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Plant Protease(PU)

Definition	Plant Protease (PU) is an enzyme such as papain, ficin, and bromelain which are obtained fro m plants. Dilutant or stabilizer can be added for the purpose of activity adjustment and quality preservation.

[Compositional Specifications of Plant Protease(PU)]

Content	Plant Protease (PU) contains 90 ~ 130% of the indicated activity as Plant Protease (PU).
Description	Plant Protease (PU) is white ~ pale yellow ~ brown powder, granule, lump or transparent ~ bro wn liquid.
Identification	
Purity	(1) Arsenic : 0.25 g of Plant Protease (PU) is placed in a platinum, quartz, or porcelain crucibl e. 10 ml of magnesium nitrate in ethyl alcohol (1→50) is added to the crucible and then alcoho l is ignited. It is then reduced to ash by heating at 450-550°. If carbonaceous substance persis ts, it is wetted with minute amount of nitric acid, which is further heat treated at 450-550°. Afte r cooling, 3 ml of hydrochloric acid is added to the residue, which is then dissolved by heating i n a water bath. When this test solution proceed as directed under Arsenic, it should be approp riate and should not be more 4ppm. (2) Heavy Metals : 0.5g of Plant Protease (PU) is carbonized by heating mildly in a quartz or p orcelain crucible. After cooling, add 2 ml of nitric acid and 5 drops of sulfuric acid, it is heated until white smoke disappears, which is then reduced to ash by further heating at 450-550°. Aft er cooling, 2 ml of hydrochloric acid is added, which is then evaporated to dryness in a water b ath. 3 drops of hydrochloric acid and 10 ml of hot water are added to the resulting residue, whi ch is then heated for 2 minutes. After cooling, 1 drop of phenolphthalein indicator solution is a dded, then ammonia solution is added until the color of the solution becomes pale red. The re sulting solution is transferred into a Nestler cylinder by rinsing with water. 50 ml of test solution is prepared by adding 2 ml of diluted acetic acid (1→20) and water. When this solution procee d as directed under heavy metals, the content should not be more than 40ppm. Color standard solution is prepared by the following procedure. 2 ml of nitric acid, 5 drops of sulfuric acid, and 2 ml of hydrochloric acid are added and evaporated to dryness in a crucible that is made of th e same material used for test solution preparation. 3 drops of hydrochloric acid are added to th e residue, which is then transferred into another Nestler cylinder as described above. Finally, 2 ml of lead standard solution, 2 ml of diluted acetic acid (1→20), and water are added to mak e the total volume to 50 ml. (3) Lead : 0.8g of Plant Protease(PU) (if it is liquid, it is concentrated by evaporation in a wat er bath) is slowly carbonized by heating, which is reduced ash by further heat treatment at a te mperature below 500°. Carefully 20 ml of diluted nitric acid is added to the ash, which is then g ently boiled for 5 minutes. It is then filtered (if necessary), the residue is washed with water, w hich is then added to the filtrate. Water is added so that total volume of this solution becomes 50 ml. When this test solution proceed as directed under lead, the detected amount of lead sh ould not be more 10ppm. (4) Coliform Group : When Plant Protease (PU) proceed as directed under Microbe Test Met hods for [Coliform Group] in General Test Methods in Food Code, it should not contain more t han 30 per 1 g of this product. (5) Salmonella : When Plant Protease (PU) proceed as directed under Microbe Test Method

s for [Salmonella] in General Test Methods in Food Code, it should be negative (-).

Assay(activity)

Application and Principle : This test is to measure the protein-decomposing activity of papain, f icin, and bromelain. Activity test is based on hydrolysis of casein substrate for 60 minutes, at p H 6.0, 40°). Unhydrolyzed casein is precipitated with trichloroacetic acid and removed by filtrat ion. The amount of casein dissolved in the filtrate is determined by the absorption measureme nt.

- Preparation of Test Solution : Sample is ground in a mortar with phosphate cysteine EDTA b uffer solution. It is then transferred into a volumetric flask and filled with the same buffer soluti on. The concentration of 2 ml sample is adjusted so that the absorption (measured as describ ed in Test Procedure) to be measured will be within a range of 0.2 to 0.5.

Test Procedure : 5 ml each of casein substrate solution is added to a 25 × 150 mm test tube, 3 for enzyme test and 6 for papain standard curve). Tubes are isothermalized for 15 minutes i n a 40 ± 0.1° water bath. 2 ml of Test Solution and 2 ml of Standard Solution are added to eac h tube, which is mixed by shaking and again isothermalized for 60 minutes in a water bath. 3 ml of trichloroacetic acid solution is added to each solution. Separately, 5 ml of substrate soluti on and 3 ml of trichloro acetic acid solution are mixed in 9 test tubes for enzyme blank test. 2 ml of Test Solution and 2 ml of corresponding standard solution are added to each test tube. A ll the tubes are again isothermalized for 30 minutes in a water bath to coagulate the precipitate d protein completely. It is then filtered through a Whatman No.42 filter paper or its equivalent. First 3 ml of the filtrate is discarded. Absorption of the clear filtrate is measured at 280 nm with 1 cm path length using each blank test solution as a reference. A standard curve of absorption of the filtrate vs. concentration of standard solution (mg/ml) is prepared. The concentration of the filtrate from Test Solution is obtained by interpolation on the standard curve. Enzyme activ ity is calculated from the following equation.

$$PU/mg = A \times C \times 10/W$$

A : Activity of USP papain standard (PU/mg)
C : Concentration of enzyme Test Solution obtained from standard curve (mg/ml)
W : Weight of sample contained in 2 ml of Test Solution (mg)

Definition of Activity : 1 Papain unit(PU) is an amount of enzyme that frees 1 μg equivalent of t yrosine in 1 hour under the above test conditions.

[Solutions]
- Sodium Phosphate Solution (0.05 M) : 7.1 g of sodium phosphate, dibasic (anhydrous) is dis solved in 500 ml of water, which is diluted to 1,000 ml with water. 1 drop of toluene is added a s a preservative.
- Citric Acid (0.05 M) : 10.5 g of citric acid (1 hydrate) is dissolved in 500 ml of water, which is diluted to 1,000 ml with water. 1 drop of toluene is added as a preservative.
- Phosphate Cysteine EDTA Buffer Solution : 7.1 g of sodium phosphate is dissolved in appro ximately 800 ml of water, where 14.0 g of EDTA (2 hydrate) and 6.1 g of cysteine hydrochlorid e (1 hydrate) are added and dissolved. pH of the resulting solution is adjusted to 6.0 ± 0.1 with 1 N hydrochloric acid or 1 N sodium hydroxide solution. The total volume of the solution is ma ke to 1,000 ml with water.
- Trichloroacetic Acid : 30 g of trichloroacetic acid is dissolved in water to make total volume t o 100 ml.
- Substrate Solution : 1 g of casein (Hammarsten) as a dried material is dissolved in 50 ml of s odium phosphate solution, which is heated for 30 minutes in a boiling water bath while shaking occasionally. It is then cooled while continuously shaking and its pH is adjusted to 6.0 ± 0.1 wit

	h citric acid solution (note : if the solution is shaken continuously and rapidly, precipitates are n ot formed.). The resulting solution is diluted to 100 ml with water. ◦ Standard Solution, Undiluted : 100 mg of USP papain standard is dissolved in phosphate cy steine EDTA buffer solution to make total volume to 100 ml. ◦ Standard Solution : 2, 3, 4, 5, 6, and 7 ml each of undiluted Standard Solution is placed in 10 0 ml volumetric flask. Each of the flask is filled with phosphate cysteine EDTA buffer solution.
Preservation of Plan t Protease(PU)	Plant Protease(PU) is strongly hygroscopic, so should be stored in a hermetic container in a c old dark place.