

## Isolation, Purification and Characterization of Trypsin Inhibitor (IETI) from the Seeds of *Inga Edulis* (Leguminosae- Mimosaceae)

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**Introduction:** Peptidases Inhibitors (PIs) isolated from leguminous seeds have been studied for their therapeutic properties, as well as plant defense mechanisms against insects, pests and pathogens. *Inga edulis* is a tree species widely distributed in South and Central America. This plant is commonly found in Amazon and Cerrado regions of the Brazil. **Objective:** To purify and characterize a peptidase inhibitor isolated from the seed *I. edulis*. **Material and Methods:** The seeds were ground; the material was delipidated and submitted to the extraction step. The crude extract was subjected to Sephadex G100, and applied to Trypsin-Sepharose 4B. The active fraction was subjected onto C-18 HPCL analytic column with a non-linear acetonitrile gradient (45-60%). The protein profile was evaluated by SDS-PAGE. Residual antitryptic activity is measured using BAPNA substrate for trypsin. Stoichiometry and stability trial were conducted according antitryptic test ranging concentrations of inhibitor (0 and 0.08675 nM, 30 min, at 37°C) and DTT (1 and 10 mM, 15-60 min, at 37°C), temperature (30-100°C for 30 min) and pH (2-10 for 30 min, at 37°C). **Results and Discussion:** IETI purified showed a relative mass of approximately 23.47 kDa. We observe that the pH-optimum of maximal activity (pH 8). The thermostable IETI still had 80% of its initial activity. This is one good characteristic of the proteinase inhibitors. Addition of 1 mM DTT, for 15 min at 37°C, it affects 50% of the inhibitory activity. Disulfide bonds are involved in the structural stability and regulation of protein activity. Stoichiometry of trypsin–protease inhibitor interaction was 1:1 and 0.0347 nM of inhibitor effected 65% inhibition. Similar characteristics of stoichiometry may be observed for of the Kunitz-type inhibitors. **Conclusions:** According to these results, IETI might contribute greatly to the development of a promising tool of biotechnological function.

**Keywords:** Peptidase inhibitor, *Inga edulis*, Protein Purification and Characterization

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