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GELATIN DIGESTION UNIT ANALYTICAL METHOD (GDU)

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A. Purpose: This procedure is used to determine the proteolytic activity of Bromelain.

B. Equipment:

1. pH Meter
2. Constant Temperature Water Bath at 45.0° ± 0.1°C
3. Analytical Balance
4. Volumetric flasks
5. Volumetric pipettes
6. Timer
7. Digital Burette (Accuracy of 0.1 ml)
8. Fume hood
9. Automatic Pipetters

C. Safety Precautions: This test must be performed in the fume hood.

1. Utilize standard laboratory safety practices.
2. Formaldehyde: Prepare and keep in a fume hood at all times. Known carcinogen and teratogen.
3. Peroxide: Strong oxidizer

D. Reagents and Reagent preparations:

1. Distilled water: approximately 500 ml: adjust to a pH of 4.5 with 0.1 N HCI.
2. Gelatin substrate:
   a. Dissolve 25 grams of gelatin (Mikrobiologie. 1.04070) in 375 ml hot water, bring to a boil. Cool to 45°C.
   b. Adjust the pH to 4.5 with 0.1 N HCI and dilute to 500 ml.
   c. Keep the gelatin substrate at 45°C.
3. Buffer Solution:
   a. Add 15 gm NaCl slowly to 40-50 ml water in a 150ml beaker and stir to dissolve.
   b. Add 0.570 ml acetic acid.
   c. Adjust the pH to 4.5 with 0.2N NaOH (volume will be greater than 100ml.)
4. 3 % Hydrogen peroxide:
   a. Pipette 2.5 ml of 30% Hydrogen peroxide (stock solution) into a 25 ml volumetric flask and dilute to volume with pH 4.5 distilled water
5. 37 % Formaldehyde pH 9.0:
   a. Adjust a sufficient volume (100 ml) of formaldehyde to pH 9.0 with 0.1 N NaOH (approximately 20 ml of formaldehyde per sample prior to pH adjustment.)

6. 0.100 N NaOH: (Purchased standardized)
   a. Stock solution: standardized at 0.100 N

E. Procedure:

1. Enzyme Preparation:
   a. Calculating Enzyme Preparation

      Weight = 100/Target activity (approximately 0.05 g or 50 mg)

   A. Weigh the enzyme precisely into 50 ml volumetric flask
   B. Add 8.3 ml of buffer solution.
   b. Let stand for 30 minutes at room temperature.
   c. Dilute to 50 ml using pH 4.5 distilled water, add a small stir bar and stir for an additional 10 to 15 minutes
2. Enzyme Procedure:
   a. Pipette 25 ml of Gelatin substrate into each of two 100 ml beakers containing stir bars and place them in a water bath at 45°C for 5 minutes, one for the Test Solution and the other for the Blank Solution.
   b. Test Solution:
      1) Add 1.0 ml of bromelain solution into the beaker designated for the test solution, start timing and swirl.
      2) After exactly 20 minutes of incubation at 45°C, add 0.1 ml of 3 % hydrogen peroxide and swirl.
      3) Incubate for an additional 5 minutes.
      4) Remove the beaker from the water bath and with constant stirring insert the pH probe.
      5) Record the pH after 10 seconds (Initial pH)
      6) Adjust to pH 6.0 with 0.1 N NaOH. (Approximately 2-4 ml)
         *Note: When adjusting the pH to 6.0 be cautious at pH 5.8; the pH increases rapidly and minute additions of NaOH at this point will significantly increase the pH.
      7) Continuing constant stirring, add 10 ml of 37 % formaldehyde pH 9.0.
      8) Record the pH after 10 seconds and 1 minute.
      9) Titrate to pH 9.0 with 0.1 N NaOH.
      10) Record the titration volume. This is the test titer, T.
   c. Blank Solution: The Blank Solution should be run concurrently with the Test solution. This is accomplished by starting the Blank Solution determination 12 minutes after the Test Solution is started. That gives you time to complete the assay on the Test Solution before having to proceed with the Blank Solution.
      1) Add 0.1 ml of 3 % hydrogen peroxide to the beaker designated for the blank solution and swirl.
      2) After exactly 20 minutes of incubation at 45°C add 1.0 ml of bromelain solution and swirl.
      3) Incubate for an additional 5 minutes.

4) Remove the beaker from the water bath and with constant stirring insert the pH probe.
5) Record the pH after 10 seconds (Initial pH)
6) Adjust to pH 6.0 with 0.1 N NaOH. (Approximately 2-4 ml)*See Note above.
7) Continuing constant stirring, add 10 ml of 37 % formaldehyde pH 9.0.
8) Record the pH after 10 seconds and 1 minute.
9) Titrate to pH 9.0 with 0.1 N NaOH.
10) Record the titration volume. This is the blank titer, B.

F. Calculation:

1. Definition: One Gelatin Digestion Unit is that amount of enzyme which will liberate, after 20 minutes digestion at 45°C, 1 mg of amino nitrogen from a standard gelatin solution at pH 4.5

$$GDU/g = \frac{(T-B) \times 14 \times N \times 50}{Wt\ (g)}$$

Where:

T = Test titer (ml 0.1 N NaOH)
B = Blank titer (ml 0.1 N NaOH)
N = Normality of standardized NaOH (i.e. 0.100)
Wt (g) = Initial weight of enzyme

G. Testing Parameters:

1. Enzyme preparations are diluted to a concentration of approximately 0.001 g/ml (1 mg/ml) or for Bromelain concentrate approximately 1-2 GDU/ml.
A Reference Material is assayed with each run to ensure method accuracy.

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