Angiotensin Converting Enzyme (ACE) inhibitory activity in the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) protein hydrolysates

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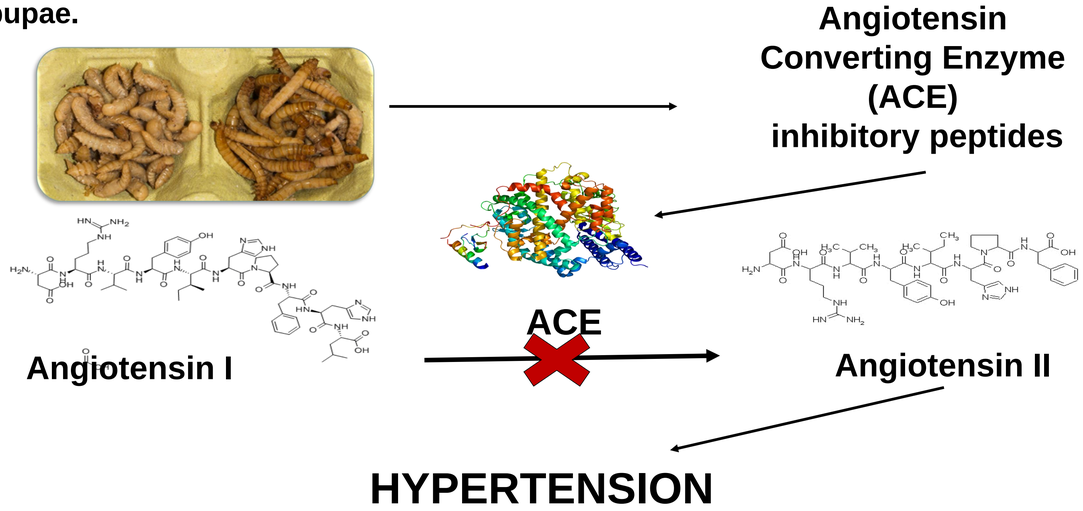
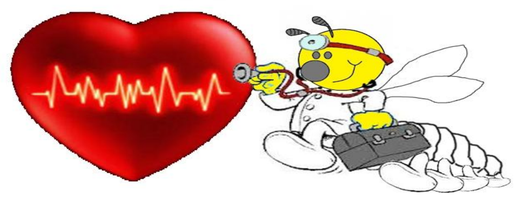
The purpose of this study was to investigate the ACE inhibitory activity of the protein hydrolysates derived from the larval and pupal stages of the edible insect *Tenebrio molitor* (Coleoptera: Tenebrionidae). The insect protein extracts were hydrolyzed by the gastrointestinal enzymes (pepsin, trypsin and α-chymotripisn) to simulate gastrointestinal digestion and compared to the crude extract. ACE inhibitory activity, determined by reverse-phase high performance liquid chromatography, significantly increased after gastrointestinal digestion of the insect protein extracts. *T. molitor* larvae resulted to be the most promising development stage for the purification and identification of bioactive ACE inhibitory peptides as they showed a significantly higher ACE inhibitory activity than pupae.





INTRODUCTION

Hypertension is well known as one of the major risk factor for cardiovascular disease. The Angiotensin Converting Enzyme (ACE) plays a key role in blood pressure regulation process. Hypertension treatment by synthetic ACE inhibitors (e.g. captopril, lisinopril, enalapril) is effective but their use can cause serious side effects, such as hypotension, cough, reduced renal function and angioedema. Therefore, research was focused on natural ACE inhibitory peptides sources such as foodstuffs and also some edible insect species recently promoted as a more environmentally sustainable, nutritious and functional alternative food to conventional livestock for human consumption.

MATERIALS AND METHODS

➢Dried *T. molitor* larvae and pupae (5 g) were homogenized at 13,500 rpm for 5 min in a IKA Labortechik blender T25 basic model with 5 ml of Tris/HCl buffer 50 mM pH 7.4, centrifuged at 5,000 rpm for 10 min and the supernatant collected and filtered.

➢The digestion that occurs in the stomach was simulated by lowering the pH of the crude protein extract to 2 with HCl 1M. Pepsin, previously dissolved in HCl 10 mM, was added to the crude protein extract (enzyme/substrate 1:250 w/w) and the mixture incubated for 2 h 30 min at 37 °C. To simulate the digestive process that occurs in the small intestine, the crude protein extract was then incubated for 2 h 30 min at 37 °C with trypsin and α-chymotrypsin (1:1 enzyme/substrate 1:250 w/w), after raising pH to 6.5 by adding NaOH 5 M. The samples were centrifuged at 5,000 rpm for 10 min and the supernatant filtered and kept at –20 °C until use.

➢ACE inhibitory activity was detected in crude protein and in the corresponding hydrolyzed protein extracts and measured by an indirect assay method based on the quantity of Hyppuric acid (HA) released by ACE from Hyppuryl-L-histidyl-leucine (HHL). Different concentrations of each sample (from 10 mg/ml to 1 µg/ml) were prepared in 0.1 M borate buffer containing NaCl 0.3 M at pH 8.3. 40 µl of each sample were pre-incubated with 25 µl of ACE 0.1 mU/ml, dissolved in the same buffer for 10 min at 37 °C; 40 µl of HHL 6.5 mM were then added and the samples incubated for 30 min at 37 °C. Enzyme reaction was terminated by adding 85 µl of HCl 1 M. Blank sample was obtained replacing sample solution with borate buffer. Captopril was prepared at different solutions (from 0.5 mg/ml to 0.1 ng/ml) and used as positive control. All samples were centrifuged at 5,000 rpm for 20 min and filtered before RP-HPLC analysis.

➢ ACE inhibitory activity was detected by an Agilent 1260 Infinity series liquid chromatograph system including a vacuum solvent degassing unit, a binary high-pressure gradient pump, an auto sampler and an UV-DAD detector. Chromatographic separation was performed by injections of 20 µl of each sample into a ready-to-use prepacked LC-18 column (Phenomenex Ultracarb, 4.6 x 150 mm, 5 µm). The column was eluted by a mixture of ACN (25%) and H_2O containing 0.05% CH_3COOH (75%) at a flow rate of 0.8 ml/min. The elution profile was monitored at 228 nm to detect HHL and HA.

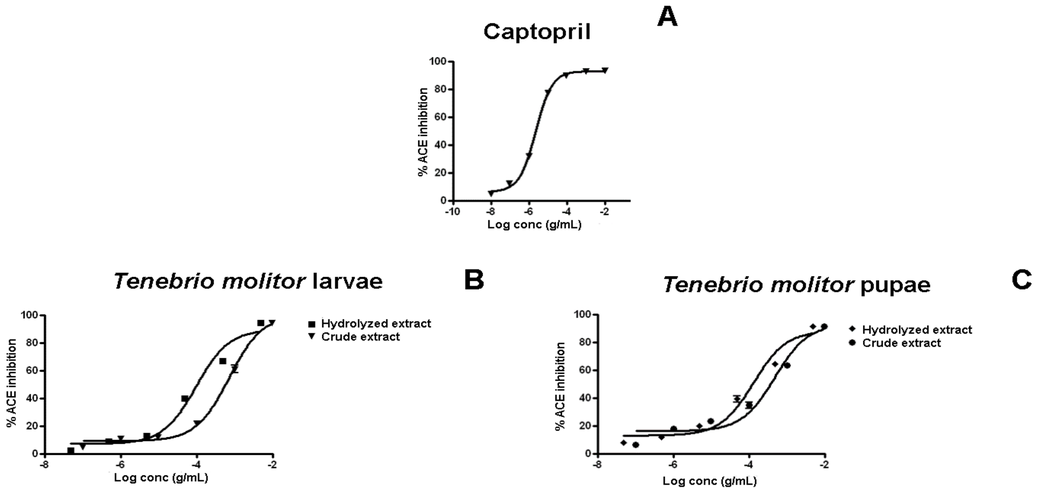


Figure 1. ACE inhibiton activity of Captopril, (A), *Tenebrio molitor* larvae (B) and pupae (C)

RESULTS

The ACE inhibition was expressed as the concentration of protein sample that inhibit 50% of ACE activity (IC_{50}), assuming that the activity of the blank is equal to 100%. The IC_{50} value of captopril was 2.6 x 10^{-6} mg/mL. A significantly lower IC_{50} was detected after gastrointestinal hydrolysis of the protein extracts obtained from larvae (0.720 *vs* 0.097 mg/mL after gastrointestinal hydrolysis; $P < 0.01$) and pupae (0.484 *vs* 0.132 mg/mL after gastrointestinal hydrolysis; $P < 0.05$). IC_{50} was significantly lower in hydrolyzed larvae than pupae ($P < 0.05$).

CONCLUSIONS

Based on experimental data, *T. molitor* larvae represent the most promising development stage for the purification and identification of bioactive ACE inhibitory peptides, confirming the potential benefits of this coleopteran for human health. The ability to inhibit ACE activity detected in their protein enzymatic hydrolysates deserves to be further investigated through the purification and identification of the peptide/s responsible of ACE inhibition.

REFERENCES

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