# OPTIMIZATION AND CHARACTERIZATION OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY PEPTIDE FROM BLOOD COCKLE (*Anadara granosa)* HYDROLYSATE

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**Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the School of Food Science and Technology Universiti Malaysia Terengganu**

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# DEDICATION

**To lecturers and teachers**

*for guiding me through this path*

**To my beloved family**

*for continuous support, understanding, inspiration*

**To best friends / seniors / juniors**

*Thanks a lot and best wishes*

**To my husband**

*for being the best supporter besides me*

**To readers**

*hopefully something benefits you*

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This study reported on the optimization and characterization of *angiotensin converting enzyme* (ACE) inhibitory peptide from blood cockle hydrolysate. Firstly, preliminary screening of commercial proteinases was carried out to select the most appropriate proteinase and hydrolysis time (0-8 hrs) to prepare the highest ACE inhibitory activity. It was found that Protamex$^{TM}$ gave the highest ACE inhibitory activity at 6 hours hydrolysis time as compared to Alcalase®, Neutrase®, pepsin, papain, trypsin and α-chymotrypsin. Next, further optimization of enzymatic hydrolysis condition (i.e. hydrolysis time, pH, hydrolysis temperature and enzyme to substrate (E/S) ratio) of blood cockle with Protamex$^{TM}$ using a central composite design (face-centered) was employed to obtain maximum ACE inhibitory activity. The range of hydrolysis conditions used were 4-8 hours of hydrolysis time,

temperature of 35-60$^{o}$C, pH 5.5-7.5 and E/S of 0.5-1.5%. It was found that quadratic model ($p < 0.0001$) can be used to explain the relationship between all variables in blood cockle meat hydrolysis with ACE inhibitory activity. The optimum hydrolysis condition was obtained at 59.62$^{o}$C, 4 hrs and 40 min of hydrolysis time, pH of 5.59 and E/S ratio of 0.93%. The predicted ACE inhibitory activity was 99.2%, which was close to that of experimental value of 97.81%. This study found that the $IC_{50}$ of crude (unpurified) ACE inhibitory peptide in blood cockle meat hydrolysate prepared at optimum condition using Protamex$^{TM}$ was 0.35 mg/ml. It was found that the crude ACE inhibitory peptide maintained high ACE inhibitory activity against gastrointestinal enzymes (pepsin and trypsin), and was stable at low temperature (4$^{o}$C) and acidic pH (pH 2) within 3.5 hrs. Purification of ACE inhibitory peptide from blood cockle meat hydrolysate was accomplished via a series of ultrafiltration membrane (10 kDa, 5 kDa and 3 kDa), ion exchange chromatography and reversed-phase HPLC. The blood cockle meat hydrolysate prepared under optimum hydrolysis condition (i.e. at 59.62$^{o}$C, 4 hrs and 40 min hydrolysis time, pH 5.59 and 0.93% E/S) was fractionated through 3 kDa ultrafiltration membrane followed by HiPrep DEAE FF 16/10 anion exchange chromatography and finally by reversed-phase $C_{18}$ column. The purification result shows the decrease in $IC_{50}$ of crude peptide in the form of hydrolysate ($IC_{50}$ = 0.35 mg/ml) after the three purification steps using 3 kDa ultrafiltration membrane ($IC_{50}$ = 0.27 mg/ml)on a HiPrep DEAE FF 16/10 anion exchange column ($IC_{50}$ = 0.014 mg/ml) and $C_{18}$ column ($IC_{50}$ = 0.009 mg/ml), resulting in 38.9-fold purification. The purified ACE inhibitory peptide was identified as VNDLLSGSFKHFLY with a molecular weight of 1621.88 Da. This study suggested that the ACE inhibitory peptide from blood cockle meat

that was hydrolyzed using Protamex$^{TM}$ has big potential as nutraceutical food ingredient.