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Bromelain

Definition Bromelain is a proteolytic enzyme preparation derived from the fruits and rhizoma of pineapples. It may contain lactose or dextrin.

Enzyme Activity The enzyme activity of Bromelain is not less than 500,000 units per gram.

Description Bromelain occurs as white to light yellowish brown powders. It is odorless or has a slight characteristic odor.

Identification Proceed as directed under Identification (1) for Papain.

Purity (1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 µg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As₂O₃ (0.50 g, Method 3, Apparatus B).

(4) Cyanide Weigh 5.0 g of Bromelain, transfer into a distillation flask, add 2 g of tartaric acid and 50 ml of water, and add 1 drop of silicone resin if necessary. Connect the flask with distillation apparatus, which is joined to a receiver with a condenser containing 2 ml of 1 mol/l sodium hydroxide solution and 10 ml of water. Distill until 20 ml of distillate is obtained and add water to the distillate to make 50 ml. To 25 ml of this solution, add 0.5 ml of ferrous sulfate TS, 0.5 ml of ferric hydrochloride solution (0.18 100) and 1 ml of dilute sulfuric acid. No blue color develops.

Microbial Limits Proceed as directed under the Microbial Limit Tests. The total viable aerobic count is not more than 50,000/g and no *Escherichia Coli* (coliform) is observed.

Enzyme Activity Determination

(i) Sample solution

Dissolve 5.27 g of L-cystein hydrochloride, 2.23 g of disodium ethylenediamine-tetraacetate and 23.4 g of sodium chloride in water. Adjust the pH to 4.5 with 1 mol/l sodium hydroxide TS and add water to make 1,000 ml. Use this solution as the diluent.

Weigh accurately about 0.1 g of Bromelain, transfer into a mortar, add the diluent and mix. Add the diluent to make exactly 100 ml. Centrifuge this solution if necessary. Dilute the supernatant liquid with the diluent to prepare a solution containing 30 to 50 units per ml.

(ii) Procedure

Measure exactly 1 ml of the sample solution, transfer into a test tube, and warm

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for 5 minutes at 37 ± 0.5 . Add 5 ml of casein TS (pH 7.0), previously warmed to 37 ± 0.5 , shake immediately and react for 10 minutes at 37 ± 0.5 . Add 5 ml of trichloroacetic acid TS, and shake. Allow to stand for 40 minutes at 37 ± 0.5 , and filter through a filter paper for quantitative analysis (5C). Discard the first 3 ml of the filtrate and measure the absorbance (A) of the subsequent filtrate at 275 nm, using water as the reference.

Measure exactly 1 ml of the sample solution, add 5 ml of trichloroacetic acid TS, and shake well. Add 5 ml of casein TS (pH 7.0), shake well, allow to stand for 40 minutes at 37 ± 0.5 . Measure the absorbance (A_0) of this solution, proceeding in the same manner as for the measurement of absorbance A.

Separately, measure the absorbances (A_S and A_{S0}) of Tyrosine Standard Solution and 0.1 mol/l hydrochloric acid at 275 nm using water as the reference.

Calculate the enzyme activity by the formula below. One unit of the enzyme activity is the quantity of enzyme which produces amino acids equivalent to 1 μg of tyrosin per minute when the test is performed under the conditions of the procedure.

$$\text{The enzyme activity of Bromelain (units/g)} = \frac{(A - A_0) \times 50}{A_S - A_{S0}} \times \frac{11}{10} \times \frac{1,000}{W},$$

where W = weight (mg) of Brommelain in 1 ml of sample solution.