LIPASE FROM *FUSARIUM HETEROSPORUM* EXPRESSED IN *OGATAEA POLYMORPHA*

New specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

SYNONYMS Triglyceride lipase; tributyrase; butyrinase; glycerol ester hydrolase; tributyrinase; triacylglycerol ester hydrolase

SOURCES Produced by submerged straight-batch or fed-batch fermentation of a genetically modified non-pathogenic, non-toxigenic strain of *Ogataea polymorpha* which contains a synthetic gene coding for the lipase from *Fusarium heterosporum.* The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass along with the solid waste slurry carrying the residual microorganism from the enzyme by centrifugation and/or filtration. The liquid enzyme filtrate is concentrated by ultrafiltration followed by polish filtration. Food-grade preservatives are added to the liquid enzyme concentrate before spray drying or agglomeration and the product is formulated to the desired activity with food-grade ingredients.

Active principles Triacylglycerol lipase

Systematic names and numbers Triacylglycerol acylhydrolase; EC 3.1.1.3; CAS No. 9001-62-1

Reactions catalyzed Hydrolysis of ester bonds, primarily the 1 and 3 position of triglycerides (yielding di- or monoglycerides plus free fatty acids)
Hydrolysis of SN-1 ester bonds of diacyl-phospholipids and diacyl-galactolipids (yielding monoacyl-phospholipids or monoacyl-galactolipids and fatty acids, respectively)

Secondary enzyme activities No significant levels of secondary enzyme activities

DESCRIPTION Off-white to light yellow powder

FUNCTIONAL USES Enzyme preparation
Used as a processing aid in the manufacture of bakery products, pasta and noodles, in egg yolk and in oil degumming

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

CHARACTERISTICS

IDENTIFICATION

Lipase activity The sample shows lipase activity.
See description under TESTS.

TESTS

Lipase activity

Principle

Lipase activity is determined by measuring the rate of release of free fatty acid that results from the hydrolysis of lecithin, used as a substrate. Continuous titration of the liberated free fatty acid with 0.05 M sodium hydroxide enables the determination of the lipase activity from the consumption of base as a function of time. Lipase of known activity is used as control sample.

The lipase activity is expressed in Titratable Phospholipase Units (TIPU). One TIPU is defined as the amount of enzyme which liberates 1 µmol free fatty acid per minute at the specified conditions.

Apparatus

Water bath with external circulation at 37.0°
Thermostable holder (stand) with the corresponding glass beakers
Homogeniser (Ultra Turrax or equivalent)
pH-stat titrator (Radiometer Phm 290 or equivalent)

Reagents and solutions

Stock solution $CaCl_2$ 0.6 M: Dissolve 8.8 g $CaCl_2$. 2 H_2O in demineralised water and make up to 100 ml.

Substrate

4% lecithin, 4% Triton X-100, and 6 mM $CaCl_2$:
Disperse 12 g lecithin (Sigma product P3644 - Phosphatidylcholine content ≥ 30%) and 12 g Triton X-100 in approx. 200 ml demineralised water with a magnetic stirrer. Add 3.0 ml of 0.6 M $CaCl_2$. Adjust the volume to 300 ml with demineralised water and homogenise the emulsion with the homogeniser (20,000 rpm, 20 sec). Prepare the substrate fresh every day.

Sample preparation

Prepare an enzyme solution to give a slope on the titration curve between 0.06 and 0.18 ml/min, with an addition of 300 µL enzyme solution. Weigh an amount of enzyme equal to (1800/expected activity enzyme)g in order to prepare 100 ml of enzyme solution. Dissolve the enzyme in 50 ml of demineralised water in a beaker and stir the solution for approx. 15 min. Transfer to a 100 mL volumetric flask and make up to the final volume.

Control sample

Prepare a solution of a control enzyme sample of known activity in demineralised water.

Procedure (carry out in duplicate):

1. Add 25.0 ml substrate to the thermostable glass beaker and thermostat to 37.0°. Thermostating takes ca. 10 min.

2. Adjust the pH of the substrate to 7.0 with 0.05 M NaOH. Stir the solution continuously. Start the pH-stat titrator and add 300 µl enzyme solution.

3. After 8 min stop the titration and calculate the slope (α) of the titration curve between 5 and 7 min.

Measure a control sample of known activity prepared in the same way as the sample.

The detection limit is 3 TIPU/ml enzyme solution.

Calculations

Calculate the lipase activity, expressed in TIPU/g enzyme:

$$\text{TIPU/g} = \frac{\alpha \cdot N \cdot 10^3 \cdot V_1}{m \cdot V_2}$$

where

α is the slope of the titration curve between 5 and 7 min of reaction time (ml/min)
N is the normality of the NaOH used (mol/l)
V_1 is the volume in which the enzyme is dissolved (ml)
m is the amount of enzyme added to V1 (g)
V2 is the volume of enzyme solution added to the substrate (ml)