INVERTASE from *SACCHAROMYCES CEREVISIAE*

New specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001); previously prepared at the 15th JECFA (1971) as part of the specifications for "Carbohydrase from Saccharomyces species", published in FNP 52. The use of this enzyme was considered to be acceptable by the 57$_t^h$ JECFA (2001) if limited by Good Manufacturing Practice.

SYNONYMS INS No. 1103

SOURCES Produced by the controlled submerged aerobic fermentation of a non-pathogenic and non-toxigenic strain of *Saccharomyces cerevisiae* and extracted from the yeast cells after washing and autolysis.

Active principles β-Fructofuranosidase (synonym: invertase, carbohydrase, saccharase)

Systematic names and numbers β-Fructofuranosidase (EC 3.2.1.26; C.A.S. No. 9001-57-4)

Reactions catalysed Hydrolyses sucrose to yield glucose and fructose

DESCRIPTION Typically white to tan amorphous powders or liquids that may be dispersed in food grade diluents and may contain stabilisers; soluble in water and practically insoluble in ethanol and ether.

FUNCTIONAL USES Enzyme preparation
Used in confectionery and pastry applications

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

CHARACTERISTICS

IDENTIFICATION The sample shows invertase activity
See description under TESTS

TESTS

Invertase activity

Principle

Invertase hydrolyses the non-reducing β-d-fructofuranoside residues of sucrose to yield invert sugar. The invert sugar released is then reacted with 3.5 dinitrosalicylic acid (DNS). The colour change produced is proportional to the amount of invert sugar released, which in turn is proportional to the invertase activity present in the sample.
The absorbance is measured at 540 nm and converted into micromoles of reducing sugar produced using a calibration curve. One invertase unit is the amount of enzyme which will produce 1 micromole of reducing sugar (expressed as invert sugar) per minute under the conditions specified in this procedure.

Apparatus

Spectrophotometer set at 540 nm
Water bath set at 30°±1.0°

Stopwatch
Boiling water bath
Ice water bath
Mixer

Reagents and solutions

0.05 M Sodium acetate buffer, pH 4.7: Adjust the pH of 200 ml of 0.05 M sodium acetate (4.1 g of sodium acetate anhydrous in 1000 ml of water) to pH 4.7 ±0.05 with 0.05M acetic acid (2.85 ml of glacial acid in 1000 ml of water).
0.3M sucrose (5.13 g sucrose in 50.0 ml of water)
20 mM Tris HCl buffer, pH 7.0: Dissolve 2.42 g of tris (hydroxymethyl) aminomethane in about 800 ml of water. Adjust pH to 7.0 using 5% hydrochloric acid (5 ml of conc. hydrochloric acid in 100.0 ml of water).
DNS solution: Weigh 300 g of potassium sodium tartrate tetrahydrate into a one litre conical flask. Add 16 g of sodium hydroxide and 500 ml of water and dissolve by heating gently. When the solution is clear, add slowly 10 g of 3,5-dinitrosalicylic acid (DNS). Keep covered to protect from light until the DNS is totally dissolved. Cool to room temperature and make up to 1 litre with water. Store in a tightly stoppered dark container. Protect from light and carbon dioxide.
Invert sugar standard (0.01M): Dry glucose to constant weight at 105° and dry fructose to constant weight at 70° under vacuum. Dissolve 0.9 g of glucose and 0.9 g of fructose in 1000 ml of 0.1% benzoic acid (1 g of benzoic acid in 1000 ml of water).

Calibration curve

Prepare a series of test tubes, in duplicate, according to the table below. The calibration curve must include at least 4 suitable standards.

Tube no.	1	2	3	4	5	6	blank
Invert sugar standard (ml)	0.1	0.3	0.5	0.8	1.0	1.2	0.0
Water (ml)	2.4	2.2	2.0	1.7	1.5	1.3	2.5
Acetate buffer (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Content of invert sugar (micromoles)	1.0	3.0	5.0	8.0	10	12	0.0

Reaction and measurement

Mix and incubate for exactly 10 min at 30±0.1°. Add 2.0 ml of DNS solution to each tube, cover tubes and place all tubes in a boiling water bath for exactly 10 min. Cool rapidly in an ice water bath and add 15 ml of water to each tube. Mix thoroughly. Measure the absorbance at 540 nm of each sample using the blank to zero the spectrophotometer. Plot the absorbance against content of invert sugar.

Sample preparation

Accurately weigh about 1 g of the sample and dissolve in 10 ml of 20 mM Tris HCl buffer. For powder samples it may be necessary to use a magnetic stirrer for up to 10 min. Dilute the sample with 20 mM Tris HCl buffer to obtain a solution for which the measured absorbance will fall within the linear range of 0.14 and 0.30.

Procedure

Into each of a series of 30 ml test tubes, pipette, in quadruplicate, 1.4 ml of water, 0.5 ml of acetate buffer and 0.1 ml of diluted enzyme. Equilibrate the tubes in a 30° water bath. Add 1 ml of 0.3 M sucrose solution to 3 of the 4 tubes. Use the fourth tube as an enzyme blank, adding 2 ml of DNS solution before adding 1.0 ml of 0.3M sucrose solution. Prepare a reagent blank using 0.1ml of water in place of diluted enzyme. Continue as described under 'Reaction and measurement'. Read the respective contents of invert sugar from the calibration curve.

Calculation

Activity for powders (units/minute/g) =

$$\frac{(C_S - C_B) \times 10 \times \text{dilution}}{W \times 10}$$

Where

C_S = Content of invert sugar in sample solution (micromoles)

C_B = Content of invert sugar in enzyme blank solution (micromoles)

W = Weight of sample (g)

Activity for liquids (units/minute/ml) =

$$\frac{(C_S - C_B) \times 10 \times \text{dilution} \times \text{S.G.}}{W \times 10}$$

Where

C_S = Content of invert sugar in sample solution (micromoles)

C_B = Content of invert sugar in enzyme blank solution (micromoles)

W = Weight of sample (g)

S.G. = Specific gravity of sample (g/ml)