



Enzymatic Assay of PECTINESTERASE (EC 3.1.1.11)

PRINCIPLE:



CONDITIONS: T = 30°C, pH = 7.5

METHOD: Titrimetric

A. 100 mM Sodium Chloride Solution (NaCl)

(Prepare 300 ml in deionized water using Sodium Chloride.)

B. 1.0% (w/v) Pectin Solution (Pectin)

(Prepare 250 ml in Reagent A using Pectin. Dissolve the Pectin into approximately 150 ml of Reagent A which has been heated to boiling and stir with a magnetic stirrer. Cool to room temperature upon solubilizing and dilute to 250 ml with Reagent A.)

C. 20 mM Sodium Hydroxide Solution - Standardized (NaOH)

(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous.

Standardize according to the ACS Reagent Procedure.2)

D. 1.7 M Sodium Chloride Solution (Enzyme Diluent)

(Prepare 50 ml in deionized water using Sodium Chloride.)

E. Pectinesterase Enzyme Solution

(Immediately before use, prepare a solution containing 10 - 15 units/ml of Pectinesterase in cold Reagent D.)

PROCEDURE:

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

	Test	Blank
Reagent B (Pectin)	20.00	20.00
Equilibrate to 30. C. Adjust to pH 7.5 with Reagent C (NaOH). Then add:		



Reagent E (Enzyme Solution)	0.20	
Reagent D (Enzyme Diluent)		0.20

Run the reaction for 1-5 minutes. Record the time when the pH reaches 7.5.

Maintain the pH of the reaction mix at pH 7.5 by the addition of small volumes (0.05 ml) of Reagent C (NaOH). Record the volume of Reagent C (NaOH) used to maintain the pH at 7.5 and the time required.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{\frac{\text{ml NaOH for Test} - \text{ml NaOH for Blank}}{(M) \quad T} \quad (1000) \text{ (df)}}{(T)(0.20)}$$

M = Molarity of NaOH solution

1000 = Conversion from milliequivalent to microequivalents

df = Dilution factor

T = Time required to maintain the pH at 7.5

0.2 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will release 1.0 microequivalent of acid from pectin per minute at pH 7.5 at 30EC.

INITIAL ASSAY CONCENTRATIONS:

In a 21.00 ml reaction mix, the initial concentrations are 176 mM sodium chloride, 0.95% (w/v) pectin, and 2 - 3 units pectinesterase.



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REFERENCES:

(1993) Reagent Chemical ACS Specification, 8th ed., 95.

Kertesz, Z.I. (1955) Methods in Enzymology, Vol. I, 158-162.

NOTES:

1. The solution must be agitated during preparation to prevent pectin from adhering to the bottom of container and being burned.

2. Standardization of NaOH solution is described in (1993) Reagent Chemical ACS Specification, 8th ed., 95.



源叶