Tannase (TAH)

(Industrial Grade)

from *Aspergillus oryzae*

Tannin acylhydrolase, EC 3.1.1.20

Digallate + H_2O ⟶ 2 Gallate

SPECIFICATION

Appearance	white lyophilizate
Activity	≧500 U/g
Stabilizer	glucose
Storage	desiccated and below 10°C
Use	food processing

PROPERTIES

Molecular weight	*ca*. 310 kDa (gel filtration)	
Structure	*ca*. 59 kDa (SDS-PAGE)	
Isoelectric point	4.1	
Michaelis constants	4.2×10^{-4} M (tannic acid)	
	9.4×10^{-3} M (methyl gallate)	
pH Optimum	5.0–5.5	(Fig. 1)
pH Stability	3.5–5.5	(Fig. 2)
Optimum temperature	*ca*. 40°C	(Fig. 3)
Thermal stability	below 40°C	(Fig. 4)
Stability (powder form)	stable at 30°C for at least six months	
		(Fig. 5)
Specificity	tannic acid (100), methyl gallate (20)	
	ethyl gallate (9), *n*-propyl gallate (10)	
	isoamyl gallate (9)	

PRODUCT

Name	Tannase-KTFH	Tannase-KT05	Tannase-KT50
Code	60554	60551	—
Activity	500 U/g or higher	5,000 U/g or higher	50,000 U/g or higher
Package	1.0 kg/bag	1.0 kg/bag	Consult us

ASSAY PROCEDURE

Principle

The degree of hydrolysis of ester bond in tannic acid is estimated by spectrophotometrically measuring the disappearance of absorbance at 310 nm according to the slightly modified method of Iibuchi *et al*.

Definition of unit

One unit (U) is defined as the amount of enzyme which hydrolyzes 1 μmol of the ester bond in tannic acid per min at 30°C and pH 5.5 under the conditions described below.

Reagents

A. Citrate buffer, 50 mM; pH 5.5: dissolve 10.5 g of citric acid in 800 ml of distilled water, adjust to pH 5.5 with 1 N NaOH and dilute with distilled water to 1000 ml.
B. Substrate (tannic acid) solution, 0.35%: 175 mg of tannic acid/50 ml of citrate buffer (Reagent A). (Prepare freshly before measurement. The solution may be slightly turbid, but use it without filtration.)
C. Ethanol solution, 90%: mix 900 ml of ethanol (99.5%) and 95 ml of distilled water.

Sample: dissolve the lyophilized enzyme to a volume activity of 2.6~2.9 U/ml with ice-cold citrate buffer (Reagent A) immediately before measurement.

Procedure

1. Pipette 1.0 ml of substrate solution (Reagent B) into a test tube.
2. Equilibrate at 30°C for about 5 min.
3. Add 0.25 ml of sample and incubate for 15 min at 30°C [test].
   The blank solution is prepared by adding citrate buffer (Reagent A) instead of sample [blank].
4. Add 5.0 ml of ethanol solution (Reagent C) and mix thoroughly to stop the reaction.
5. Pipette 0.25 ml of the test and blank mixture into respective test tubes.
6. Add 5.0 ml of ethanol solution (Reagent C) and mix thoroughly.
7. Read the absorbance at 310 nm in a cuvette (light path: 1 cm) [test: A_S, blank: A_0].

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_0 - A_S) \times 20.3 \times 1.0\ (\text{ml}) \times 1.04 \times df}{0.71 \times 0.25\ (\text{ml}) \times 15\ (\text{min})} = \Delta A \times 7.93 \times df$$

Weight activity (U/g) = (U/ml) × 1/C

20.3 : Micromoles of tannic acid in 1.0 ml of substrate solution (Reagent B)
0.71 : Change in absorbance after complete hydrolysis of 20.3 μmol of tannic acid under the assay conditions
1.04 : A factor for correction between the method of Iibuchi *et al*. and the present method
df : Dilution factor
C : Content of tannase preparation in sample (g/ml)

APPLICATIONS

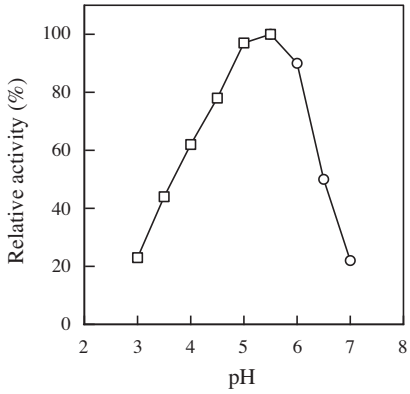
This enzyme is useful for the treatment of tea extract. It breaks down any turbidity complex in the tea extract to produce clearer and more delicious drinks with higher concentrations of ingredients and high antioxidative activity.

REFERENCES

Iibuchi, S. *et al*., *Agric. Biol. Chem*., **31**, 513–518 (1967).
Mizusawa, K., *Monthly Food Chemical*, **1994–2**, 36–41 (1994).
Lanc, R. W. *et al*., *Food and Chemical Toxicology*, **35**, 207–212 (1997).
Nakamori, K. and Kawazoe, T., *Foods and Development*, **32**(**12**), 14–16 (1997).
Arai, A. and Nakamori, K., *Foods and Development*, **38(2)**, 70–72 (2003).

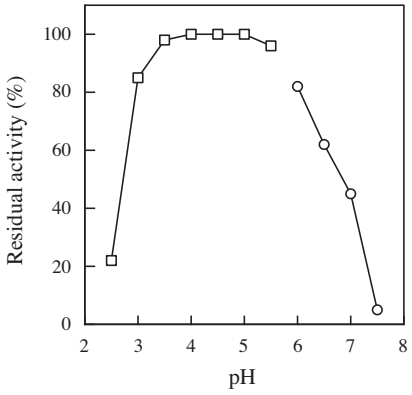
EXPERIMENTAL DATA

Fig. 1 pH Optimum



□: 50 mM citrate buffer
○: 50 mM phosphate buffer

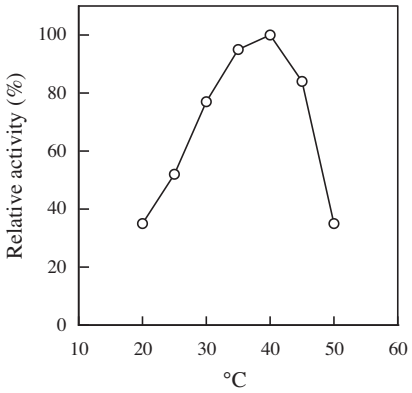
Fig. 2 pH Stability



Treatment: 30°C, 1 h

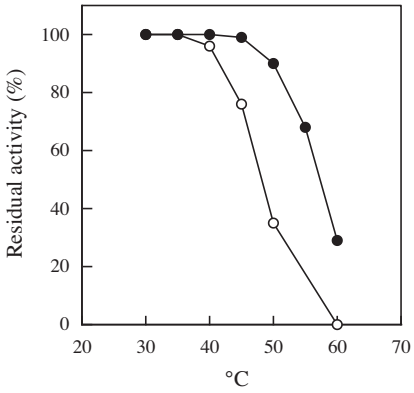
□: 50 mM citrate buffer
○: 50 mM phosphate buffer

Fig. 3 Optimum temperature



Buffer: 50 mM citrate buffer, pH 5.5

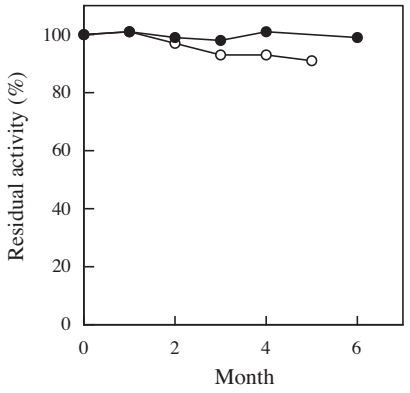
Fig. 4 Thermal stability



Treatment: 10 min

●: 50 mM citrate buffer, pH 4.5
○: 50 mM citrate buffer, pH 5.5

Fig. 5 Stability (powder form)



〔Kept under dry conditions
Treatment: 30°C (●), and 40°C (○)〕