UNIVERSITI
MALAYSIA
KELANTAN

Characterization of chitosan extract from Etok shell (*Corbicula Fluminea*)

Syazwin Atika Binti Hasrin
J20A0633

A reported submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Technology Bioindustry) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY
UMK

2024

DECLARATION

I declare that this thesis entitled "Characterization of chitosan extract from etok shells (*Corbicula Fluminea*)" is the results of my own research except as cited in the references.

Signature : 

Student's Name : Syazwin Atika binti Hasrin

Date : 8 February 2024

Verified by:

Signature : _____

Supervisor's Name : Dr. Zubaidah Aimi binti Abdul Hamid

Stamp : _____

Date : _____

ACKNOWLEDGEMENT

I am profoundly grateful to Allah SWT for the strength and ability granted to me, enabling the successful completion of my study project. I am delighted to acknowledge the assistance and valuable suggestions I received from those around me during the completion of this thesis.

My deepest and sincerest appreciation goes to my research supervisor, Dr. Zubaidah Aimi binti Abdul Hamid. Her guidance throughout this project has significantly enriched my knowledge and practical skills, particularly in lab work field. It was an honor and privilege to learn under her tutelage.

I extend my heartfelt gratitude to my parents, Hasrin bin Suyuti and Azilah binti Aris, for their unwavering love, prayers, and support, both mentally and physically. Their support was not limited to the duration of this thesis but extended throughout my entire academic journey. I also wish to thank my siblings for their support and cherished prayers.

I am deeply appreciative of the invaluable assistance provided by the laboratory assistants. Their support played a crucial role in the successful completion of this project. Their guidance, patience, and dedication significantly enhanced my understanding and experience. I am thankful for their steadfast support and encouragement.A special acknowledgement to myself for not giving up, for enduring the cries and stresses, knowing that the sweetness of accomplishment awaited at the end.

Lastly, ultimate thanks to everyone who helped me out directly or indirectly throughout the completion of this project. May this research benefit our society, Insha'Allah.

ABSTRACT

Chitosan is a linear polysaccharide derived from chitin by the deacetylation process, with a wide range of potential commercial and medicinal applications. In this study, chitosan was extracted from etok shell (Corbicula Fluminea) through the chemical extraction process. Deproteination, demineralization, depigmentation and deacetylation were involved in the extraction process and the purity of chitosan produced from was determined through its degree of deacetylation. Characterization of chitosan has been done using elemental analysis (CHNS) to determined the carbon, hydrogen, sulfur and oxygen and other analysis using Fourier Transform Infrared (FTIR), Differential scanning calorimetry (DSC), Thermogravimetric analysis (TGA), X-ray diffraction (XRD) and solubility test. The degree of deacetylation obtained from elemental analysis was found to be very low with 15.92%. While preliminary results of FTIR revealed that different functional groups of C-O, NH2 and amide group were observed in the chitosan extracted from etok shells. DSC showed endothermic peak (692°C to 726°C) and exothermic peak (718°C to 759°C). The TGA reveals the onset of the first phase was observed at approximately 83.75 °C with weight loss 6.86%, while the second phase commences around 571.25 °C with weight loss of 40.36%. The characteristics XRD showed very weak peaks in the spectra at 10.67° and 20.52° which suggest a low crystallinity and an amorphous nature of the product. Solubility tests indicate that chitosan is 99% soluble in acetic acid and 5.55% insoluble in distilled water.

Keywords: Chitosan, extraction, etok shells, characterization, degree deacetylation.

ABSTRAK

Kitosan ialah polisakarida linear yang diperoleh daripada kitin melalui proses deasetilasi, dengan pelbagai potensi aplikasi komersial dan perubatan. Dalam kajian ini, kitosan telah diekstrak daripada kulit etok (Corbicula Fluminea) melalui proses pengekstrakan kimia. Penyahproteinan, penyahmineralan, depigmentasi dan deasetilasi telah terlibat dalam proses pengekstrakan dan ketulenan kitosan yang dihasilkan akan ditentukan melalui tahap diasetilasinya. Pencirian kitosan telah dilakukan menggunakan analisis unsur (CHNS) untuk menentukan karbon, hidrogen, sulfur dan oksigen dan analisis lain iaitu, Spektoskopi Inframerah Transformasi Fourier (FTIR), pembezaan pengimbasan Kalorimetri (DSC), analisis thermogravimetri (TGA), pembelauan Sinar X (XRD) dan ujian keterlarutan. Tahap deasetilasi yang diperoleh daripada analisis unsur didapati sangat rendah iaitu 15.92%. Manakala keputusan awal FTIR mendedahkan bahawa kumpulan fungsi C-O, NH2 dan kumpulan amida yang berbeza diperhatikan dalam kitosan yang diekstrak daripada cengkerang etok. DSC menunjukkan puncak endotermik (692°C hingga 726°C) dan puncak eksotermik (718°C hingga 759°C). TGA menunjukkan permulaan fasa pertama diperhatikan pada kira-kira 83.75 °C dengan penurunan berat kitosan 6.86%, manakala fasa kedua bermula sekitar 571.25 °C dengan penurunan berat kitosan sebanyak 40.36%. Ciri-ciri XRD menunjukkan puncak yang sangat lemah dalam spektrum pada 10.67^{o} dan 20.52^{o} yang menunjukkan kehabluran yang rendah dan sifat amorfus produk. Ujian keterlarutan menunjukkan bahawa kitosan adalah 99% larut dalam asid asetik dan 5.55% tidak larut dalam air suling.

Kata kunci : Kitosan, pengekstrakan, cengkerang etok, pencirian, tahap diatelasi.

Table of Contents

DECLARATION ..... **i**

ACKNOWLEDGEMENT ..... **ii**

ABSTRACT ..... **iii**

ABSTRAK ..... **iv**

TABLE OF CONTENT ..... **v**

LIST OF TABLES ..... **viii**

LIST OF FIGURES ..... **ix**

LIST OF ABBREVIATIONS ..... **x**

LIST OF SYMBOLS ..... **xi**

CHAPTER 1 INTRODUCTION **1**

1.1 Background of study 1

1.2 Problem statement 3

1.3 Objectives 3

1.4 Scope of study 3

1.5 Significance of study 4

CHAPTER 2 LITERATURE REVIEW **5**

2.1 Background of chitosan 5

2.2 Background of etok (*Corbicula Fluminea*) 6

2.3 Chitosan extraction from etok shell 7

2.4 Characterization of chitosan extract 8

2.4.1 Elemental analysis 8

2.4.2 Fourier transform infrared spectroscopy (FTIR)..... 9
2.4.3 Differential scanning calorimetry (DSC)..... 11
2.4.4 Thermogravimetric analysis (TGA)..... 11
2.4.5 X-ray diffraction (XRD)…….. 12
2.4.6 Solubility test……... 12
2.5 Application of chitosan..... 13

CHAPTER 3 MATERIALS AND METHOD..... 14
3.1 Materials..... 14
3.2 Methods..... 14
3.2.1 Preparation of etok shell 14
3.2.2 Deproteination..... 14
3.2.3 Demineralization..... 14
3.2.4 Depigmentation..... 15
3.2.5 Deacetylation..... 15
3.3 Characterization method..... 15
3.3.1 Elemental Analysis..... 15
3.3.2 Fourier transform infrared spectroscopy (FTIR)..... 16
3.3.3 Differential scanning calorimetry (DSC)..... 16
3.3.4 Thermogravimetric analysis (TGA)..... 16
3.3.5 X-ray diffraction (XRD)…….. 17
3.3.6 Solubility test……... 17

CHAPTER 4 RESULTS AND DISCUSSION..... 18
4.1 Results……..... 18
4.1.1 Elemental Analysis..... 18
4.1.2 Fourier transform infrared spectroscopy (FTIR)..... 19

4.1.3 Differential scanning calorimetry (DSC)..... 21

4.1.4 Thermogravimetric analysis (TGA)..... 22

4.1.5 X-ray diffraction (XRD)…….. 23

4.1.6 Solubility test……... 24

CHAPTER 5 CONCLUSION AND RECOMMENDATION..... 26

5.1 Conclusion……... 26

5.2 Recommendation……. 27

REFERENCES..... 28

APPENDIX A..... 33

APPENDIX B..... 36

APPENDIX C..... 40

LIST OF TABLES

Table 2.1:	The degree of deacetylation comparison from various type of clam shell……	9
Table 2.2:	FTIR results from various clam…….	10
Table 4.1:	Elemental analysis of chitosan sample and its degree of deacetylation……...	18
Table 4.2:	FTIR spectral peaks of commercial chitosan and extracted chitosan from etok shells……...	19
Table 4.3:	Solubility of chitosan from different solvent, expressed in percentage……...	25

LIST OF FIGURES

Figure 2.1:	The chemical structure of chitosan……	5
Figure 2.2:	Complete life cycle of etok……	7
Figure 4.1:	FTIR spectra of commercial chitosan (a) and chitosan extracted from etok shells (b)..……..	20
Figure 4.2:	DSC thermogram of commercial chitosan and chitosan extracted from etok shells..……...…….....	22
Figure 4.3:	TGA analysis of commercial chitosan and chitosan extracted from etok shells.…….…….…….…….	23
Figure 4.4:	X-ray Diffraction spectra of chitosan extracted from etok shell…...	24

LIST OF ABBREVIATIONS

NaOH	Sodium hydroxide	1
NaOCl	Sodium hypochlorite	1
FTIR	Fourier Transform Infrared Spectroscopy	4
TGA	Thermogravimetric Analysis	4
DSC	Differential Scanning Calorimetry	4
XRD	X-ray Diffraction	4
HCl	Hydrochloric acid	7
DD	Degree of deacetylation	8
TS	Thermal Stability	11
TM	Melting Temperature	11
MW	Molecular weight	14

LIST OF SYMBOLS

%	Percentage	2
β	Beta	5
˚C	Degree Celsius	7
$C0_2$	Carbon Dioxide	8
cm	Centimetre	10
kg	Kilogram	14
g	Gram	14
C	Carbon	16
N	Nitrogen	16
θ	Tetra	17
rpm	Revolutions Per Minute	20
ml	Millilitre	20

CHAPTER 1

INTRODUCTION

1.1 Background of study

Chitosan is a linear polysaccharide that is derived from chitin through a process called deacetylation. Chitin is a structural component found in the crustacean's exoskeletons such as lobster, shrimp, and crab. The chemical structure of chitosan consists of repeating units of glucosamine, a derivated of glucose. Amino groups, denoted as NH_2, are vital in bestowing a positive charge and unique characteristics to chitosan. The molecular weight and the degree of deacetylation of chitosan molecules can differ, reflecting the level of acetyl group removal from chitin. The degree of deacetylation has a significant impact on the solubility and other attributes of chitosan. Typically, the protonation of amino groups in the chitosan structure improves its solubility in acidic solutions. (Ibrahim et al., 2015).

There are four steps that are involved in the extraction of chitosan namely deproteinization, demineralization, depigmentation (optional) and deacetylation.

Deproteinization is the process of separating the proteins, which is commonly accomplished by treating the shells with an alkaline solution such as sodium hydroxide or potassium hydroxide. The alkaline solution helps to break down and dissolve the proteins, however chitin remains insoluble.

The process is then continued by demineralization, which involves removing inorganic minerals from natural sources (for example, invertebrate exoskeletons). In this procedure, the shells were treated with hydrochloric acid to dissolve the minerals (e.g., calcium carbonate), leaving in a chitin structure. Depigmentation is an optional procedure that removes the chitin colour from the etok shell. This method employs a bleaching chemical, such as sodium hypochlorite (NaOCl) (Salsabila et al., 2022). Finally, deacetylation transforms chitin to chitosan by eliminating acetyl groups. Deacetylation is commonly accomplished by treating the chitin with an alkaline solution under controlled conditions, such as high temperature and

extended reaction times. In the deacetylation procedure, increased temperatures (105-110˚C) were employed in concentrated base solutions (40% to 50%) for enhanced conversion (Ahyat et al., 2017).

The versatility of chitosan, a biopolymer derived from crustaceans, has made it a popular commodity for a variety of industrial applications. It is anticipated that the chitosan market will witness substantial growth, with an estimated revenue of 26,272.32 million by 2028. The global chitosan market consists of biomedical & pharmaceutical, water treatment, food & beverage, cosmetics, and others. In 2021, the application of chitosan in water treatment emerged as the dominant sector within the chitosan market, and this trend is expected to persist. This can be attributed to the continuous expansion of water treatment operations globally, driven by the rising output of industries such as biomedical, cosmetic, and food and beverage. These sectors have witnessed significant growth, consequently creating a greater demand for water treatment solutions, thereby promoting the development of the chitosan market (GlobeNewswire, 2016).

The etok, also referred to as asian clams, is scientifically known as *Corbicula Fluminea*. These freshwater organisms are originally found in Eastern and Southern Asia. However, they pose a significant threat as a non-indigenous invasive species, as they tend to establish themselves in new habitats. This is primarily due to their quick growth rate, early attainment of sexual maturity, relatively short lifespan, remarkable reproductive capacity, and their affinity for areas influenced by human activities (McMahon, 2002). In general, etok is considered a hermaphrodite. (Sousa et al., 2008). Etok are normally found at the base of freshwater rivers in Malaysia, in the states of Kelantan and Terengganu. Besides river estuaries and deltas, it is also found near seacoasts. However, etok occurs more near paddy fields in rivers. Furthermore, the dry season, which occurs from March to August, has been reported to be the ideal season to harvest etok. Etok is a popular Malaysian cuisine, which is found only in the Kelantan region, therefore, it has been given the title of the "unique heritage food of Kelantan" (Nazri et al., 2021).

Chitosan extract from etok imparts it various properties that make it useful in different applications, such as its ability to form films, gels, and complexes with other substances. These properties have led to its wide use in industries such as food, agriculture, textiles, and biomedicine.

In this study, chitosan was extracted from etok shell and purity of chitosan were characterized by its physical and chemical properties using elemental analysis, FTIR, DSC, TGA, XRD and its solubility test. The aim is to discover a novel source of chitosan from etok shells that may be used in a variety of applications.

1.2 Problem statement

Previously, chitosan was discovered in the structural component of marine life in crustacean exoskeletons such as lobster, prawns, and crab. Thus, its potentially to observe the chitosan in the etok shells. Besides, the utilization of etok shells leads to the generation of waste.

However, there is a lack of research on the characterization of chitosan extracted from etok shells and further understanding of this chitosan is limited. Therefore, there is a constraint to get a better understanding of the chitosan properties from etok shell in order to explore new source of chitosan from etok shells that can be used in various applications such as antimicrobial films, medicine and food packaging and coatings for biofilm in food industries (Martău et al., 2019).

1.3 Objectives

This study aimed to investigate the potential of etok shells as a viable source of chitosan and sought to contribute to the field of polymer science and sustainable materials. The specific objectives of this study were stated below.

1. To extract the chitosan from etok shell.
2. To determine the degree of deacetylation of chitosan extracted from etok shell.
3. To characterize the chemical and physical properties of chitosan extracted from etok shell.

1.4 Scope of study

Etok are collect as the raw material in this project. This clam is chosen as the raw material as it is easy to be found in Kelantan and far cheaper than other sources such as shrimp and crab, plus its characteristic is suitable to be used as the source to chitosan extraction.

Analytical techniques are used for chitosan characterization which are degree of deacetylation is determined in this study to analyse the percentage composition of NH_2 group in chitosan. Next, to identify the functional groups in chitosan, Fourier Transform Infrared Spectroscopy (FTIR) will be used. Plus, Thermogravimetric analysis (TGA) to measure the physical changes of chitosan when placed under heat (Kandile et al., 2018). The heat properties of chitosan and its morphology were investigated using differential scanning calorimetry (DSC). Additionally, the crystalline nature of chitosan was determined by evaluating it through X-ray diffraction (XRD). Lastly, solubility test to understand its behavior in different solvents.

1.5 Significance of study

The result of this study will help in adding new information to be one of the sources for future knowledge especially for the novel source of chitosan from etok shells. Etok shells represent a specific source of chitosan that has not been extensively explored**.** The purpose of this study is to explore a new and potentially valuable source of chitosan from etok shells by determined the degree of deacetylation. Characterization could give further information about the properties of chitosan that has been extracted from etok shells. Through this study, the chitosan extracted from etok shells can then be compared to chitosan from other sources, which provides valuable information on the similarities, differences, and potential advantages of using etok shells. Researchers and industry can benefit from this comparative analysis in determining the best chitosan sources for their use.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of chitosan

Chitosan, a natural polysaccharide is an example of value-added biomaterial. In 1859, Rouget discovered a substance through the application of mild alkali to chitin. Nevertheless, it was not until 1950 that the chemical composition of this substance was finally ascertained (Hahn et al., 2020). Chitosan made up of both 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose as well as 2-amino-2-deoxy-β-D-glucopyranose (Ibrahim et al., 2015). Chitosan, a renewable natural polymer sourced from chitin, can be discovered in numerous invertebrate organisms. This biopolymer possesses non-toxic properties, as well as biocompatibility and biodegradability, rendering it an environmentally friendly material of high interest (Martău et al., 2019). Chitosan finds extensive applications in the realms of food, pharmaceuticals, and agriculture, owing to its remarkable biological attributes, including biodegradability within the human body and effective antibacterial properties (Kandile et al., 2018). Figure 2.1 illustrates the chitosan chemical structure.

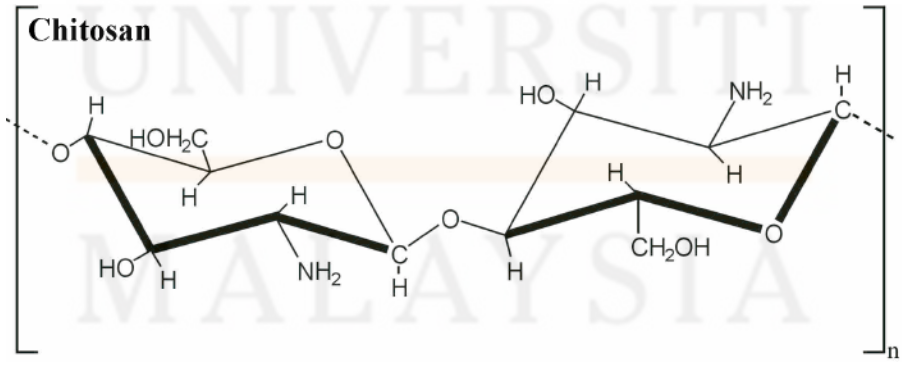


Figure 2.1: The chemical structure of chitosan.

(Source : (López-García et al., 2014)

2.2 Background of Etok (*Corbicula Fluminea*)

Etok refers to a species of freshwater clam known as *Corbicula fluminea*, which inhabits sandy substrates and can be commonly observed in rivers located within the geographical region of Malaysia (Han et al., 2020). Based on the previous study, etok comes from a mollusc species (Ramli et al., 2020) and is commonly consumed as a popular snack known as etak, in Kelantan, Malaysia . This asiatic clam demonstrates an accelerated rate of growth and reaches sexual maturity quickly, coupled with a significant reproductive capacity and extensive dispersal capabilities (Nazri et al., 2021). The life cycle of etok, which comprises four distinct phases. The initial phase, referred to as the inner demibranch with larvae, represents the protective period when the larvae is released into water. In the subsequent phase, etok juveniles exhibit small dimensions, typically around 250 meters. However, they possess fully developed characteristics, including a adductor muscles, well-developed shell, gills, foot, statocyst, and digestive system, as well as the characteristic D-shaped morphology. Phase three of the life cycle is marked by the occurrence of small adults before the specimens reach full adulthood. The proliferation of this particular species yields advantageous outcomes for the ecosystems it inhabits, as it offers diverse organisms a means of shelter and support. For instance, the vacant shells provided by this species serve as suitable substrates or protective havens for gastropods, algae and freshwater organisms. (Sousa et al., 2008). According on the previous study from Ismail et al., (2014) etok be the best option for eliminating Cd ions from contaminated waters. The growth of this species give positive effects to the ecosystems in which this species can provide habitats to other organisms. For instance, empty shells provide substrate or refuge for algae, freshwater and gastropods (Sousa et al., 2008). Figure 2.2 illustrates the complete life cycle of etok.

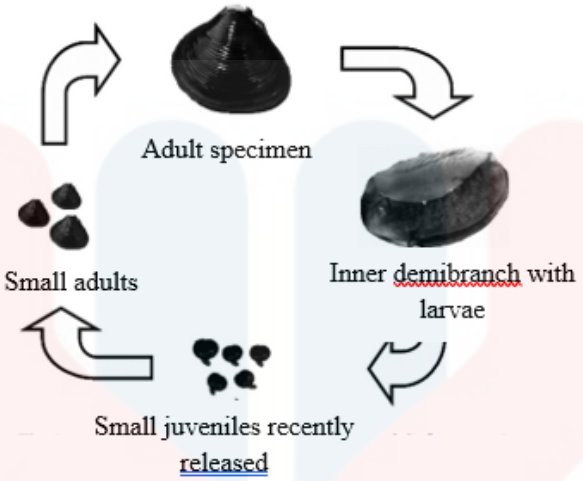


Figure 2.2: Complete life cycle of *etok*

(Source: Sousa et al., 2008)

2.3 Chitosan extraction from etok shell

The extraction of chitosan can be carried out chemically through several major steps including deproteination, demineralization, depigmentation, and deacetylation. The process of deproteination is usually used to reduce the protein content of shells. In this process, a chemical reagent such as a strong alkaline solution, specifically Sodium hydroxide (NaOH) at concentrations ranging from 3 to 6 N. Sodium hydroxide (NaOH) at a ratio of 3:1 (mL NaOH/g shell) is used to break the protein bonds after being heated at 80º C degrees Celsius for 30 minutes. The alkaline solution facilitates the dissolution of proteins contained within the shells, resulting in Na-proteinate. After that, the solution was filtered and neutralized, then dried in an oven at 60º C. Sodium hydroxide is typically preferred over alternative solutions like potassium carbonate or potassium hydroxide due to its lower cost. The effectiveness of the deproteinization process can be observed through the reduction of protein content. This is indicated by a significant decrease in the colour intensity in the solution. This can be observed where a colourless filtrate can be seen and indicates the successful removal of proteins from the shell. To eliminate the presence of inorganic salts or mineral content, a process called demineralization is employed on the sample, specifically using a strong acid such as hydrochloric acid (HCl). This method is designed to eliminate mineral salts, like calcium carbonate (CaCO3), from the crushed shells. Past research has employed a 3:1 ratio (mL HCl/g shell) with a 1.25 N HCl concentration. The demineralization procedure entails exposing the sample to a temperature of 75°C for an hour. To expedite the mineral destruction process, it is

necessary to heat the shell powder in the presence of HCl at a specific temperature. Stirring is crucial during the heating process to prevent the overflow of CO_2 (carbon dioxide) gas. The demineralization process is discernible by the formation of CO_2 gas bubbles, which serve as an indicator of the reaction between HCl, and mineral salts present in the shell. Once the reaction is identified, the solution can be filtered and subsequently neutralized and dried in an oven at 60°C until all moisture is removed. After the demineralization process, chitin will be produced, and the color or pigment of chitin will be removed through process depigmentation by soaking the chitin powder using 4% sodium hypochlorite with a ratio of 2:3 w/v, then heated for 1 hour while stirring every 10 minutes. Using the Knorr method, chitin is deacetylated with a 50% NaOH solution using a ratio of 20:1 (mL NaOH/g chitin). Chitin undergoes deacetylation, losing the acetyl group (CH3-CO), which converts it into chitosan with a free amine group (-NH). Previous studies reveal that squid gladius have the highest chitin content of 31.2%, followed by shrimp shells ranging from 26.98% to 36.43%. Meanwhile, mussel shells which from clam species contain only 16.73% to 23.25% chitin content (Iber et al., 2022). 50% NaOH is used to achieve high purity in chitosan production as it results in the highest degree of deacetylation. The process involved stirring and heating the mixture within a temperature range of 90-100°C for a duration of 60 minutes. Subsequently, the resulting residue was neutralized after cooling and subjected to drying at a temperature of 60°C. (Salsabila et al., 2022; Wulandari et al., 2020). In a previous study conducted by Salsabila et al. (2022), the extraction of chitosan from blood clam shells was determined. The results indicated that the chitin powder obtained after the demineralization process exhibited a grayish-white color, with a dense and solid texture. The degree of deacetylation of chitosan from blood cockle shell is 63.18%. Furthermore, the subsequent deproteination and depigmentation processes yielded a pure white powder, which demonstrated ease of drying.

2.4 Characterization of chitosan extract

2.4.1 Elemental analysis

The production of chitosan is largely dependent on the degree of deacetylation (DD), which significantly influences the physical and chemical properties of the material. Depending on its source and preparation method, the DD of chitosan typically varies between 40% and 98%. (Wulandari et al., 2020). Several techniques have been employed by researchers to determine the degree of deacetylation (DD) of chitosan. One commonly used method is the

elemental composition of chitosan that can be initially determined using a Carlo–Erba 1108 Elemental Analyzer. This analysis provides quantitative data on the carbon, hydrogen, and nitrogen content of the sample. The DD can then be calculated based on the carbon-to-nitrogen ratio (C/N) using the following formula in equation 2.1 (Kasaai et al., 2000).

$$\text{Degree of deacetylation: } \frac{\frac{C}{N} - 5.145}{6.861 - 5.145} \times 100\% \qquad \text{Equation. 2.1}$$

Previous research studies have shown that donacid clam (*Donex scortum*) provided a higher DD of 74.35%. In addition, the deacetylation rate of blue mussel shells was 69.60%, exceeding the 49% value observed for green mussel shells. Also, horse mussel shell and blood clams had DD values of 57.43% and 63.18%, respectively. These results indicate that the chitosan obtained from these sources has a deacetylation degree higher than 40%, making it suitable for various applications. Table 2.1 shows that the comparison between result the degree of deacetylation from various clam shells.

Table 2.1: The degree of deacetylation comparison from various type of clam shell.

Raw material	Degree of deacetylation (%)	Source
Donacid clam (*Donex Scortum*)	74.35%	(Shanmugam et al., 2012)
Blue Mussel (*M. Edulis*)	69.60%	(Varma et al., 2020)
Green Mussels Shell (*Perna Viridis L*)	49%	(Wulandari et al., 2020)
Horse Mussel (*Modiolus modiolus*)	57.43%	(Varma et al., 2020)
Blood clamshell (*Anadara granosa*)	63.18%	(Ma'ruf et al., 2022)
Mussel shell	60.69%	(Abdulkarim et al., 2013)

2.4.2 Fourier transform infrared spectroscopy (FTIR)

The determination of chitosan functional groups is accomplished through the application of Fourier transform infrared spectroscopy (FTIR). In accordance with a prior investigation, the infrared absorption spectrum was documented within the range of 4000-400 cm^{-1} (Ma'ruf et al., 2022). Based on the previous study results, a comparative analysis of FTIR

results of chitosan from various clam revealed discrepancies in the identification and peak intensities of certain functional groups. The Fourier Transform Infrared (FTIR) analysis of chitosan derived from the blood cockle shell shows an absorption peak at 872 cm-1, which is attributed to the stretching vibrations of the N-H bonds. Additionally, a range of 1600 – 600 cm-1 indicates the carboxyl bond (O-C) associated with the presence of calcium carbonate (CaCO3). Another significant absorption band was noted at 1419.61 cm-1, which corresponds to the stretching vibrations of the C-H bonds, and 3356.14 cm-1, which is a typical absorption of N-H vibration of chitosan. These observations strongly imply the existence of these functional groups in the chitosan structure. FTIR analysis of various shell mussel species, particularly green mussel shells, revealed the NH functional group at specific bending frequencies. For *Perna Viridis*, it was found at 3641.60 cm^{-1} and 712.50 cm^{-1}, for *Mytillus Virdis Linnaeaus* at 3445.89 cm^{-1}, and for Horse Mussel shells at 711 cm^{-1}. Next, chitosan extracted from *Perna Viridis* green mussel shells exhibited C=O stretching vibrations associated with the amide I group. Similar peaks were observed at 1784.15 cm^{-1} and 1798.40 cm^{-1}, indicating structural similarities in chitosan. Meanwhile, the bending 1082,73 cm^{-1} was assigned to CH_2 bending and C–O stretching vibration in alcohol. Chitosan from Clam *Coelomactra antiquata* representing the stretching of O-H groups (3458 cm^{-1}), as well as the stretching of C-H (2987 cm^{-1}) in the aliphatic hydrocarbon side chain, respectively Table 2.2 show the FTIR result comparation between functional group and plane bending from various clamshell.

Table 2.2: Observation the functional group of various clam using FTIR .

Raw material	Functional group	Bending (cm^{-1})	Source
Blood clamshell (*Anadara granosa*)	N-H O-C	872 1600 - 600	(Siswoyo et al., 2023)
Blood clamshell (*Anadara granosa*)	C-H N-H	1419.61 3356.14	(Ma'ruf et al., 2022)
Green Mussels Shell (*Perna Viridis L*)	NH C=O	3641.60 1784.15	(Wulandari et al., 2020)
Green Mussels Shell (*Perna Viridis*)	C=O C-O NH	1798.40 1082.73 712.50	(Kaewprachu et al., 2023)
Green Mussels Shell (Mytilus virdis linneaus)	N-H O-H C-H	3445.89 2521.97 2927.03 – 1466.89	(Sinardi et al., 2013)
Horse Mussel (*Modiolus modiolus*)	NH C-H	711 1174	(Varma et al., 2020)

	C-N	863	
Clam Coelomactra antiquata	O-H C-H	3458 2987	(Chen et al., 2022)

2.4.3 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a widely used technique for analysing the heat flow in response to temperature changes over time. It is particularly useful in assessing the thermal properties of chitosan, such as its thermal stability (TS) and melting temperature (TM). The thermal stability of chitosan can be characterized by endothermic (water loss) or exothermic (dehydration and decomposition of monomers) peaks. Previous research has demonstrated that chitosan derived from shrimp shells exhibits an endothermic peak within the temperature range of 77.78 to 80.44°C, while the exothermic peak occurs between 303.77 and 304.28°C. The findings, illustrated in Figure 2.4, highlight the DSC thermogram of chitosan extracted from shrimp shells. In its solid form, chitosan possesses a disordered structure and a strong affinity for water, which makes it easily susceptible to drying processes (Dey et al., 2016). Furthermore, Differential Scanning Calorimetry (DSC) can determine the melting point of chitosan, which signifies the temperature at which chitosan experiences a change from a solid form to a liquid state. Previous research has indicated that chitosan derived from crab shells exhibits an endothermic peak in the range of 152.3 to 159.2 degrees Celsius. In contrast, chitosan obtained from *Donacid Clam* displays an endothermic peak at approximately 151.6°C (Shanmugam et al., 2012). This analytical technique allows for the precise identification and characterization of chitosan's melting behavior, aiding in its practical applications.

2.4.4 Thermogravimetric analysis (TGA)

The thermogravimetric analysis (TGA) technique was employed to investigate the thermal degradation characteristics of chitosan. Due to the interaction of amino groups, the chitosan degradation process begins with the formation of unsaturated structures. The breakdown of polysaccharides during pyrolysis causes the glycosidic linkages to randomly split, releasing acetic and butyric acids as well as various kinds of lower fatty acids such C2, C3, and C6 (Dey et al., 2016). According to studies on the thermogravimetric analysis of chitosan, the degradation of chitosan in a nitrogen atmosphere occurs with only one reaction, and as the heating rate increases, the degradation temperature of chitosan also increases (Hong et al., 2007). Previous study by using flower crabs (*Portunus pelagicus*), the thermogravimetric analysis revealed a two-stage degradation pattern for chitosan. During the initial phase of

deterioration, which took place within the temperature interval spanning from 30°C to 123°C, a reduction in weight amounting to approximately 12% was observed. The decrease in weight can be ascribed to the removal of water molecules, given that chitosan structures have a high tendency to attract water and undergo dehydration in this phase. Following this, the second phase of chitosan degradation was noted between 148°C and 392°C, leading to a weight reduction of approximately 48%. This significant weight loss indicates further decomposition and degradation of the chitosan structure (Ahyat et al., 2017). In the case of chitosan derived from shrimp shells, a notable fraction of the total weight loss, approximately 10%, can be attributed to the evaporation of moisture occurring within the temperature range of 22 to 100 °C during the initial phase. Subsequently, when chitosan is subjected to a nitrogen atmosphere, it undergoes a non-oxidative thermal degradation process in the temperature range of 248 to 600 °C. This phenomenon can be attributed to the deacetylation of chitosan, as well as the elimination of byproducts and the evaporation of volatile substances (Dey et al., 2016).

2.4.5 X-ray Diffraction (XRD)

The examination of the crystalline structure of extracted chitosan is facilitated through the utilization of X-ray diffraction (XRD) analysis. This non-destructive technique serves as a dependable methodology for ascertaining the existence of crystalline phases within the material, thereby imparting significant elucidation on its chemical composition (Azahar et al., 2021). By conducting a comparative analysis of the acquired data with well-established reference databases, the identification of these crystalline phases is effectively achieved. Previous investigations have reported that the XRD pattern of chitosan derived from chiton exhibited discernible peaks at 2θ values ranging from 30º to 35º, with a count rate of 625 (Rasti et al., 2017). Likewise, the chitosan derived from the horse mussel *M. modiolus* showed a significant peak at an angle of 20.04º, with a count rate of 113.92. Furthermore, the more pronounced peaks seen at 20.92 º for the chitosan procured in this study strongly suggest a more densely packed and compact crystalline structure. (Varma et al., 2020).

2.4.6 Solubility Test

Solubility is an important property of chitosan, for its potential applications in various fields. Chitosan, made from chitin, gets soluble in acidic water because it has lots of protonated -NH2 groups (Kou et al., 2021). Testing the solubility of chitosan is necessary to determine its

suitability for different applications, such as biomedical, pharmaceutical, agricultural, and environmental fields (Aranaz et al., 2021). The dissolution of chitosan in aqueous solutions is facilitated by acidic solvents. This method involves the addition of acids like acetic acid, formic acid, or citric acid to water, thereby reducing the solution's pH. This procedure leads to the protonation of the amino groups in chitosan, which facilitates its solubility. The degree to which chitosan dissolves depends on various factors, including the concentration and acid used. Additionally, aspects like the molecular weight and the degree of deacetylation of the chitosan molecule greatly affect its solubility in these acidic conditions (Sakai et al., 2001; Yusharani et al., 2019). Previous study, the solubility assessment of chitosan derived from Green Mussel shells demonstrated distinct solubility values. Specifically, chitosan extracted from smaller shells exhibited a solubility of 81.30%, while its counterpart sourced from larger shells displayed a notably higher solubility of 90.00%. Additionally, chitosan derived from alternative sources, namely shrimp shells, exhibited a solubility of 70%, while that from crab shells displayed a slightly lower solubility at 60%. (Kaewprachu et al., 2023). These findings indicate variations in chitosan solubility based on the shell source, with Green Mussel shells yielding chitosan with higher solubility compared to shrimp and crab shells.

2.5 Application of chitosan extract

There are several qualities that make chitosan an industrially significant material, it is water insoluble, it behaves like a cationic biopolymer, and it forms gel easily. This makes it appealing for medicine, food, and agricultural uses. Recent years, the use of chitosan in water treatment industry has dominate the chitosan market. It is because the composition that environmentally friendly by removing toxic metal. Next, it also used on active pharmaceutical ingredients which serve as medication delivery systems. Due to positively charged amino groups on its surface, it is widely used as an antimicrobial agent (Santos et al., 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Etok shells was obtained from a stall in Pasir Mas, Kelantan. Hydrochloric acid (HCl) (CAS number 7647-01-0; MW of 36.46 g/mol), Sodium hydroxide (NaOH) (CAS number 215-185-5; MW of 40 g/mol) and Sodium hypochlorite (NaOCl) (CAS number 7681-52-9; MW of 74.44 g/mol) were used in this study.

3.2 Methods

3.2.1 Preparation of etok shell

1 kg of etok was obtained at "Gerai Etak" Pasir Mas, Kelantan. Etok meat was removed, and the shell was dried under the sun for 3 days. Then, etok shells was crushed using a mortar and pestle before ground into fine powder to pass through 0.3 mm sieve.

3.2.2 Deproteination

Etok powder (300 g) was mixed with 3% NaOH with a ratio of 1:3. Then, the mixture was constantly stirred at a temperature of 80°C for 2 hours. Next, the mixture was washed using distilled water under stirring condition and change the water every 1 hour until it reaches pH 7. Neutral mixture was filtered and dried in an oven for 24 hours at a temperature of 40° C.

3.2.3 Demineralization

The dried deproteinized of etok powder (184 g) was mixed with 1.25 N hydrochloric acid at a ratio of 1:3 to produce chitin. Then, the chitin was stirred at a temperature of 75°C for 2 hours followed by washed using distilled water and change the water every 1 hour until it

reaches Ph 7. Neutral mixture was filtered and dried in an oven for 24 hours at a temperature of 40° C.

3.2.4 Depigmentation

141 g of chitin powder that obtained from demineralization process was mixed with the 4% sodium hypochlorite with the ratio 1:5 and stirred for 6 hours. Next, the chitin mixture was filtered and washed using distilled water and change the water every 1 hour until it reaches Ph 7. Neutral mixture was filtered and dried in an oven for 24 hours at a temperature of 40° C.

3.2.5 Deacetylation

The deacetylation process was conducted by adding 50 g chitin powder into a 60% NaOH solution with a weight-to-volume ratio of 1:10. Next, the samples was heated at 100°C for 5 hours followed by washed using distilled water and change the water every 1 hour until it reaches Ph 7. Neutral mixture was filtered and dried in an oven for 24 hours at a temperature of 50° C.

3.3 Characterization Methods

Chitosan obtained prof above extraction process was characterized the chemical and physiochemical properties.

3.3.1 Elemental analysis

The CH-NS Elemental Analyzer (CHNS Microanalysis / EA1112 CHNS - O Analyzer) was employed to determine the degree of deacetylation (DD) in chitosan samples. The degree of deacetylation of a chitosan sample was computed using equation 3.1 (Kasaai et al., 2000.)

$$\text{Degree of deacetylation: } \frac{\frac{C}{N} - 5.145}{6.816 - 5.145} \times 100\% \qquad \text{Equation 3.1}$$

The peak area of the carbon to nitrogen ratio (C/N) is determined by measuring the elemental composition of the chitosan samples.

3.3.2 Fourier transform infrared spectroscopy (FTIR)

The functional group of chitosan extracted from etok shells was evaluated using a Thermo Scientific Nicolet iZ10 FT-IR spectrometer. A sample of 0.5 g chitosan powder was meticulously positioned in the sample holder, and its FTIR infrared absorption spectrum was documented within the wavenumber range of 4000-400 cm-1 at a resolution of 4 cm-1 (Ahyat et al., 2017).

3.3.3 Differential Scanning Calorimetry (DSC)

The chitosan sample, weighing between 25 mg and 30 mg, underwent controlled heating from 35 to 1000°C with heating rate 15°C/min. The heating process occurred in a nitrogen atmosphere with a flow rate of 20 ml/min.

3.3.4 Thermogravimetric analysis (TGA)

Thermogravimetric measurements were conducted using the TA-Instrument SDT-Q600 system. The chitosan sample, was weighed between 25 mg and 30 mg, underwent controlled heating from 35 to 1000°C with heating rate 15°C/min. The heating process occurred in a nitrogen atmosphere with a flow rate of 20 ml/min, ensuring an inert environment for accurately evaluating the sample's thermal behavior and weight changes across the specified temperature range. Analysis of the recorded data provided valuable insights into the chitosan sample's thermal stability and decomposition characteristics.

3.3.5 X-ray diffraction (XRD)

XRD analysis was performed on the 8 g of chitosan samples using a Siemens D500 equipment, which uses Cu-k-alpha X-rays at 40 kV and 25 mA. The specimens, which measured 1515 mm2, were carefully placed for scanning and examination. XRD analysis offers extensive information about the crystal structure and composition of the samples, assisting in the characterization and comprehension of the materials under study.

3.3.6 Solubility test

A 0.1 g sample of chitosan was placed in a centrifuge tube and dissolved in a solution of 10 ml of 40% acetic acid and distilled water. This was done for 1 hour in an incubator shaker

set at a temperature of 40°C and a speed of 150 rpm. Following this, the sample was subjected to a water bath for 10 minutes at 27°C and then centrifuged at a speed of 6000 rpm for 20 minutes. The supernatant was then carefully discarded, leaving only the pellet behind. The tube was subsequently dried in an oven set at 60°C for a period of two days. After drying, the tube was weighed to calculate the solubility of chitosan using equation 3.2v(Alabaraoye et al., 2018).

$$\text{Solubility (\%)}: \frac{\text{Wi}-\text{Wf}}{\text{Wi}-\text{Wo}} \times 100\% \qquad \text{Equation 3.2}$$

Where;

Wi = Initial weight of the tube with added chitosan
Wf = Final weight of tube after drying
Wo = Initial weight of the tube

CHAPTER 4

RESULT AND DISCUSSION

4.1 Results

4.1.1 Elemental analysis

Elemental analysis plays a crucial role in quantifying the degree of deacetylation of chitosan, which is essential for understanding its physicochemical properties of chitosan. According to Table 4.1, the weight percent of carbon (13.18%) and nitrogen (2.011%) was used to calculate the degree of deacetylation using Equation 2.1. In contrast to the literature, which reports a degree of deacetylation of 60% for chitosan derived from mussel shells (Abdulkarim et al., 2013), chitosan extracted from etok shells indicates a significantly lower DD of 15.92%. The degree of deacetylation (DD) value is influenced by various factors, including the origin of the sample, the sample preparation method, and the type of analytical instruments used (Amor et al., 2023). The low DD in this study may be attributed to the inefficient extraction process as indicated by the presence of calcium carbonate ($CaCO_3$) in the FTIR results at a wavenumber 712.59 – 700.04, suggests that the extraction process may not have been fully efficient. Calcium carbonate is typically removed during the demineralization step of chitin extraction (Salsabila et al., 2022), and its presence can imply incomplete removal of minerals from the chitin can interfere with the deacetylation process, potentially leading to a lower DD in the final chitosan product (Amor et al., 2023; Mathaba et al., 2020).

Table 4.1: Elemental analysis of chitosan sample and its degree of deacetylation.

Name of sample	Percent of elements (%)			Degree of deacetylation (%)
	N	C	H	
Chitosan	2.01	13.18	0.06	15.92

4.1.2 Fourier Transfom Infrared Specstroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy is a characterization method used to identify the functional groups of chitosan within the frequency range of 4000 to 400 cm^{-1}. The FTIR spectra of chitosan, derived from etok shells, were analyzed and compared to the commercial chitosan. Figure 4.2 showed the spectrum of commercial chitosan displays peaks at 3289.39 cm^{-1} and 2871.57 cm^{-1}, representing the stretching of O-H and N-H groups, as well as the stretching of C-H in the aliphatic hydrocarbon side chain, respectively (Chen et al., 2022).

Table 4.2: FTIR spectral peaks of commercial chitosan and extracted chitosan from etok shells.

Wavenumber (cm^{-1})		Functional group
Commercial chitosan	**Extracted chitosan from etok shells**	
3289.39	-	O-H and N-H stretching
2871.57	-	Aliphatic C-H strecthing
-	2522.31	O-H stretching vibration
-	1791.21	C=O stretching
1646.91	1647.00	NH primary amine
1457.03	1446.41	CH_2 bending
1059.36	1082.95	C-O stretching
893.00	875.49	NH_2 vibrations
-	854.44	C-N stretching
-	712.59 – 700.04	Carboxyl bond
552.14 - 517.76	526.19	CO out of plane bending

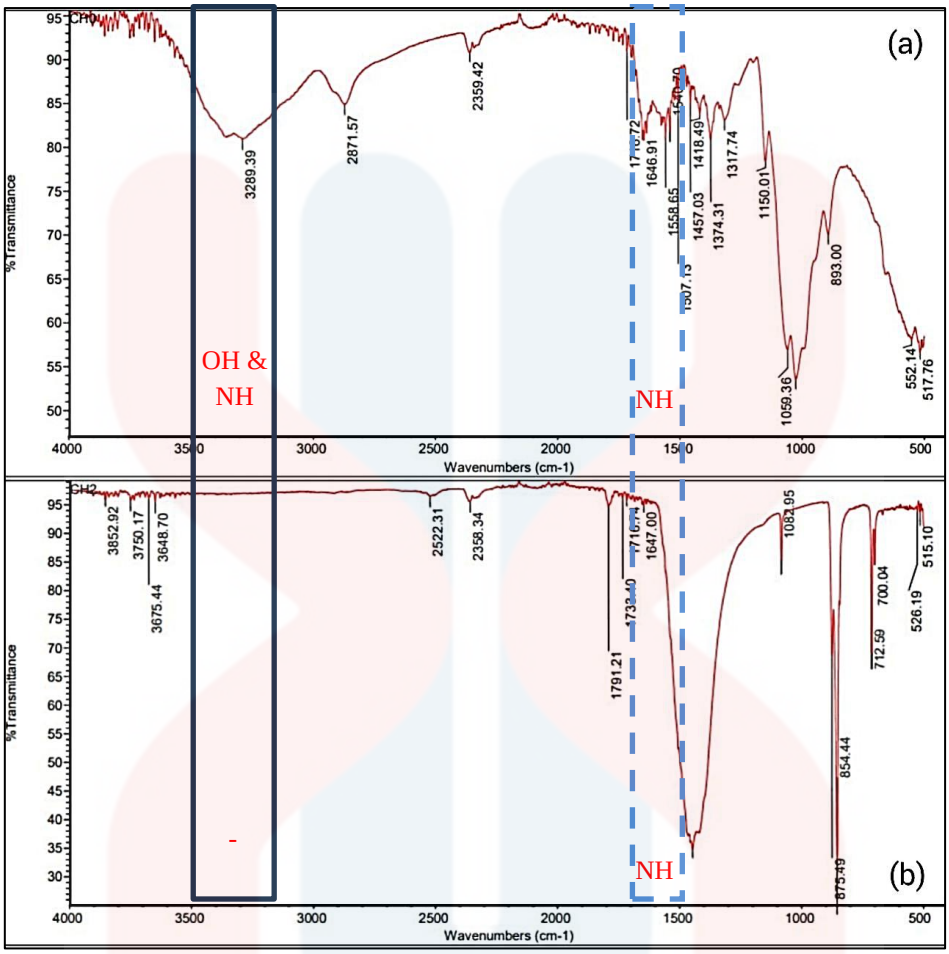


Figure 4.1: FTIR Spectra of commercial chitosan (a) and chitosan extracted from Etok shells (b).

The absence of prominent peaks within the broadband range of 3000 cm^{-1} to 3500 cm^{-1} in the FTIR spectrum of chitosan extracted from etok shells implies potential inadequacies in the extraction process. This absence may indicate incomplete removal of proteins or other impurities (Islam et al., 2022). The observed peaks at 1646.91 cm^{-1} for commercial chitosan and 1647.00 cm^{-1} for chitosan extracted from etok shells signify the presence of NH primary amine bends. These peaks are reminiscent of the 1638 cm^{-1} peak observed in *S. pharaonis*, which is associated with the identification of primary amines (Achsan, 2021; Varma et al., 2020). The wavenumbers of 1457.03 cm^{-1} and 1059.36 cm^{-1} for commercial chitosan, 1446.41 cm^{-1} and 1082.95 cm^{-1} for extracted chitosan from etok shells respectively, was assigned to CH_2 bending and C–O stretching vibration in alcohol, respectively (Kaewprachu et al., 2023). The small and thin peak observed at the wavenumber 875.49 cm^{-1} in the extracted chitosan from etok shells is the typical absorption of NH_2 vibrations of chitosan (Siswoyo et al., 2023). In the spectrum, a noticeable peak is seen at 854.44 cm^{-1} in the extracted chitosan from etok shells, which is linked to the C−N bond's stretching (Varma et al., 2020). Moreover, other peaks

ranging from 712.59 cm^{-1} to 700.04 cm^{-1} are associated with the carboxyl bond (O-C) indicates the presence of calcium carbonate ($CaCO_3$) (Siswoyo et al., 2023). Furthermore, FTIR spectra of the extracted chitosan from etok shells were similar to those of chitosan from green mussel, horse mussel and blood cockle shell. Previous study by Sinardi et al.,2013 indicate that the FTIR spectrum of the chitosan extracted from green mussel shells showed almost similar trend that, revealed at 2522.31 cm^{-1} representing the O-H stretching vibration. In the obtained spectra, the presence of peaks at 1791.21 cm^{-1} of extracted chitosan from etok shells indicates the C=O stretching vibration associated with the amide I group. This observation mirrors findings from chitosan extracted from green mussel shells, where similar peaks were identified at 1798.40 cm^{-1} and 1797.57 cm^{-1}, indicating analogous structural characteristics in chitosan (Kaewprachu et al., 2023).

4.1.3 Differential Scanning Calorimetry (DSC)

The DSC thermogram of commercial chitosan and chitosan extracted from etok shells were shown in figure 4.2. Chitosan's thermal behavior involves both exothermic and endothermic processes, which are crucial for understanding its applications in various fields. In exothermic reactions, heat is released, usually due to stronger bond formation or energy release. On the other hand, endothermic reactions absorb heat, often breaking weaker bonds or changing solids to liquids or gases (McMurrv et al., 2024). The commercial chitosan shows the exothermic peak at 318 °C with onset temperature 292 °C. The thermal analysis of chitosan extracted from etok shells revealed an initial endothermic behaviour spanning from 692°C to 726°C. The onset of this endothermic peak corresponds to the pressure buildup resulting from water evaporation. In the solid state, the disordered structures of polysaccharides exhibit a pronounced affinity for water, facilitating easy hydration (Muley et al., 2018). Subsequently, an exothermic peak emerged within the temperature range of 718°C to 759°C. Notably, the chitosan sample exhibited consistent heat flow, followed by the appearance of an exothermic peak at 732°C. This exothermic peak signifies the thermal decomposition process of chitosan, encompassing depolymerization, saccharide ring dehydration, and the degradation of both deacetylated and acetylated chitosan units (Acosta-Ferreira et al., 2020).

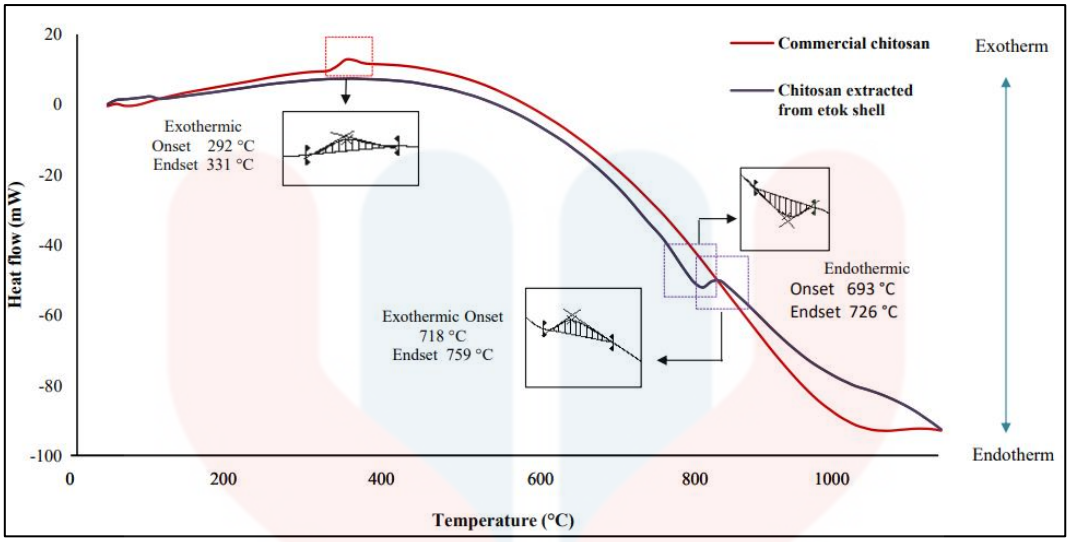


Figure 4.2: DSC thermogram of commercial chitosan and chitosan extracted from etok shells.

4.1.4 Thermogravimetric analysis (TGA)

TGA analysis was conducted to examine the thermal stability of chitosan extracted from etok shells. Figure 4.3 presents the TGA curves of the extracted chitosan in comparison with a commercial chitosan sample, heated at a rate of 15 K/min. The commercial chitosan exhibits the initiation of the first phase at a lower temperature of 45 °C with weight loss 7.4%, and the second phase begins at 278.75 °C with weight loss of 45.40%. In contrast, the TGA curves of chitosan extracted from etok shell distinctly show a slight initial weight gain at the beginning from a temperature of 35 °C up to 83.75 °C. The observed increase in weight can be attribute to the residual moisture present in the sample. This is due to the sample's interaction with atmospheric air during the weighing process, which could lead to moisture absorption, thereby causing the sample to become moist. The thermal degradation of chitosan occurs in two distinct phases. The chitosan extracted from etok shells, the onset of the first phase is observed at approximately 83.75 °C with weight loss 6.86%, while the second phase commences around 571.25 °C with weight loss of 40.36%. The first phase degradation could be due to the loss of water molecules, as chitosan structures have a strong attraction to water, leading to dehydration (Ahyat et al., 2017). The second stage of degradation is attributed to the thermal decomposition of the primary chitosan chain, which involves the vaporization and elimination of volatile products (Bashandy et al., 2017). The total weight loss of the chitosan extracted from etok shell at about 932 °C is 47.21%. The 52.79% remaining residue observed in chitosan extracted from etok shells primarily results from the formation of inorganic complexes, including minerals such as calcium carbonate ($CaCO_3$). These impurities do not undergo complete decomposition and persist as ash.

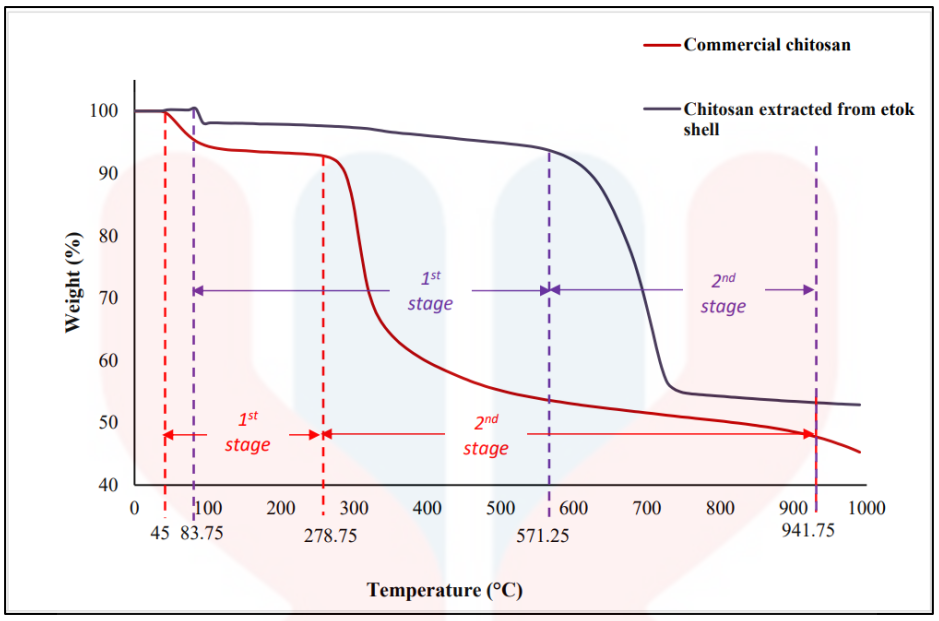


Figure 4.3: TGA analysis of commercial chitosan and chitosan extracted from etok shells.

4.1.5 X-ray diffraction (XRD)

The crystalline structure of chitosan extracted from the etok shells were analyzed by X-ray diffraction and is shown in Figure 4.4. The characteristics peaks of chitosan usually present only two XRD sharp peaks at approximately 9° - 11° and 19° - 21° around 2θ value (Ibitoye et al., 2018). According to studies in the literature, pure chitosan showed very broad peaks at 2θ values 10° and 20° (Ramya et al., 2012). However, these two peaks at 2θ values 10.67° and 20.52° corresponding to count indexes (113) and (56) respectively are very weak in the spectra of chitosan extracted from etok shells, which suggest a low crystallinity and an amorphous nature of the product (Badawy et al., 2019). In previous studies, the weak reflection peak at 26° in X-ray diffraction (XRD) patterns has been commonly used as a reference peak for α-chitin (Ahyat et al., 2017). This peak was observed in the chitosan derived from *P. pelagicus.* In contrast, a sharp peak with high count indexes at 26.17° (520) was observed in the chitosan extracted from etok shells. This observation suggests that the chitosan may retain some characteristics of the original chitin structure. The high reflection at the 26° peak implies that the chitosan sample might not be fully deacetylated, indicating the presence of some N-acetyl-D-glucosamine units from the original chitin. This could be correlated with the low degree of deacetylation (15.92%), which influences the XRD pattern of chitosan. Next, a pronounced peak was observed at 29.34° with a high-count index of 571, indicating the successful synthesis of chitosan. This result is consistent with findings from previous studies on chitosan extracted

from Scylla olivacea, where analogous peaks were identified at 2θ = 29.3398°. This evidence strongly suggests that the conversion of chitin to chitosan has been successfully achieved (Yanti et al., 2021). On the other hand, a peak found at 33º - 37º indicates the presence of hydroxyapatite materials that is commonly found in chitosan extracted from crustaceans (da Silva Lucas et al., 2021; Kaewprachu et al., 2023) The diffraction patterns of chitosan extracted from etok shells were different from pure chitosan and chitosan extracted from P. pelagicus and green mussel shells due to the presence of some minerals and a difference in the origin starting material.

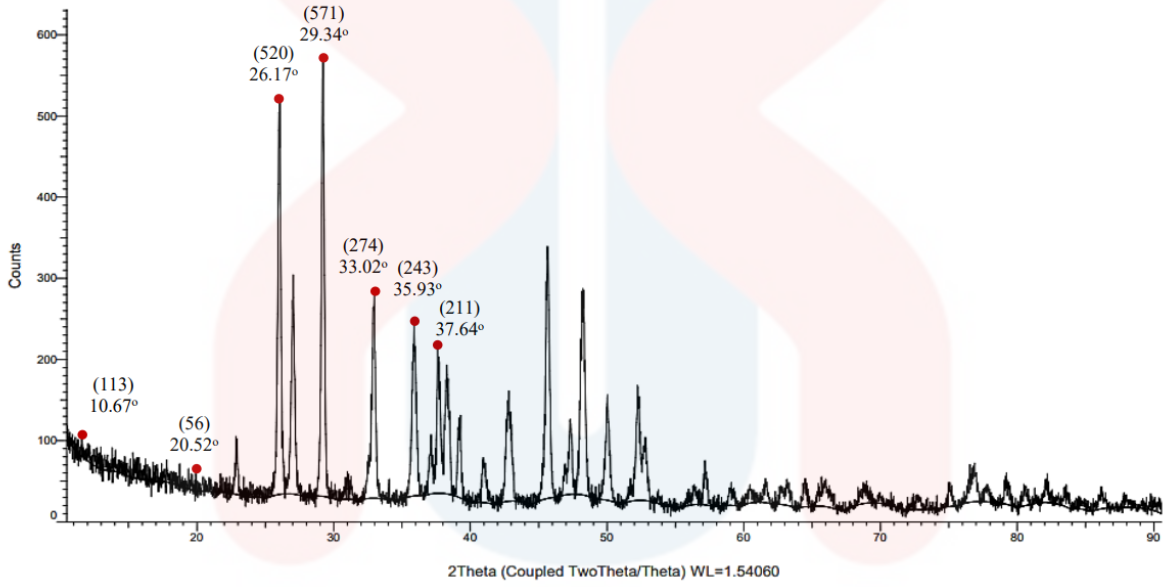


Figure 4.4: X-ray Diffraction spectra of chitosan extracted from etok shells.

4.1.6 Solubility Test

The results have revealed significant differences in the solubility of chitosan extracted using various solvents. Table 4.3 presents the solubility of chitosan from different solvents, expressed as a percentage. In acetic acid, chitosan has a high solubility of almost 99%. This may be due to the strong interaction between chitosan and acetic acid molecules. Not only that, this high solubility can also be ascribed to the protonation of amino groups in chitosan by acetic acid, which cause the formation of soluble chitosan acetate (Pardo-Castaño et al., 2019). The solubility of chitosan samples in distilled water is relatively low, at just 5.55%. Chitosan is insoluble in both organic and water solvents, and only dissolves in an aqueous acidic solution (Budiman et al., 2022). This limited solubility can be attributed to the ineffective interaction

between water molecules and chitosan molecules, which is not as efficient as that with acetic acid. Consequently, this results in a lower level of solubility.

Table 4.3: Solubility of chitosan from different solvent, expressed in percentage.

	Mass of tube (g)	Initial mass of tube with chitosan (g)	Final mass of tube with chitosan (g)	Percentage (%)
Acetic Acid	6.88	6.98	6.87	99
Distilled water	6.93	7.03	7.02	5.55

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Chitosan with low degree of deacetylation was produced from chitin which was extracted from etok shell by chemical extraction. Elemental analysis result confirmed the chitosan extracted exhibited a low degree of deacetylation (15.92%), which may impact its potential applications. FTIR analysis confirmed the peaks around 700 cm^{-1} indicate the presence of calcium carbonate ($CaCO_3$) suggests the possibility of incomplete impurity removal during chitosan extraction. DSC showed endothermic peak (692°C to 726°C) corresponds to the pressure buildup resulting from water evaporation and exothermic peak (718°C to 759°C) signifies the thermal decomposition process of chitosan, encompassing depolymerization, saccharide ring dehydration, and the degradation of both deacetylated and acetylated chitosan units. The TGA reveals the onset of the first phase was observed at approximately 83.75 °C with weight loss 6.86% may attribute to the loss of water molecules because the chitosan structures have strong affinity towards water then lead to dehydration and thermal decomposition of chitosan (Ahyat et al., 2017), while the second phase commences around 571.25 °C with weight loss of 40.36% is attributed to the thermal decomposition of the primary chitosan chain, which involves the vaporization and elimination of volatile products (Bashandy et al., 2017). X-ray diffraction analysis indicates five sharp peaks at 26.17^o, 29.34^o, 33.02^o, 35.93^o and 37.64^o due to the presence of some minerals and a difference in the origin starting material. The very weak peaks at 2θ values 10.67^o and 20.52^o suggested chitosan extracted from etok shells is predominantly amorphous with low crystallinity. Solubility test results that chitosan's solubility is high in acetic acid (99%) due to strong interactions, but low in distilled water (5.55%) due to weaker interactions, highlighting the importance of solvent selection. Characterization of chitosan via FTIR, elemental analysis, DSC, TGA, and XRD confirmed etok shells as a viable source for chitosan extraction, yielding chitosan with unique properties compared to other shells.

5.2 Recommendations

Considering the experimental results, the following improvements are suggested to improve the overall results of the experiment. Increasing the acid concentration as well as the demineralization period by ensuring an accurate calculation of the solvent-sample ratio is essential to ensure complete impurity removal. Next, to increase the deacetylation level of chitosan, optimize the parameters of the deacetylation process, such as temperature, time, and concentration of sodium hydroxide. It is advised to optimize the temperature and sodium hydroxide concentration to enhance chitosan deacetylation. However, caution is recommended with the duration of heating, as it could inversely impact the deacetylation degree.

In the conducted research, etok shells were obtained from a local vendor in Pasir Mas, Kelantan. However, the specific species of etok was not identified, which may introduce variability as different species could be utilized over time. This lack of identification could potentially influence the outcomes, particularly if diverse etok species are employed across various experimental methods. For future studies, it is strongly recommended to identify the etok species in use beforehand. Additionally, establishing independent sources for etok shells could help minimize variations induced by differences in etok breeds. This approach would ensure a more controlled and consistent raw material supply, thereby enhancing the reliability and reproducibility of the research findings. It would also provide a clearer understanding of how species-specific characteristics may impact the extraction and deacetylation processes. This, in turn, could lead to more precise optimization strategies for chitosan production.

Chitosan extraction from crustacean sources harms the environment by involving harsh processing steps such as alkali deproteinization. Addressing the limitations inherent in traditional chitosan extraction methodologies, future research endeavours are encouraged to explore alternative techniques for environmentally sustainable chitosan production. Specifically, investigations into methods leveraging biological processes, such as fermentation and enzymatic processing of raw materials, present promising avenues for exploration. These innovative approaches offer the advantage of a more controlled extraction environment, potentially yielding more consistent results. Consequently, the pursuit of this eco-friendly extraction methodology holds significant potential for advancing the field of chitosan production, enhancing efficiency, and promoting sustainability.

REFERENCES

Abdulkarim, A., Isa, M. T., Abdulsalam, S., Muhammad, A. J., & Ameh, A. O. (2013). Extraction and characterisation of chitin and chitosan from mussel shell. Extraction, 3(2), 108-114.

Achsan, K. F. (2021). A Review on the Synthesis of Chitosan From Different Waterbodies Exoskeleton Species and Its Application in Treating Water/Wastewater. Progress in Engineering Application and Technology, 2(2), 721–731-721–731.

Acosta-Ferreira, S., Castillo, O. S., Madera-Santana, J. T., Mendoza-García, D. A., Núñez-Colín, C. A., Grijalva-Verdugo, C., Villa-Lerma, A. G., Morales-Vargas, A. T., & Rodríguez-Núñez, J. R. (2020). Production and physicochemical characterization of chitosan for the harvesting of wild microalgae consortia. Biotechnology Reports, 28, e00554.

Ahyat, N. M., Mohamad, F., Ahmad, A., & Azmi, A. A. (2017). Chitin and chitosan extraction from Portunus pelagicus. Malaysian Journal of Analytical Sciences, 21(4), 770-777.

Alabaraoye, E., Achilonu, M., & Hester, R. (2018). Biopolymer (Chitin) from various marine seashell wastes: isolation and characterization. Journal of Polymers and the Environment, 26, 2207-2218.

Amor, I. B., Hemmami, H., Laouini, S. E., Abdelaziz, A. G., & Barhoum, A. (2023). Influence of chitosan source and degree of deacetylation on antibacterial activity and adsorption of AZO dye from water. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-023-03741-9

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Heras Caballero, A., & Acosta, N. (2021). Chitosan: An overview of its properties and applications. Polymers, 13(19), 3256.

Azahar, S. S., Hamidon, T. S., Latip, A. F. A., & Hussin, M. H. (2021). Physicochemical and conductivity studies of chitosan-tapioca flour-LiBF4 gel polymer electrolytes. Chemical Physics Impact, 3, 100055.

Badawy, M. E., Lotfy, T. M., & Shawir, S. (2019). Preparation and antibacterial activity of chitosan-silver nanoparticles for application in preservation of minced meat. Bulletin of the National Research Centre, 43(1), 1-14.

Bashandy, A., Raffat, E., Ibrahim, H., El Tayeb, T., & Gamal, R. (2017). Characterization of Chitosan Produced from Fermented Shrimp Shell Waste by Bacillus subtilis NA12 Using Gamma Radiation. Arab Journal of Nuclear Science and Applications, 50(1), 120-130.

Budiman, A., Nurfadilah, N., Muchtaridi, M., Sriwidodo, S., Aulifa, D. L., & Rusdin, A. (2022). The Impact of Water-Soluble Chitosan on the Inhibition of Crystal Nucleation of Alpha-Mangostin from Supersaturated Solutions. Polymers (Basel), 14(20). https://doi.org/10.3390/polym14204370

Chen, G.-L., Cai, H.-Y., Chen, J.-P., Li, R., Zhong, S.-Y., Jia, X.-J., Liu, X.-F., & Song, B.-B. (2022). Chitosan/Alginate Nanoparticles for the Enhanced Oral Antithrombotic Activity of Clam Heparinoid from the Clam Coelomactra antiquata. Marine Drugs, 20(2), 136.

da Silva Lucas, A. J., Oreste, E. Q., Costa, H. L. G., López, H. M., Saad, C. D. M., & Prentice, C. (2021). Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. Food Chemistry, 343, 128550.

Dey, S. C., Al-Amin, M., Rashid, T. U., Sultan, M. Z., Ashaduzzaman, M., Sarker, M., & Shamsuddin, S. M. (2016). Preparation, characterization and performance evaluation of chitosan as an adsorbent for remazol red. International Journal of Latest Research in Engineering and Technology, 2(2), 52-62.

Hahn, T., Tafi, E., Paul, A., Salvia, R., Falabella, P., & Zibek, S. (2020). Current state of chitin purification and chitosan production from insects. Journal of Chemical Technology & Biotechnology, 95(11), 2775-2795.

Han, D. K., Appalasamy, S., Nasir, S. N. A. M., Abdullah, F., Nor, M. M., & Rak, A. E. (2020). High quality of bacterial dna extraction from Corbicula fluminea (Muller, 1774) tissue in Kelantan. Malaysian Journal of Fundamental and Applied Sciences.

Hong, P. Z., Li, S. D., Ou, C. Y., Li, C. P., Yang, L., & Zhang, C. H. (2007). Thermogravimetric analysis of chitosan. Journal of applied polymer science, 105(2), 547-551.

Iber, B. T., Kasan, N. A., Torsabo, D., & Omuwa, J. W. (2022). A review of various sources of chitin and chitosan in nature. Journal of Renewable Materials, 10(4), 1097.

Ibitoye, E., Lokman, I., Hezmee, M., Goh, Y., Zuki, A., & Jimoh, A. (2018). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. Biomedical Materials, 13(2), 025009.

Ibrahim, H., & El-Zairy, E. (2015). Chitosan as a biomaterial—structure, properties, and electrospun nanofibers. Concepts, compounds and the alternatives of antibacterials, 1(1), 81-101.

Islam, N., Hoque, M., & Taharat, S. F. (2022). Recent advances in extraction of chitin and chitosan. World Journal of Microbiology and Biotechnology, 39(1), 28. https://doi.org/10.1007/s11274-022-03468-1

Kaewprachu, P., & Jaisan, C. (2023). Physicochemical Properties of Chitosan from Green Mussel Shells (Perna viridis): A Comparative Study. Polymers, 15(13), 2816. https://doi.org/10.3390/polym15132816

Kandile, N. G., Zaky, H. T., Mohamed, M. I., Nasr, A. S., & Ali, Y. G. (2018). Extraction and characterization of chitosan from shrimp shells. Open Journal of Organic Polymer Materials, 8(3), 33-42.

Kasaai, M. R., Arul, J., & Charlet, G. (2000). Intrinsic viscosity–molecular weight relationship for chitosan. Journal of Polymer Science Part B: Polymer Physics, 38(19), 2591-2598.

Kou, S. G., Peters, L. M., & Mucalo, M. R. (2021). Chitosan: A review of sources and preparation methods. International journal of biological macromolecules, 169, 85-94.

López-García, J., Lehocký, M., Humpolíček, P., & Sáha, P. (2014). HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. Journal of functional biomaterials, 5(2), 43-57.

Ma'ruf, A., & Hartati, S. (2022). Production and Characterization of Nano-Chitosan from Blood Clamshell (Anadara granosa) by Ionic Gelation. Nature Environment and Pollution Technology, 21(4), 1761-1766.

Martău, G. A., Mihai, M., & Vodnar, D. C. (2019). The use of chitosan, alginate, and pectin in the biomedical and food sector—biocompatibility, bioadhesiveness, and biodegradability. Polymers, 11(11), 1837.

Mathaba, M., & Daramola, M. O. (2020). Effect of Chitosan's Degree of Deacetylation on the Performance of PES Membrane Infused with Chitosan during AMD Treatment. Membranes, 10(3), 52. https://www.mdpi.com/2077-0375/10/3/52

McMahon, R. F. (2002). Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences, 59(7), 1235-1244.

McMurrv et al. (Ed.). (2024). Fundamentals of general organic and biological chemistry.

Muley, A. B., Chaudhari, S. A., Mulchandani, K. H., & Singhal, R. S. (2018). Extraction and characterization of chitosan from prawn shell waste and its conjugation with cutinase

for enhanced thermo-stability. International journal of biological macromolecules, 111, 1047-1058.

Nazri, Z. M., Rafi, Y. M., Merican, A. R., Farizan, M. W. S., Ismail, M., Zulhazmsan, H., Ariff, A. Z., & Aweng, E. (2021). The link between seller and supplier for creating business model of Etak-The heritage food revival sustainability in Kelantan, Peninsular Malaysia. AIP Conference Proceedings,

Pardo-Castaño, C., & Bolaños, G. (2019). Solubility of chitosan in aqueous acetic acid and pressurized carbon dioxide-water: Experimental equilibrium and solubilization kinetics. The Journal of Supercritical Fluids, 151, 63-74.

Ramli, M. Z., Ayyapan, V., Yusoff, A., Rak, A. E., & Lee, S. W. (2020). Phenotype and Genotype Characterisation of the Asian Clam of the Genus Corbicula Megerle von Mühlfeld, 1811 (Venerida, Cyrenidae) from the East Coast of Peninsular Malaysia. Borneo Journal of Resource Science and Technology, 10(1), 24-36.

Ramya, R., Sudha, P., & Mahalakshmi, J. (2012). Preparation and characterization of chitosan binary blend. Int. J. Sci. Res. Publ, 2(10), 1-9.

Rasti, H., Parivar, K., Baharara, J., Iranshahi, M., & Namvar, F. (2017). Chitin from the mollusc chiton: extraction, characterization and chitosan preparation. Iranian journal of pharmaceutical research: IJPR, 16(1), 366.

Sakai, Y., Hayano, K., Yoshioka, H., & Yoshioka, H. (2001). A novel method of dissolving chitosan in water for industrial application. Polymer Journal, 33(8), 640-642.

Salsabila, C., Wahyuningsih, C., Fitriana, D. A., Asih, R. S., Nida, K., & Ferniah, R. S. (2022). Semi-Manual Processing Of Blood Clamps Waste into Chitosan Powder. International Journal of Research in Community Services, 3(1), 8-12.

Santos, V. P., Marques, N. S. S., Maia, P. C. S. V., Lima, M. A. B. d., Franco, L. d. O., & Campos-Takaki, G. M. d. (2020). Seafood Waste as Attractive Source of Chitin and Chitosan Production and Their Applications. International Journal of Molecular Sciences, 21(12), 4290. https://www.mdpi.com/1422-0067/21/12/4290

Shanmugam, A., Subhapradha, N., Suman, S., Ramasamy, P., Saravanan, R., Shanmugam, V., & Srinivasan, A. (2012). Characterization of biopolymer “chitosan” from the shell of donacid clam Donax scortum (Linnaeus, 1758) and its antioxidant activity. Int. J. Pharm. Pharm. Sci, 4, 460-465.

Sinardi, P. S., & Notodarmojo, S. (2013). The chemical characteristics of chitosan extracted from green mussels shell (Mytilus virdis linneaus) and its potential application as a

natural coagulant. The Second International Conference on Sustainable Infrastructure and Built Environment,

Siswoyo, E., Zahra, R. N., Mai, N. H. A., Nurmiyanto, A., Umemura, K., & Boving, T. (2023). Chitosan of blood cockle shell (Anadara granosa) as a natural coagulant for removal of total suspended solids (TSS) and turbidity of well-water. The Egyptian Journal of Aquatic Research.

Sousa, R., Antunes, C., & Guilhermino, L. (2008). Ecology of the invasive Asian clam Corbicula fluminea (Müller, 1774) in aquatic ecosystems: an overview. Annales de Limnologie-International Journal of Limnology,

Varma, R., & Vasudevan, S. (2020). Extraction, Characterization, and Antimicrobial Activity of Chitosan from Horse Mussel Modiolus modiolus. ACS Omega, 5(32), 20224-20230. https://doi.org/10.1021/acsomega.0c01903

Wulandari, W. T., Puspitasari, R., & Aprilia, A. Y. (2020). Antioxidant Activity of Chitosan from the Waste of Green Mussels Shell (Perna Viridis L). 2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019),

Yanti, P. H., & Pebrianti, R. (2021). Microwave-assisted of synthesis and characterization of nanocomposite hydroxyapatite-chitosan. Journal of Physics: Conference Series,

Yusharani, M. S., Ulfin, I., & Ni'mah, Y. L. (2019). Synthesis of water-soluble chitosan from squid pens waste as raw material for capsule shell: temperature deacetylation and reaction time. IOP Conference Series: Materials Science and Engineering,

APPENDIX A

Appendix A.1: Calculation of 1Liter of 3% NaOH solution for deproteination process.

1 L = 1000 ml

3% × 1000 ml = 30 g

Amount of distilled water (DH2O):

1000 - 30 = 970 ml

Appendix A.2: Calculation of 1 Liter of 1.25 N HCl solution for demineralization process.

$N_1 V_1 = N_2 V_2$

$N_1 = 12$ N

$V_1 = ?$

$N_2 = 1.25$ N

$V_2 = 1000$ ml

$12 \times V_1 = 1.25 \times 1000$

$12\ V_1 = 1250$ ml

$$V_1 = \frac{1250 \text{ ml}}{12}$$

$V_1 = 104.20$ ml (Volume of HCl)

Volume of distilled water (DH2O):

1000 ml – 104.20 ml = 896.00 ml

Appendix A.3: Calculation of 4 % NaOCl solution from 10% NaOCl for depigmentation process.

$C_1 V_1 = C_2 V_2$

C_1 = 10% (initial concentration)

V_1 = Volume of the initial solution needed

C_2 = 4% (final concentration)

V_2 = Final volume 1 L

V_1 = $\frac{C2 \times V2}{C1}$

= $\frac{4\% \times 1L}{10\%}$

= 0.4 L

0.4 L × 1000 ml = 400 ml

Volume of distilled water (DH2O):

1000 ml – 400 ml = 600 ml

Appendix A.4: Calculation of 1 Liter of 60% NaOH solution for deacetylation process.

2 L = 2000 ml

60% × 2000 ml = 1200 g

Amount of distilled water (DH2O):

2000 - 1200 = 800 ml

Appendix A.5: Calculation of degree of deacetylation of chitosan extracted from etok shell.

DD (%) = $\frac{1 - \left(\frac{C}{N}\right) - 5.145}{6.816 - 5.145}$

= $1\ \frac{\left(\frac{13.18}{2.011}\right) - 5.145}{1.671}$

= $1\ \frac{6.55\ - 5.145}{1.671}$

= $1\ \frac{1.405}{1.671}$

= $1 - 0.8408$

= 15.92%

Appendix A.6: Calculation of the percentage solubility of chitosan extracted from etok shells in different solvents.

Acetic acid solvent

$$= \frac{(6.9835 - 6.8824)}{(6.9835 - 6.8815)} \times 100$$

$$= \frac{0.1011}{0.102}$$

$$= 99\%$$

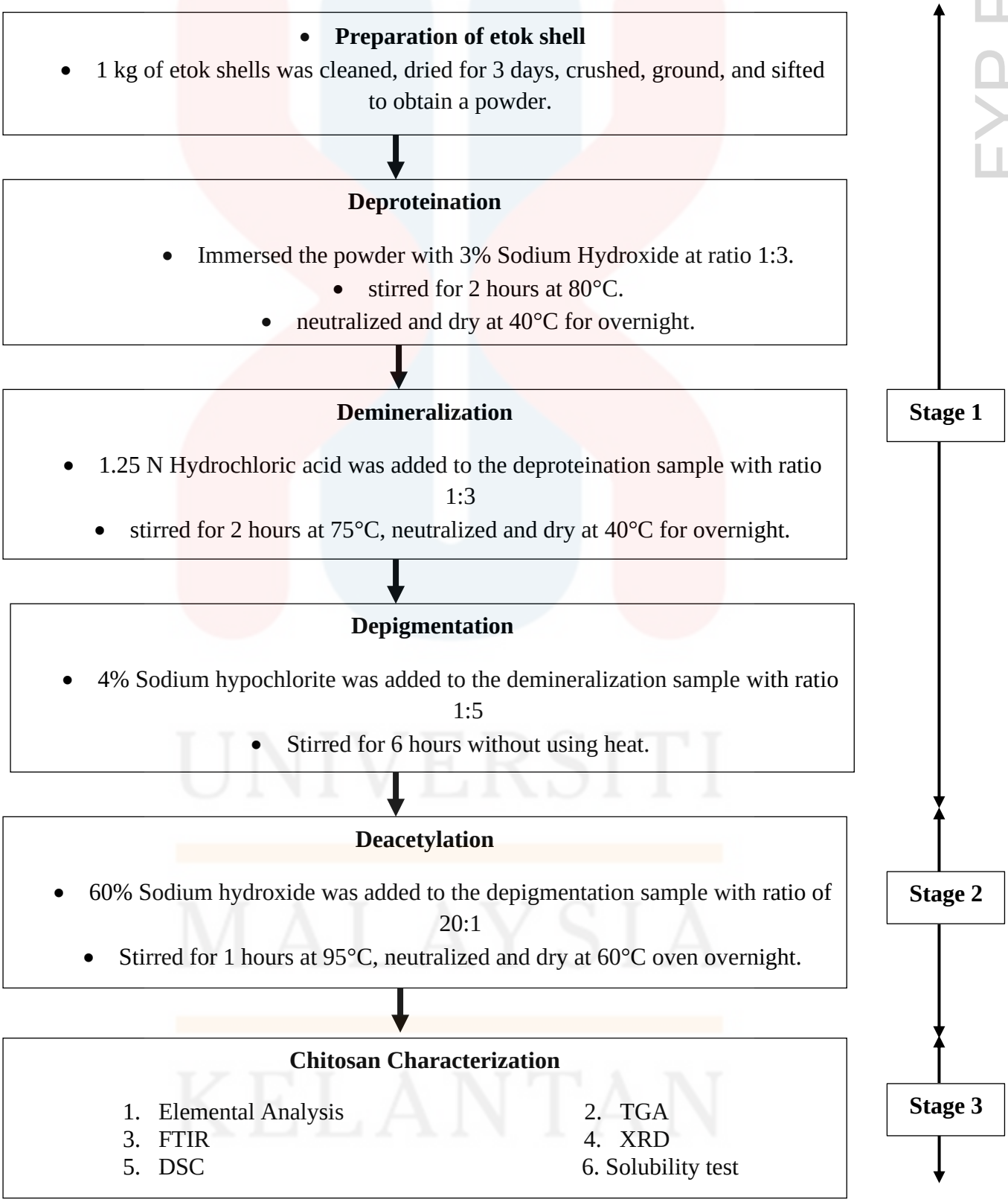
Distilled water

$$= \frac{(7.0301 - 7.0246)}{(7.0301 - 6.9311)} \times 100$$

$$= \frac{0.0055}{0.099}$$

$$= 5.55\%$$

APPENDIX B



Appendix B.1: The flowchart of chitosan extraction from etok shell.

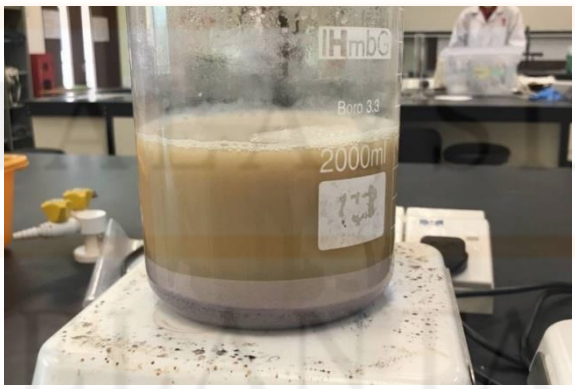
(a)

(b)

(c)

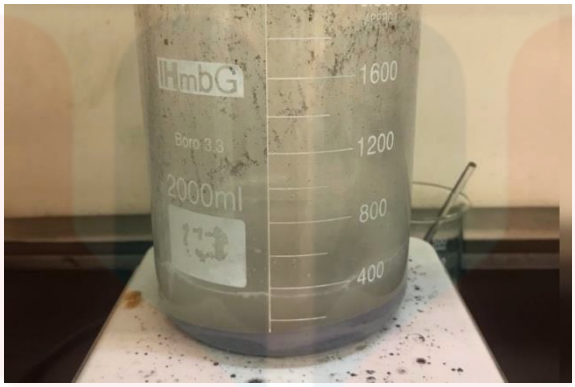
(d)

Appendix B.2: The picture depicts the process of preparation of etok powder; (a) 1 kg of etok had been separated from the fles, and (b) dried etok shell under the sun for three days. (c) The dried etok was then crushed using a mortar, blended with a machine, and (d) sieved through a 0.3 mm sieve. This process yielded 990 g of fine etok powder.

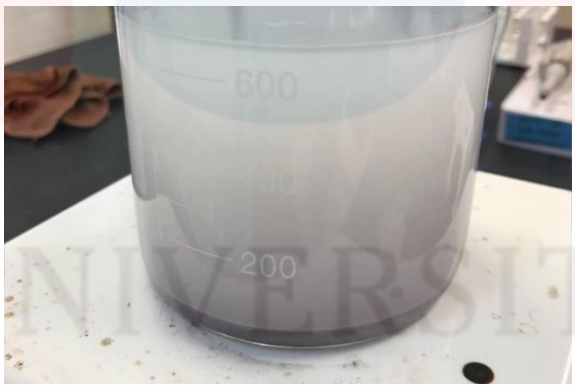


Appendix B.3: The images show the outcome after the deproteination process. 330 g of etok powder was treated with 3% NaOH for 2 hours at 80°C. The solvent turned turbid, indicating

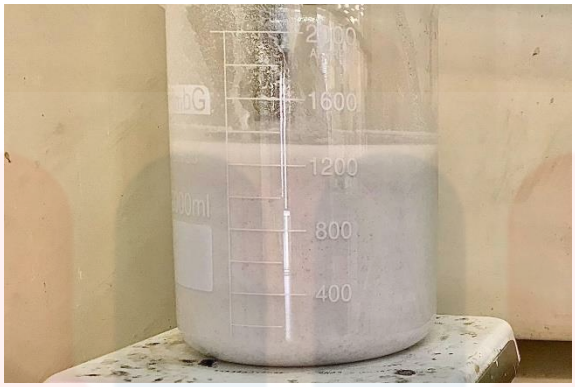
successful protein removal from the etok mixture. This process yielded 218 g of deproteinated sample.



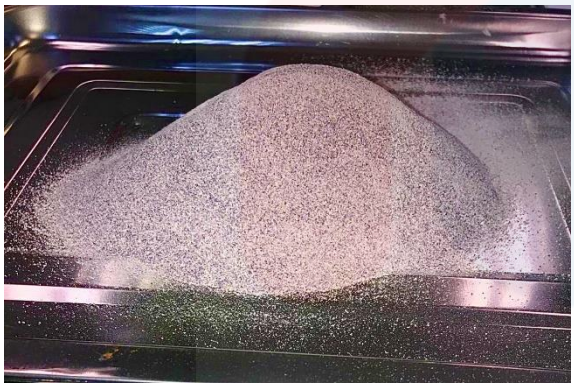
Appendix B.4: The images present the result of a demineralization process. A quantity of 218 g of deproteination sample was immersed with 1.25 N for 2 hours at 75°C. The results of the demineralization process are characterized by the presence of CO2 gas bubbles which are the indicator of the reaction between HCl and mineral salts in the shell. The yield produced from this process is 180 g of chitin sample.



Appendix B.5: The images show the result of a depigmentation process. Chitin powder was treated with 4% sodium hypochlorite, changing the etok shell powder color from purple to dull purple. This indicates a successful depigmentation process, reducing the pigment in the sample. The process yielded 90 g of depigmented chitin sample.



Appendix B.6: The images present the result of a deacetylation process which convert chitin into chitosan using 60% NaOH for one hour at 100°C. The mixture solution turns slightly white lilac after the deacetylation process with 60% NaOH due to a chemical reaction that occurs during the process. This reaction involves the removal of acetyl groups from the etok, which is facilitated by the alkaline NaOH solution. The mass yield of chitosan produced from deacetylation process is 40 g.



(a)

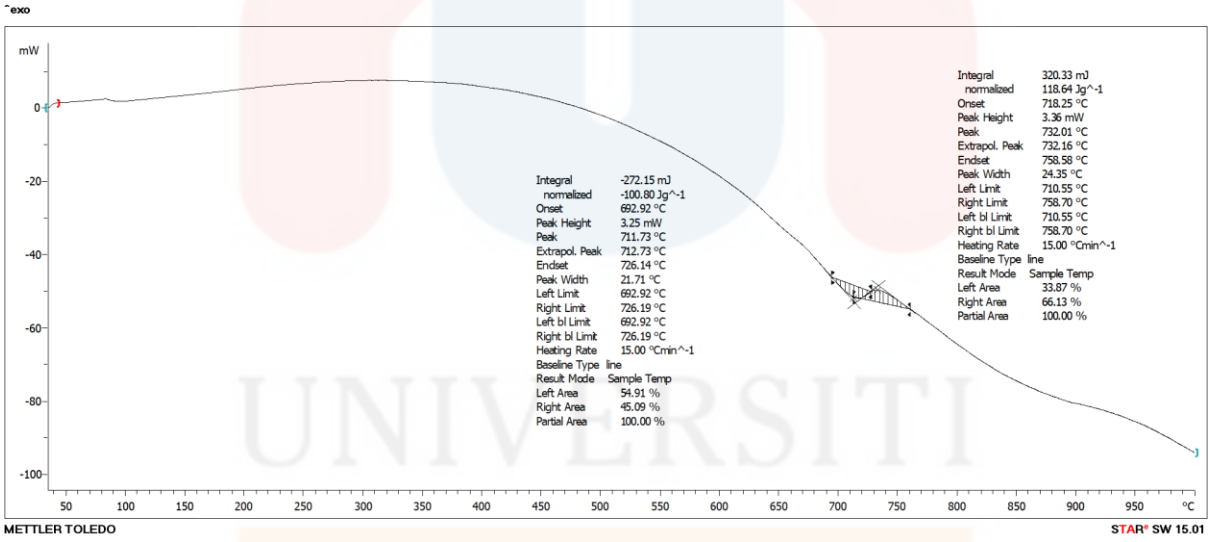
(b)

Appendix B.7: The images illustrate: (a) the chitin powder, and (b) the chitosan produced from deacetylation process. After deacetylation, the chitosan exhibited a slightly white hue, indicative of the successful removal of acetyl groups. The texture of the resulting chitosan appeared to be finer compared to the original chitin powder. From the initial 180 g of chitin powder, 40 g of chitosan was produced. This transformation underscores the efficiency of the deacetylation process.

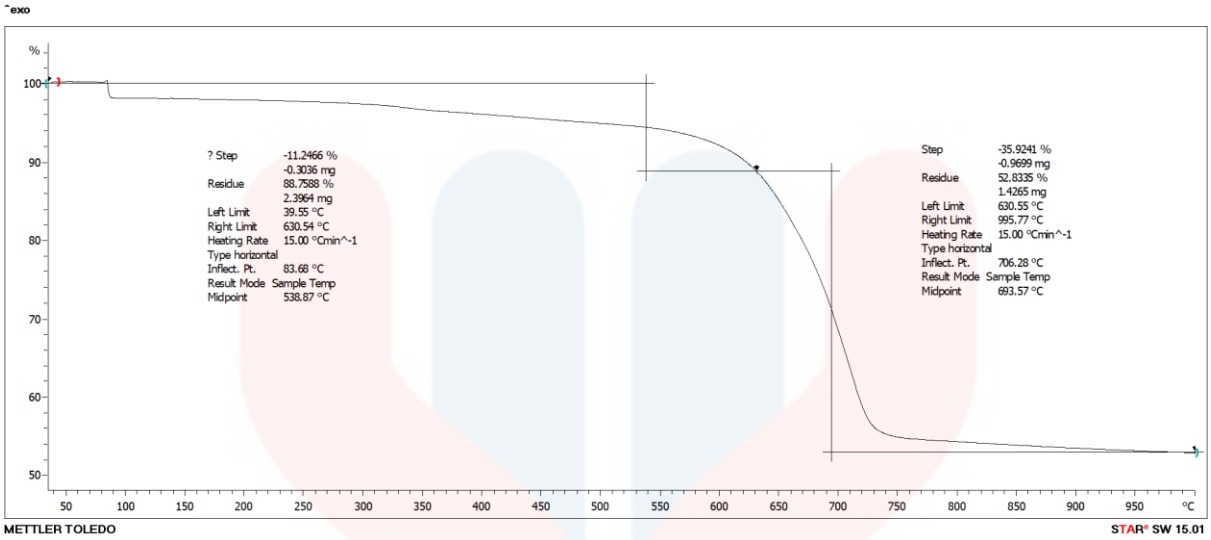
APPENDIX C

Name	Mass mg	Method	Carbon %	Hydrogen %	Nitrogen %	Sulfur %	Analysis Date	Analysis Time	Comments
Sulfa	2.084	UPM CHNS	51.14	5.127	22.48	11.25	8/1/2024 14:27	194	
C1	2.029	UPM CHNS	13.27	0.0784	1.587	0.00061	8/1/2024 14:40	194	
C1 rpt	2.089	UPM CHNS	13.09	0.0316	2.435	0.00105	8/1/2024 14:45	194	
C2	2.048	UPM CHNS	13.08	0.0595	2.864	0.00112	8/1/2024 14:50	194	
C2 rpt	2.05	UPM CHNS	13.1	0.0618	3.022	0.00098	8/1/2024 14:56	194	
C3	2.031	UPM CHNS	13.13	0.0336	3.265	0.00081	8/1/2024 15:01	194	
C3 rpt	2.049	UPM CHNS	13.15	0.0326	3.496	0.00072	8/1/2024 15:06	195	
STANDARD									
SULFAMETHAZINE			51.78	5.07	20.13	11.52			

Appendix C.1: The highlighted images show the raw data from the CHNS elemental analysis. The average data (carbon and nitrogen) was used to calculate the degree of deacetylation of chitosan extracted from etok shells.



Appendix C.2: The data above shows the results of the differential scanning calorimetry (DSC) of chitosan extracted from etok shells. The DSC technique measures the heat flow associated with the physical and chemical changes of the sample as a function of temperature.



Appendix C.2: The graph pattern above displays the data for the thermogravimetric analysis (TGA) of chitosan, showing the percentage of weight loss and its derivative as a function of temperature.

Solvent	Weight of tube (g)	Weight of tube + chitosan (g)	Weight of tube + chitosan pellet before dry (g)	Weight of tube + chitosan pellet after dry (g)
Acetic Acid	6.8815	6.9835	6.8824	6.8824
Distilled water	6.9311	7.0301	7.0246	7.0246

Appendix C.3: The table above shows the data of the solubility test. The highlighted data was used to calculate the percentage of chitosan extracted from etok shells.