DETERMINATION OF THE EFFECTS OF DIFFERENT STORAGE CONDITIONS AND CHEMICAL TREATMENTS AFFECTING PROTEIN PROFILE AND ALLERGENICITY OF *Cerithidea obtusa*

SITI RUKAIYAH BINTI ABD FATAH

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

DISSERTATION SUBMITTED IN FULLFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2019

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

UPSI/IPS-3/BO 32
Pind : 00 m/s: 1/1

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

Please tick (√)
Project Paper
Masters by Research
Master by Mixed Mode PhD

√

INSTITUTE OF GRADUATE STUDIES

DECLARATION OF ORIGINAL WORK

This declaration is made on the 24th day of December 2018

i. Student's Declaration:

I, SITI RUKAIYAH BINTI ABD FATAH, M20142002614, FSM (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled DETERMINATION OF THE EFFECTS OF DIFFERENT STORAGE CONDITIONS AND CHEMICAL TREATMENTS AFFECTING PROTEIN PROFILE AND ALLERGENICITY OF *Cerithidea obtusa* is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

Signature of the student

ii. Supervisor's Declaration:

I DR ROSMILAH BINTI MISNAN (SUPERVISOR'S NAME) hereby certifies that the work entitled DETERMINATION OF THE EFFECTS OF DIFFERENT STORAGE CONDITIONS AND CHEMICAL TREATMENTS AFFECTING PROTEIN PROFILE AND ALLERGENICITY OF *Cerithidea obtusa* (TITLE) was prepared by the above named student, and was submitted to the Institute of Graduate Studies as a * ~~partial~~/full fulfillment for the conferment of DEGREE OF MASTER OF SCIENCE (BIOLOGY) (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

24th December 2018
Date

Signature of the Supervisor

UPSI/IPS-3/BO 31
Pind.: 01 m/s:1/1

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

INSTITUT PENGAJIAN SISWAZAH /
INSTITUTE OF GRADUATE STUDIES

BORANG PENGESAHAN PENYERAHAN TESIS/DISERTASI/LAPORAN KERTAS PROJEK
DECLARATION OF THESIS/DISSERTATION/PROJECT PAPER FORM

Tajuk / *Title*: DETERMINATION OF THE EFFECTS OF DIFFERENT STORAGE CONDITIONS AND CHEMICAL TREATMENTS AFFECTING PROTEIN PROFILE AND ALLERGENICITY OF *Cerithidea obtusa*

No. Matrik /*Matric No.*: M20142002614

Saya / *I* : SITI RUKAIYAH BINTI ABD FATAH

(Nama pelajar / *Student's Name*)

mengaku membenarkan Tesis/Disertasi/Laporan Kertas Projek (Kedoktoran/Sarjana)* ini disimpan di Universiti Pendidikan Sultan Idris (Perpustakaan Tuanku Bainun) dengan syarat-syarat kegunaan seperti berikut:-
acknowledged that Universiti Pendidikan Sultan Idris (Tuanku Bainun Library) reserves the right as follows:-

1. Tesis/Disertasi/Laporan Kertas Projek ini adalah hak milik UPSI.
   The thesis is the property of Universiti Pendidikan Sultan Idris
2. Perpustakaan Tuanku Bainun dibenarkan membuat salinan untuk tujuan rujukan dan penyelidikan.
   Tuanku Bainun Library has the right to make copies for the purpose of reference and research.
3. Perpustakaan dibenarkan membuat salinan Tesis/Disertasi ini sebagai bahan pertukaran antara Institusi Pengajian Tinggi.
   The Library has the right to make copies of the thesis for academic exchange.
4. Sila tandakan (√) bagi pilihan kategori di bawah */ Please tick (√) from the categories below:-*

☐	**SULIT/*CONFIDENTIAL***	Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972. / *Contains confidential information under the Official Secret Act 1972*
☐	**TERHAD/*RESTRICTED***	Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. / *Contains restricted information as specified by the organization where research was done.*
☑	**TIDAK TERHAD / *OPEN ACCESS***	

(Tandatangan Pelajar/ Signature)

(Tandatangan Penyelia / *Signature of Supervisor*)
& (Nama & Cop Rasmi / *Name & Official Stamp*)

ASSOC. PROF. DR. [illegible]
Faculty of Science and Mathematics
Sultan Idris Education University

Tarikh: 29/10/2019

Catatan: Jika Tesis/Disertasi ini **SULIT @ TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai **SULIT** dan **TERHAD**.

Notes: If the thesis is CONFIDENTAL or RESTRICTED, please attach with the letter from the related authority/organization mentioning the period of confidentiality and reasons for the said confidentiality or restriction.

ACKNOWLEDGEMENTS

First and foremost, I appreciatively acknowledge the technical guidance and supervision of Dr. Rosmilah Binti Misnan. Her tireless and valuable supervision from the beginning to the end of this work has not only registered success in this work but has imparted a lot of knowledge in me. Besides, I would also like to thank Dr. Remmy Keong Bun Poh for sharing their invaluable knowledge, guidance, and encouragement throughout my Master journey.

I am very grateful to MyBrain 15 Scholarship under the Education Ministry for the financial support provided to me over the past two years.

My deepest gratitude goes to all of the research participants, as well as the Institute for Medical Research and Biology Department Sultan Idris Education University for undertaking and facilitating my research. The primary motivation for completing a Master was always to try and make a difference, no matter how small, to improve the lives of allergy sufferers. With all of your help, I hope I am a step closer to achieving this.

Finally, this Master would not have been achievable without my family, who have never stopped believing in me and who I am indebted to for their constant support. To my closest friends, thank you from the bottom of my heart for your continued love and support throughout the process of writing this thesis.

ABSTRACT

This study aimed to determine the effects of different storage conditions and chemical treatments on protein profile and allergenicity of local edible sea snail, *Cerithidea obtusa*. Several snail extracts from untreated, stored snails at different times and temperatures (4°C, 25°C and -20°C) and chemical-treated snails (salting and vinegar) were extracted and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine their protein profiles. Allergenic properties of all snail extracts were then determined by immunoblotting using sera from snail-allergic patients. In SDS-PAGE, the untreated extract of snail resulted in at least 26 protein bands, between molecular weights of 8 to >250 kDa. In contrast, fewer protein bands (in range of 5-25) were seen in almost all stored snail extracts and chemical-treated extracts, except for the frozen snails which could retain their protein profiling as the untreated snail. Immunoblotting of the untreated snail extract identified numerous IgE-binding proteins at various molecular weights between 25 to 245 kDa. Four major allergens at 35, 48, 100, and 135 kDa were detected. Immunoblotting revealed that the untreated and all the frozen snail extracts had more allergenic proteins than the other stored snail extracts and chemical-treated extracts. The vinegar-treated extract was demonstrated to have the least allergenic proteins. As a conclusion, *C. obtusa* had four major allergens and all extracts of untreated, stored and chemical-treated snails played a crucial role in snail allergy. These results would directly contribute to advancements in diagnosis, management and therapeutic methods of snail-allergic patients worldwide.

PENENTUAN KESAN PERBEZAAN KEADAAN PENYIMPANAN DAN RAWATAN KIMIA KE ATAS PROFIL PROTEIN DAN ALERGENISITI *Cerithidea obtusa*

ABSTRAK

Kajian ini bertujuan untuk menentukan kesan-kesan keadaan penyimpanan yang berlainan dan rawatan kimia pada profil protein dan alergenisiti siput laut tempatan yang boleh dimakan, *Cerithidea obtusa*. Beberapa ekstrak siput daripada siput yang tidak dirawat, siput yang disimpan pada masa dan suhu yang berbeza (4°C, 25°C dan -20°C) dan siput yang dirawat kimia (garam dan cuka) diekstrak dan dipisahkan oleh elektroforesis gel natrium dodesil sulfat berpoliakrilamida (SDS-PAGE) bagi menentukan profil protein. Ciri-ciri alergen dari semua ekstrak kemudiannya ditentukan oleh pemblotan imuno menggunakan serum daripada pesakit alahan siput. Dalam SDS-PAGE, ekstrak siput yang tidak dirawat menghasilkan sekurang-kurangnya 26 jalur protein, antara berat molekul 8 hingga > 250 kDa. Sebaliknya, jalur protein yang sedikit (dalam julat 5-25) dapat dilihat dalam semua ekstrak siput yang disimpan dan yang dirawat kimia, kecuali siput yang telah disejukbeku didapati dapat mengekalkan profil proteinnya seperti siput yang tidak dirawat. Pemblotan imuno terhadap ekstrak siput yang tidak dirawat telah dikenalpasti banyak protein pengikat IgE pada pelbagai berat molekul antara 25 hingga 245 kDa. Empat alergen major pada 35, 48, 100, dan 135 kDa telah dikesan. Pemblotan imuno menunjukkan bahawa ekstrak siput yang tidak dirawat dan semua ekstrak siput yang telah disejukbeku mempunyai lebih banyak protein alergenik berbanding ekstrak siput yang lain. Ekstrak siput yang diberi rawatan cuka pula menunjukkan protein alergenik yang paling sedikit. Sebagai kesimpulan, siput mempunyai empat alergen major dan semua ekstrak siput yang tidak dirawat, disimpan dan dirawat dengan bahan kimia memainkan peranan penting dalam alahan siput. Dapatan kajian ini secara langsung akan menyumbang kepada kemajuan dalam diagnosis, pengurusan dan kaedah rawatan pesakit alergi siput di seluruh dunia.

TABLE OF CONTENTS

CHAPTER 2 LITERATURE REVIEW

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

2-DE	Two-dimensional electrophoresis
APS	Ammonium Persulfate
BSA	Bovine serum albumin
IgE	Immunoglobulin E
kDa	Kilo Dalton
MW	Molecular Weight
PBS	Phosphate Buffer Saline
RAST	Radio-allergosorbent tests
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel
TBS	Tris-buffered Saline
TEMED	N, N, N, N-Tetramethyl Ethylenediamine
TTBS	Tris-buffered Saline with 10 % Tween 20

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter focuses on the research background, problem statements, research objectives, research questions, research significant and research limitations. This chapter also will give a general overview of this study.

1.2 Research Background

Seafood is composed of diverse sea organisms and plays an important role in human nutrition worldwide (Lopata & Kamath, 2012) and the world economy (Wild & Lehrer, 2005), but humans are allergic to many of them (Lehrer, Ayuso, & Reese, 2003). The increasing consumption and production of seafood have resulted in more

often reports of food hypersensitivity reactions including fatal anaphylaxis. A hypersensitive reaction to seafood is one of the most common food allergies (Rahman, Helleur, Jeebhay, & Lopata, 2012).

Shellfish is one of the most important seafood groups (Lopata & Lehrer, 2009). Shellfish belongs to two major phyla which are Arthropoda and Mollusca. The Mollusca phylum is divided into eight classes including three classes that are important for human food; Gastropoda, Bivalvia, and Cephalopoda (Hickman, Roberts, Larson, I' Anson, & Eisenhour, 2004). The Arthropoda phylum contains the Crustacean class of shellfish that includes crab, lobster, shrimp, crayfish, barnacles, and prawns.

Shellfish is the most frequent causes of food-allergic reactions in both adults and children (Wild & Lehrer, 2005) and become the highest ranking in the Asia-Pacific regions as causes of food allergy in children (Lee, Gerez, Shek, & Lee, 2012). One random telephone survey completed by Sicherer et al. (2004) found that seafood allergy is reported by 3.3% of the general population and by at least one member of 5.9% of US households. In Malaysia, the prevalence of shellfish allergy was reported to be 26% among patients who had a positive skin prick test (SPT) to at least one of the five bivalve extracts tested. Besides, among the seafood allergens, the prawn was found to be the most common allergen in 48% of 24 subjects (Zailatul et al., 2015b). Shellfish is capable of inducing long-lasting allergy throughout the life of allergic subjects (Zheng, Lin, Pawar, Li, & Li, 2011).

Snail is a marine gastropod mollusk in the family Potamididae (Noor Asyikin, Rosmilah, & Zailatul, 2016). Fleshy portion of sea snails was utilized as food in Southeast Asia that particularly can cause allergy after its ingestion and tropomyosin were identified as its allergen (Van et al., 1996). Obtusa horn snail (*Cerithidea obtusa*) locally known as 'siput sedut' is among the most widely consumed snails by society and traditionally used for therapeutic purposes (Purwaningsih, 2012). Besides, this snail is also known as ''Mud Creeper'' because it is usually found in muddy coastal areas (Lee, Wong, Tan, & Yew, 2015). As well as in Southeast Asia, this snail is a delicacy eaten in Spain, France and Portugal (De la Cuesta, Garcia, Cordoba, Dieguez, & Oehling, 1989). Snail allergy can be dangerous when more case reported anaphylaxis resulted from accidental ingestion of snail in patients known to be allergic to this mollusk (Purwaningsih, 2012). In Malaysia, the immediate hypersensitivity to local sea snail, *C. obtusa* exists among local atopic patients at a low frequency of 8%. A patient with *C. obtusa* sensitization is also probably to be sensitized to other shellfish (Noorazlin et al., 2016).

In many shellfish, tropomyosin is the most important major allergen, especially in crustaceans and mollusks. Oddly, it has also been identified as a significant allergen in other invertebrates including dust mites and cockroaches (Lehrer et al., 2003). In shellfish, tropomyosin is a heat stable major allergen. Tropomyosin is composed of two identical subunits with the molecular mass of 35 to 38 kDa (Huang et al., 2010) and 34 to 38 kDa (Zailatul et al., 2010) that known as a myofibrillar protein. It is highly conserved in an amino acid sequence of various invertebrates. Thus it is implicated in IgE cross-reactivity among various invertebrates including mollusks (Taylor, 2008), crustaceans (Garcia & Lizaso, 2011), mites and

cockroaches (Ayuso, Reese, Leong-Kee, Plante & Lehrer, 2002) that regarded as a pan-allergen. So far, tropomyosin has been endorsed as the major allergen of various species of shellfish including mollusks (Zailatul et al., 2018; Zailatul et al., 2017; Zailatul et al., 2015; Zailatul et al., 2010).

There is still a lack of information on the mollusk allergens and its diagnosis although *in vitro* determination of food-specific IgE and skin prick testing (SPT) is the most common allergy test performed in an allergy clinic (Allergy UK, 2012). To date, specific immunotherapy and allergy diagnosis are accomplished using allergenic extracts containing a variety of non-allergenic and allergenic components that are too complicated to be standardized (Ciardiello et al., 2013). The result of incomplete knowledge of main allergens based on the lack of standardization of allergenic extracts for allergy tests may result in low diagnostic accuracy (Zailatul, Rosmilah, Faizal, Noormalin, & Shahnaz, 2015a).

1.3 Problem Statement

Shellfish has been recognized as one of the leading causes of food allergy in the world in both children and adults, and it has been demonstrated to be one of the highest ranking in the Asia Pacific causes of food allergy in children (Lee et al., 2012), where the supply, as well as the consumption of seafood in this region, is the top in the world. In Malaysia, obtusa horn snail (*Cerithidea obtusa*) is among the most widely consumed species (Mohd Nazllie, 2015). However, after snail's ingestion some people develop allergic reactions, mostly consisting of severe episodes of asthma

(Martins, Peltre, da Costa Faro, Pires & da Cruz Inacio, 2005). In spite of the high prevalence of snail allergy, there is limited information regarding snail allergens because it has not been well studied (Asturias et al., 2002), but one previous studies have successfully identified several major allergens including tropomyosin, actin and arginine kinase in several species of bivalves such as cockle, mussel, and gastropod including edible snails (Lopata, Kleine-Tebbe, & Kamath, 2016).

Snails are usually subjected to certain food processing treatments or preservation methods such as freezing, pickling, and salting. Besides, the effect of thermal treatments on the snails' allergenicity has also been revealed (Rosmilah et al., 2015a, 2015b). However, studies on the effect of different storage conditions and other treatments such as chemical treatments on the allergenicity of local edible snails had not been done before. Thus, this study aims to identify the effect of storage conditions and common chemical treatments on the allergenicity of a local edible snail, *C. obtusa*. Limitation of information regarding snail allergy will make a snail's allergen more complicated to manage. Besides, it is also necessary to find approaches to reduce the allergenicity of edible mollusks especially snails. So, the findings from this study may directly contribute to the advancements in diagnosis, management of allergic patients and the standardization of allergenic test products as tools in molecular allergology.

1.4 Objectives

The objectives of this research are:

1. To determine the effect of storage conditions on protein profile of local snails (*Cerithidea obtusa*).
2. To determine the effect of chemical treatments on protein profile of local snails (*C. obtusa*).
3. To determine the effect of storage conditions on allergenicity of local snails (*C. obtusa*).
4. To determine the effect of chemical treatments on allergenicity of local snails (*C. obtusa*).

1.5 Research Questions

Research questions for this research are:

1. What is the effect of storage conditions on protein profile of local snails (*C. obtusa*)?
2. What is the effect of chemical treatments on protein profile of local snails (*C. obtusa*)?
3. What is the effect of storage conditions on allergenicity of local snails (*C. obtusa*)?
4. What is the effect of chemical treatments on allergenicity of local snails (*C. obtusa*)?

1.6 Research Significant

Molluscan shellfish including snails play an important role in the world economy and human nutrition. The consumption of snail is a common cause of food hypersensitivity reactions. Snail allergy is diagnosed using food challenges as well as by skin prick tests (SPT). Snail extracts are indispensable for SPTs. However, storage condition is among the most important factor in preserving allergenicity of allergen extracts including snail allergens. So, this research will be a valuable addition to develop more effective diagnostic and therapeutic strategies for the management of patients with snail's allergy. Besides, the results of the effect of chemical treatments on the allergenicity of *C. obtusa* will be useful for both industries and consumers to identify an effective way to reduce snail allergenicity in food products. Both findings will provide useful information for researchers, clinicians, and the public about the effect of storage conditions and preservation methods on the allergenicity of local edible snails, which may be beneficial to develop a promising technology to reduce the allergenicity of this snail such as microarrays (Melioli, Riccio, Ledda, Passalacqua, & Canonica, 2017).

1.7 Research Limitations

This research is limited in determining the effect of storage conditions and chemical treatments on the allergenicity of one species of local marine snail (*C. obtusa*). The storage conditions tested were only at three temperatures of 25, 4, and -20 °C for durations of 24 hours, 3 days, one week, and one month in SDS-PAGE experiments.

However, only the untreated (0 days), 1 week and 1 month stored extracts were selected to study the effect of different storage condition in immunoblotting experiments. Meanwhile, for chemical treatments, three types of treatments were applied; acid hydrolysis by white vinegar (pH 2.4), dry salting and wet salting by sodium chloride (NaCl). The respondent is limited to only 36 snail-allergic patients which were confirmed by Specific IgE Immunocap test, skin prick tests (SPTs) and clinical history to snail allergens. Sera from these patients and five non-allergic respondents used in this study were collected from our previous study (Zailatul et al., 2015a). All 36 sera were used in immunoblotting of untreated extract, while for the treated and stored extracts, 20 selected sera were used. This is because of limitation of sera availability. Besides, molecular identifications of allergens involved in snail's allergy were only detected by SDS-PAGE and immunoblotting analysis.