

Enzymatic Assay of BROMELAIN
(EC 3.4.22.32)

PRINCIPLE:

Gelatin + H₂O Bromelain > Amino Acids + Oligopeptides

CONDITIONS: T = 45°C, pH = 4.5

METHOD: Titrimetric

REAGENTS:

- A. 100 mM Sodium Acetate Buffer with 2.6 M Sodium Chloride, pH 4.5 at 45°C
(Prepare 100 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283 and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 4.5 at 45°C with 1 M NaOH.)
- B. 5.0% (w/v) Gelatin Solution, pH 4.5 at 45°C
(Prepare 100 ml in deionized water using Gelatin, Type A from Porcine Skin, approximately 300 bloom, Sigma Prod. No. G-2500. Dissolve gelatin in approximately 40 ml boiling water. Stir until the gelatin is dissolved. Add approximately 60 ml water and place in an 80°C water bath for about 20 minutes. Cool to 45°C.)
- C. 3% (v/v) Hydrogen Peroxide Solution (H₂O₂)
(Prepare 20 ml in deionized water using Hydrogen Peroxide, 30% Solution, Sigma Prod. No. H-1009.)
- D. 0.05 N Sodium Hydroxide Standardized Solution (NaOH)
(Prepare 100 ml in deionized water using Sodium Hydroxide, Volumetric Standard, 0.5 N, Aldrich Stock No. 31,950-3.)
- E. 37% Formaldehyde Solution
(Use Formaldehyde, 37% Solution, Sigma Prod. No. F-1635.)

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REAGENTS: (continued)

F. Bromelain Enzyme Solution
(Immediately before use, prepare a solution containing
2.0-4.0 units/ml Bromelain in Reagent A.)¹

PROCEDURE:

Using a suitable pH meter in conjunction with a magnetic stirrer, pipette (in milliliters) the following reagents into a suitably thermostatted titration vessel:

	<u>Test</u>	<u>Blank</u>
Reagent B (Gelatin Solution)	25.0	25.0
Equilibrate to 45°C. Then add:		
Reagent F (Enzyme Solution)	1.0	-----
Incubate at 45°C for 20 minutes. Then add:		
Reagent C (H ₂ O ₂)	0.10	0.10
Reagent F (Enzyme Solution)	-----	1.0
Adjust both the Test and Blank to pH 6.9 with Reagent D. Then add:		
Reagent E (Formaldehyde Solution)	10.0	10.0

Titrate both the Test and Blank to pH 7.8 with Reagent D.
Record the volume of Reagent D required to titrate both
the Test and Blank from pH 6.9 to pH 7.8.

CALCULATION:

$$\text{Units/g enzyme} = \frac{(\text{Volume for Test} - \text{Volume for Blank})(N)(14)(1000)}{\text{mg enzyme/RM}}$$

N = Normality of Reagent D
14 = mg Nitrogen per millimole Nitrogen
1000 = milligrams/gram

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UNIT DEFINITION:

One unit will hydrolyze 1.0 mg of amino nitrogen from gelatin in 20 minutes at pH 4.5 at 45°C.

FINAL ASSAY CONCENTRATIONS:

In a 26.0 ml reaction mix, the final concentrationS are 3.8 mM sodium acetate, 5.8 mM sodium chloride, 4.8% gelatin and 2.0 - 4.0 units bromelain.

NOTES:

1. Allow enzyme solution to stand on ice (0-4°C) for 1 hour prior to assay.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.