

## Abstract

### Application of acid proteases from *Nepenthes* in hydrogen/deuterium exchange

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*Nepenthes* are mostly found in Borneo and Sumatra. They are one of a few carnivorous plants which produce its own proteolytic enzymes (nepenthesin I and nepenthesin II), which provide an alternative source of nitrogen and other nutrients in case that these plants grow in a soil which lacks such nutrients. These aspartate proteases are capable of proteolysis at a very low pH of a digestive gastrointestinal cavity fluid after catching insects and in a cooperation with other proteins participating in digestion process.

Processing of a digestive fluid, isolated from a digestive gastrointestinal cavities of carnivorous plants of the *Nepenthes* genus and its possible application as a tool in a protein study using hydrogen/deuterium exchange were done in this thesis. Isolates of the digestive fluids were purified from coarse-grained impurities by centrifugation, activated by acidification and concentrated by ultrafiltration. The amount of proteins and their protein profile were monitored and an activity of acidic proteases was determined by enzymatic assay. Consequently, using LC-MS/MS and model proteins, the cleavage preferences of the preparations were studied under conditions applicable for experiments using hydrogen/deuterium exchange. Selected preparations were compared to each other and also by the genus and parameters of the fluids during sampling. A comparison to pepsin, the most common protease used in hydrogen/deuterium experiments, is also provided.

Interesting slight distinctions were observed in the cleavage preferences of nepenthesin compared to pepsin, they lie in the cleavage after the basic amino acids and proline and also in the high enzymatic activity of the proteases in isolates. These attributes predetermine nepenthesines as a fitting candidate for application in hydrogen/deuterium experiments. (In Czech)

**Keywords:** nepenthesin, mass spectrometry (MS), hydrogen/deuterium exchange