

# **THESIS**

**PROTEIN HYDROLYSATE FROM TILAPIA AND PERCH FRAME :  
ANTIOXIDANT AND ACE - INHIBITOR PROPERTIES**

**CHETTHA VONGPORNIMIT**

**GRADUATE SCHOOL, INTEGRATED THESIS AND RESEARCH  
MANAGEMENT SYSTEM  
2017**



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THESIS

PROTEIN HYDROLYSATE FROM TILAPIA AND PERCH FRAME :  
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CHETTHA VONGPORNIMIT

A Thesis Submitted in Partial Fulfillment of  
the Requirements for the Degree of  
Doctor of Philosophy  
GRADUATE SCHOOL, INTEGRATED THESIS AND RESEARCH  
MANAGEMENT SYSTEM  
2017

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**THESIS APPROVAL**  
**GRADUATE SCHOOL, INTEGRATED THESIS AND RESEARCH**  
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**DEGREE:** Doctor of Philosophy  
**MAJOR FIELD:** Mathematics  
**DEPARTMENT:** Department of Chemical Technology

**TITLE:** Protein hydrolysate from tilapia and perch frame :  
antioxidant and ace - inhibitor properties

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**THIS THESIS HAS BEEN ACCEPTED BY**

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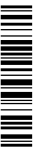
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Chettha Vongpornnimit 2017: Protein hydrolysate from tilapia and perch frame :

antioxidant and ace - inhibitor properties. Doctor of Philosophy, Major Field: Mathematics, Department of Department of Chemical Technology. Thesis Advisor: Assistant Professor Thanakorn Tawsan, Ph.D.

The optimum condition to produce protein hydrolysate from tilapia and perch frame with antioxidant (analyzed by DPPH method, metal chelating activity method and TBA assay) and ACE inhibitory properties were investigated. Minced fish frame was enzymatically hydrolyzed by using Flavourzyme 1000 L at different concentration (0, 1, 2 and 3 % w/w) and hydrolysis time (0, 1, 2 and 3 hrs). The results showed that enzyme concentration and hydrolysis time affected the % DPPH radical scavenging, % metal chelating activity, % TBA activity ratio and % ACE inhibition significantly ( $P \leq 0.05$ ). Tilapia frame protein hydrolysate obtained by using 2 % Flavourzyme 1000 L hydrolyzed for 1 hour and perch frame protein hydrolysate obtained by using 3 % Flavourzyme 1000 L for 2 hours were the selected conditions due to the high value of % DPPH radical scavenging, % metal chelating activity, % TBA activity ratio and % ACE inhibition which were 90.38, 91.80, 70.54 and 81.90 % for the selected tilapia frame protein hydrolysate, respectively. And % DPPH radical scavenging, % metal chelating activity, % TBA activity ratio and % ACE inhibition were 96.80, 92.54, 90.12 and 92.59 % for the selected perch frame protein hydrolysate, respectively. Spray-dried of the selected protein hydrolysates from tilapia and perch frame were made.

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Chettha Vongpornnimit

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## CHAPTER 1

### Introduction

The Nile tilapia, *Oreochromis nilotica* L. (Perciformes: Cichlidae), is native to Africa but has been extensively introduced and hybridised around the world for aquaculture and become an invasive pest, including within Thailand where it is widely spread and cultivated. The species and its hybrids are popular as they are easily reared, including on cheap vegetarian diets, are less polluting, grow quickly and have a good taste. Only the fillets of tilapia have a good economical value. In contrast, the tilapia by-products[1], such as frame, viscera and head, are commonly used as low market value resources or are discarded and can cause environmental pollution. These by-products still contain a large amount of good quality protein, and the hydrolysis of their products should be the method to upgrade their market value, increase their functional properties and drive towards sustainable development. The antioxidant and angiotensin I converting enzyme (ACE) inhibitor properties can be improved by enzymatic hydrolysis under controlled conditions. In recent years, the global trend in consumers' preferences has moved strongly towards the use of natural antioxidants over synthetic ones for food applications. The amino acid and peptide content formed in protein hydrolysates, including those from fish frames, have been reported to be an effective natural antioxidant suitable for food use (Klompong, Benjakul et al. 2007).[2] As different amino acids can inhibit oxidation reactions via different mechanisms, the free amino acid composition and the sequence of the peptides in the protein hydrolysate are important. For example, amino acids containing an aromatic group, such as tryptophan, tyrosine and phenylalanine, scavenge free radicals by a resonance system, whilst anionic amino acids, such as glutamic acid and aspartic acid, chelate metal ions and so retard the autoxidation induced by catalytic metal ions (Arcan and Yemenicioglu 2007, Dong, Zeng et al. 2008) Variations in the raw material used as the protein hydrolysate source, the type of enzyme used, the hydrolysis time and enzyme concentration are

all important factors that influence the free amino acid composition 3403916179 OHEC iThesis 5873010129 independent study / recv: 21052559 21:15:26 / seq: 36 2 and the size and abundance of different peptides and their amino acid sequences of the resulting **hydrolysate**. [3] Moreover, the molecular weight (MW) of the peptides, presumably as a reflection of the peptide size, also affects their functional property, where those with a MW of approximately 1.0–3.0 kDa were found to be effective antioxidants (Ahn, Kim et al. 2014). Angiotensin I converting enzyme (EC 3.4.15.1) plays an important role in regulating blood pressure, where it catalyses the conversion of a potent vasodilator (angiotensin I) to a potent vasoconstrictor (angiotensin II) and also inactivates the antihypertensive bradykinin (Ondetti, Rubin et al. 1977) and so can also generate hypertension in humans. [4] Currently, synthetic ACE inhibitors, such as captopril, enalapril or lisinopril, are used to reduce the blood pressure level in hypertension and heart failure patients. However, there is an increasing demand for natural ACE inhibitors that are effective and do not induce such side effects include a dry, persistent cough, abdominal pain, diarrhoea, rash, dizziness and fatigue. Similar to the antioxidant properties, the type and sequence of the amino acid residues in the peptides, including the size of the peptide chain, influences the complex reaction of these inhibitors with the active site of ACE (Nakajima, Yoshie-Stark et al. 2009). The active site of ACE [5] contains a narrow centre groove formed from two domains and so excludes larger peptides from access to the active site. Smaller peptides that access into this groove can interact with the reactive group of amino acid residues at the active site directly by electrostatic repulsion, hydrophobic interaction or hydrogen bonding according to their amino acid sequence. Accordingly, small peptides of the correct composition are more efficient inhibitors of ACE than larger ones. The antioxidant and ACE inhibitor properties of the protein hydrolysates derived from fish muscle have been well documented, but that for their byproducts are largely neglected. The objective of this study then was to select a suitable condition for the commercial aminopeptidase (Flavourzyme 1000 L) to produce a



tilapia frame 3403916179 OHEC iThesis 5873010129 independent study / recv: 21052559 21:15:26 / seq: 36 3 protein hydrolysate (TFPH), defined as that which yielded the highest antioxidant and ACE inhibitor levels. Accordingly, the effect of varying the enzyme concentration and hydrolysis time upon the level of antioxidant and ACE inhibition obtained in the resultant TFPH was evaluated.

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