

SCREENING OF PROTEOLYTIC ACTIVITIES EXTRACTED FROM *Caesalpinia echinata* (PAU-BRASIL) SEEDS. PURIFICATION AND PARTIAL CHARACTERIZATION OF ONE TRYPSIN-LIKE ENZYME

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Some proteases found in different longevity stages of the leguminous seeds play an important role on their viability. *Caesalpinia echinata* seeds contain high amounts of proteins, including enzymes and their inhibitors. When stored in laboratory environmental conditions for one month or at 4°C for 18 months, these seeds lose their viability. We had already identified kallikrein (CeKI) and elastase (CeEI) inhibitors on stored and freshly harvested *C. echinata* seeds. Now, we are interested to study the different proteolytic activities in *C. echinata* seeds in several longevity stages, and to purify one of them. Both saline extracts, from freshly harvested and 18-months-stored seeds, presented amyolytic activity on chromogenic substrates H-D-Pro-Phe-Arg-pNan, H-D-Val-Leu-Lys-pNan, Suc-Ala-Ala-pNan and MeO-Suc-Ala-Ala-Pro-Val-pNan. A trypsin-like protein was isolated from both saline extracts by acetone fractionation, ion exchange (DEAE-Sephadex A-50), affinity (Trypsin-Sepharose) and hydrophobic (C18) chromatographies. The enzyme activity was followed by H-D-Pro-Phe-Arg-pNan hydrolysis in all steps. It was inhibited by serine protease inhibitors (SBTI and CeKI), but not by cysteine (E-64) and metallo proteases (EDTA), nor elastase (CeEI) inhibitors. The presence of the enzyme activity on freshly harvested and stored seeds suggests that it could be important for the seed longevity but not in the viability.

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Key words: *Caesalpinia echinata*, protease, seed longevity