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Proteolytic action of proteinases of *Nepenthes distillatoria* on Natural Proteins

Isolation, purification procedure, and cleavage specificity of acid Proteinase of *Nepenthes* juice were reported previously. Stability of proteinases at different temperatures was investigated and results clearly demonstrated a similar stability of acid proteinases of *Nepenthes* at lower temperatures (4^{o}C) as well as at higher temperatures (37^{o}C & 50^{o}C). In this study the proteolytic action of acid proteinases of *Nepenthes* juice on natural proteins at different pH levels was investigated.

Crude *Nepenthes* juice was collected from *N. distillatoria* collected from the forest. Native proteins bovine serum albumin (BSA) and chicken ovalbumin samples were prepared by dissolving 10 mg of each protein in 2 ml of buffers at pH 2.0, 3.5 & 5.) Protein extract of dhal was prepared by boiling 100g of dhal in 200ml of distilled water and homogenizing. The resulting extract was centrifuged and the supernatant was dialyzed against buffers at pH 2.0- 7.0 The protein solutions at various pHs were mixed with crude *Nepenthes* juice (1:1 v/v) and incubated at 37^{o}C. Aliquots were removed at appropriate time intervals and mixed with SDS sample buffer. They were boiled for 2 minutes and subjected to sodium dodecyl sulphate poly acrylamide gel ectrophoresis (SDS PAGE).

Intensity of bands of intact proteins (BSA, ovalbumin) at SDS PAGE electrophorogram was reduced and low molecular weight protein bands appeared with time. Further, intensity of protein bands produced on SDS PAGE of dhal extracts were reduced gradually and novel bands appeared on SDS PAGE with time Proteolytic action of *Nepenthes* acid proteinases on ovalbumin was higher in acidic pH values (pH 2.0 & 3.5). However, relatively higher rate of hydrolysis was observed for BSA at pH 5.0 compared

to pH 2.0 & 3.5. A remarkable proteolytic action of Nepenthes proteinases on dhal proteins at different pH values was observed at neutral pH.

Proteolytic action on dhal proteins at higher pHs 5.0, 6.- & 7.0 is remarkable because pepsins lose their activity at higher pH levels. Effect of pH on digestion depends on the substrate used. Further studies are in progress to clarify possible application in medicine.