follows:

$\beta\text{-glucanase Activity, BGF/g} = (P \times 60 / 0.147) \times (17/2) \times (1/C) \times Sf$

Where
P is Slope from Step 5
60 is Conversion of sec to min
0.147 is Conversion Factor from definition of Enzyme Activity
17/2 is Correction Factor for incubation mixture
C is Sample Concentration from Step 6

Xylanase activity

The sample shows xylanase activity.
Method information required.