Change to read:

Bromelain— [9001-00-7]
A glycoprotein that is highly active thiol proteinase. It is found in the leaves and stems of the pineapple plant. Yellowish-white to tan powder.

ACTIVITY DETERMINATION

pH 4.5 water: Adjust with 0.1 N hydrochloric acid to a pH of 4.5.

Gelatin substrate: Dissolve 25 g of gelatin in 375 mL of hot water. Bring to a boil. Cool to 45°. Adjust the pH to 4.5 with 0.1 N hydrochloric acid. Dilute with *pH 4.5 water* to 500 mL. Keep it at 45°. This substrate should be prepared fresh daily.

Buffer solution: Add 150 mg of sodium chloride to 700 mL of *pH 4.5 water*, stir to dissolve, then add 5.7 mL of acetic acid. Adjust with 50% sodium hydroxide to a pH of 4.5, if necessary. Dilute to 1 L.

3% Hydrogen peroxide solution: Transfer 2.5 mL of hydrogen peroxide to a 25-mL volumetric flask. Dilute with *pH 4.5 water* to volume.

pH 9.0 formaldehyde solution: Adjust a 100-mL formaldehyde solution to a pH of 9.0 with 0.1 N sodium hydroxide VS.

Bromelain preparation: Weigh 100 mg of bromelain with a theoretical activity of 2400 GDU/g. If the sample activity differs by more than 10% from 2400 GDU/g, determine the sample weight:

$$\text{mg of sample} = (2400 \times 100)/\text{theoretical activity}$$

Transfer the sample to a 50-mL volumetric flask. Add 8.3 mL of *Buffer solution*. Let stand for 30 min at room temperature. Dilute with *pH 4.5 water* to volume. Add a small stir bar and stir for 10–15 min. ▲▲USP41

Procedure: Transfer 25 mL of *Gelatin substrate* to each of two 100-mL beakers containing stir bars and place them in a water bath at 45° for 5 min. One beaker is for the *Test solution* and the other for the *Blank solution*.

Test solution

Add 1.0 mL of *Bromelain preparation* into the beaker, start timing, and swirl.

After exactly 20 min of incubation at 45°, add 0.1 mL of *3% Hydrogen peroxide solution*, and swirl.

Incubate for an additional 5 min.

Remove the beaker from the water bath and, with constant stirring, insert the pH electrode. Record the pH after 10 s (initial pH).

Adjust with 0.1 N sodium hydroxide VS to a pH of 6.0. [NOTE—When adjusting the pH to 6.0 be cautious at pH 5.8; the pH increases slowly but minute additions of sodium hydroxide at this point will significantly increase the pH.]

Add 10 mL of *pH 9.0 formaldehyde solution* with constant stirring.

Titrate to a pH of 9.0 with 0.1 N sodium hydroxide VS. Record the volume of titrant used. This is the test titer, *T*.

Blank solution

Run concurrently with the *Test solution* by starting the *Blank solution* determination 12 min after the *Test solution* is started.

Add 0.1 mL of *3% Hydrogen peroxide solution* to the *Blank solution* beaker, and swirl.

After exactly 20 min of incubation at 45°, add 1.0 mL of *Bromelain preparation*, and swirl.

Incubate for an additional 15 min.

Remove the beaker from the water bath and, with constant stirring, insert the pH electrode.

Record the pH after 10 s (initial pH).

Adjust with 0.1 N sodium hydroxide to a pH of 6.0. See the *Note* under *Test solution*.

Add 10 mL of *pH 9.0 formaldehyde solution* with constant stirring.

Titrate to a pH of 9.0 with 0.1 N sodium hydroxide VS. Record the volume of titrant used. This is the blank titer, *B*.

Calculation

1 Gelatin Digestion Unit (GDU) is the amount of enzyme that, after 20 min of digestion at 45°, will liberate 1 mg of amino nitrogen from a standard gelatin solution at a pH of 4.5.

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

T = mL of 0.1 N sodium hydroxide used with the *Test solution*
B = mL of 0.1 N sodium hydroxide used with the *Blank solution*
N = actual normality of 0.1 N sodium hydroxide VS from standardization
W = weight of bromelain taken (g)

Bromine, Br—**At. Wt. 79.904** [7726-95-6]—Use ACS reagent grade.

α-Bromo-2′-acetonaphthone *(Bromomethyl 2-naphthyl ketone),* $C_{12}H_9BrO$—**249.10**—Tannish–pink crystals.

MELTING RANGE ⟨741⟩: between 81° and 83°.

***p*-Bromoaniline,** C_6H_6BrN—**172.02** [106-40-1]—White to off-white crystals. Insoluble in water; soluble in alcohol and in ether.

ASSAY: Transfer about 650 mg, accurately weighed, to a suitable container, and dissolve in 50 mL of glacial acetic acid TS. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 17.20 mg of C_6H_6BrN. Not less than 98% is found.

MELTING RANGE ⟨741⟩: between 60° and 65°, within a 2° range.

Bromofluoromethane: Use a suitable grade.

***N*-Bromosuccinimide,** $C_4H_4BrNO_2$—**177.98** [128-08-5]—White to off-white crystals or powder. Freely soluble in water, in acetone, and in glacial acetic acid. [**CAUTION:** Highly irritating to eyes, skin, and mucous membranes.]

ASSAY: Transfer 200 mg, accurately weighed, to a conical flask, add 25 mL of 0.5 N alcoholic potassium hydroxide, cover with a watch glass, heat to boiling, and boil for 5 minutes. Cool, transfer the solution to a beaker, rinsing the flask with water until the total volume of solution plus rinsings is about 100 mL, and add 10 mL of glacial acetic acid. Insert suitable electrodes, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 17.80 mg of $C_4H_4BrNO_2$. Not less than 98% is found.

Brucine Sulfate, $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$—**1013.11** [60583-39-3]—Use ACS reagent grade.

Buffers—See *Buffer Solutions* under *Solutions*.

Butane-1,2-diol *(1,2-Butanediol),* $C_4H_{10}O_2$—**90.12** [584-03-2]—Use a suitable grade with a content of NLT 97%.

Butane-1,4-diol *(1,4-Butanediol, 1,4-Butylene Glycol),* $C_4H_{10}O_2$—**90.12** [110-63-4]—Use a suitable grade with a content of NLT 98%.

Butane-2,3-diol *(2,3-Butanediol, 2,3-Butylene Glycol),* $C_4H_{10}O_2$—**90.12** [513-85-9]—Use a suitable grade with a content of NLT 97%.