

UNIVERSITI TEKNOLOGI MARA

**CHARACTERIZATION AND
FLAVOUR ENHANCEMENT OF
PROTEIN HYDROLYSATE
GENERATED FROM
ENZYMATICALLY HYDROLYZED
CLAM (*Polymesoda erosa*)**

NOORASMA BINTI MUSTAKIM

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Applied Sciences

February 2019

ABSTRACT

An enzymatic hydrolysis of the bivalve species to produce value-added hydrolysate products has emerged as a priority area for the global seafood industry development. However, research on protein hydrolysates from bivalves are still very limited and some of them have been shown to impart undesirable flavours which limit its application for human consumption. Therefore, this research was conducted to characterize the mud clam (*Polymesoda erosa*) hydrolysates produced using two different enzymes; alcalase and flavourzyme. The selected hydrolysate was then modified by Maillard reaction and analysed. Mud clam was hydrolysed using alcalase at pH 8.5, 60°C, 3% enzyme substrate (ES) ratio and flavourzyme at pH 7, 55°C, 3% ES ratio for 2 hours. The hydrolysate was then characterized for physicochemical, functional and sensory properties. The sensory properties of hydrolysate were evaluated through Quantitative Descriptive Analysis (QDA), volatile compounds, amino acids and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The result showed that the type of enzyme used gave significant impact on the hydrolysate properties. Hydrolysis using alcalase resulted in 20.28% DH, 25.06% yield and 45.37% protein while flavourzyme hydrolysis resulted in 22.93% DH, 46.67% protein and 30.68% yield. Better emulsifying properties, foaming properties and water and oil holding capacity were exhibited by flavourzyme hydrolysate compared to alcalase hydrolysate. Alcalase hydrolysate was more bitter compared to flavourzyme based on QDA result, the presence of more bitter like amino acids and higher content of 2-piperidinone volatile compound. Based on these observations, alcalase hydrolysate was produced and further subjected to Maillard reaction process. The Maillard reaction products (MRPs) from alcalase hydrolysate produced in the presence of xylose (AH-x) and alcalase hydrolysate with the presence of xylose and L-cysteine (AH-mx) model system were evaluated and compared to the original alcalase hydrolysate. Findings from amino acid analysis showed that AH-x and AH-mx were more umami and bitter taste was reduced. 2-piperidinone that contributed to bitter taste was reduced in AH-x and not detected in AH-mx. Furthermore, the QDA result showed that AH-mx had greater umami taste and reduced in bitter taste and fishy flavour compared to alcalase hydrolysate and AH-x due to highest content of furfural. The MRPs also showed the increased in DPPH scavenging activity and reducing power antioxidant activity. Therefore, MRPs alcalase hydrolysate could potentially be used as flavour enhancers with antioxidant additive as a new marketable seafood product.

ACKNOWLEDGEMENT

In the name of Allah and entire praise for only Almighty Allah who has given me the ability to embark on my master research study and for completing this challenging journey successfully.

I would like to express my profound gratitude and sincere regard to my esteemed supervisor, Dr Normah Ismail for her unbound enthusiasm and rationalist ideas and excellent guidance.

I would express my heartfelt gratitude to my respected and superwoman mom, and my beloved brothers and sisters for their great support in leading me to proper intellectual pursuit. Their immeasurable love affection gave me courage to complete this study.

It is my pleasure to express my gratefulness and thanks to all Staff member of Food Science & Technology department, Universiti Teknologi MARA, UiTM for kind helps in several occasion during my research.

Finally, I shall also like to express my thanks to all my friends especially Nurul Fasihah, Siti Hafsa, Norakma, Siti Raihan, Dian Nashiela, Siti Sarah, Izzatul Adyani, Nurul Farhana, and Aziatul Akma for their unfailing affection suggestion, encouragement and cooperation in my study. Their valuable criticize helped me and made it possible for me to complete the work embedded in this research. A warm salute to all who were directly or indirectly interconnected during my research work. Thank You, may Allah always bless us.

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF PLATES	xii
LIST OF ABBREVIATIONS	xiii
CHAPTER ONE: INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Objectives	4
1.4 Scope of Study	4
1.5 Significance of Study	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Mud Clam (<i>Polymesoda erosa</i>)	6
2.2 Protein Hydrolysate	7
2.3 Enzymatic Protein Hydrolysate	8
2.3.1 Alcalase	8
2.3.2 Flavourzyme	9
2.4 Utilization of Protein Hydrolysate	10
2.5 Degree of Hydrolysis	11
2.6 Functional Properties of Fish Protein Hydrolysate	12
2.6.1 Solubility	13

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Mud clam (*Polymesoda erosa*) is edible marine bivalves widely distributed throughout the west indo-pacific region including Peninsular Malaysia and Sarawak (Ingole et al., 2002). In peninsular Malaysia, this species are normally found in Selangor, Johor, and Perak. In Southern Asia the clam are often harvested but not on commercial scale for food. The clam can be found buried in the stiff mud of mangroves (Bayen et al., 2005). This clam is able to live in low tide for long period and has the ability to resume filter-feeding rapidly when inundated (Ng, Corlett, and Tan, 2011). Clam production and aquaculture are important economic resources worldwide. In 2009, clam fisheries along with cockles and arkshells amounted almost 740 thousand tonnes, which corresponded to less than 1% of the world total capture of marine organisms (FAO, 2010). Concerning the overall economic value, those catches were estimated to account around 725 million US\$, which was also under 1% of the total economic value of fisheries worldwide (FAO, 2010).

Hydrolysate is any compound produced by hydrolysis process. Meanwhile, protein hydrolysate is a mixture of amino acids prepared by splitting a protein with acid, alkali or enzyme (Adler-Nissen, 1986). Protein hydrolysates are widely used in the food industry for various applications, such as milk replacers, protein supplement, beverage stabilizers and flavour enhancers (dos Santos et al., 2011; Thorkelsson and Kristinsson, 2009; Laohakunjit, Selamassakul, and Kerdchoechuen, 2014). Due to these applications, various resources of marine protein such as fish, fish waste, and recently from edible bivalve were widely studied in order to produce protein hydrolysate. Bivalve protein hydrolysate such as green mussel and anglewing clam was successfully produced by enzymatic hydrolysis (Normah, Siti Hafsa, and Nurul Izzaira, 2013; Normah and Nurul Fasihah, 2016). Enzymatic hydrolysis of protein is a chosen option because it works without destructing amino acids and by avoiding the extreme temperatures and pH levels as required for chemical hydrolysis, the nutritional properties of the protein hydrolysates remains largely