PRODUCTION OF CHITOSAN FROM MARINE CRAB, *Scylla serrata*

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2008

Production of chiosan from marine crab, Scylla serrata / Siti Asmaa' Baharudin.

PRODUCTION OF CHITOSAN FROM MARINE CRAB, *Scylla serrata*

By

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**Research Report submitted in partial fulfillment of
the requirements for the degree of
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JABATAN SAINS MARIN
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PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **Production of chitosan from marine crab, *Scylla serrata*** oleh **Siti Asmaa' binti Baharudin** No. Matrik **UK 12059** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini telah dikemukakan kepada Jabatan Sains Marin sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah **Sarjana Muda Sains (Biologi Marin)**, Fakulti Pengajian Maritim dan Sains Marin, Universiti Malaysia Terengganu.

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LIST OF ABBREVIATIONS

cm	-	centimeter
g	-	gram
M	-	molarity
min	-	minute
ml	-	mililiter
μm	-	micrometer
R_f	-	retardation factor
R_{glc}	-	glucose retention time
ºC	-	degree celcius
$	-	dollar
%	-	percentage
μL	-	microlitre

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ABSTRACT

This study was conducted to determine the production of chitosan from marine crab, *Scylla serrata*. Crab shell need to undergo of dimineralization (DM), deproteinizition (DP), and deacetylation (DA) process in order to produced chitosan. Purpose of this study was to produce chitin and chitosan from the crab shell, with two different method been compared (reversing method and traditional method. Physicochemical and functional properties effect of sample due to the changes in modification or reversing method, where demineralization initiate the method but not in deproteinization process which commonly practiced in traditional method. The chitosan yield from modification method (DMPA) is higher with 53.7% compared with 53.0% from traditional method (DPMA). The result from physicochemical analysis (moisture and ash content) shown that moisture content in traditional method is lower than modification with 1.3 % to 1.7% respectively. The results from both samples are acceptable. For ash content, the percentage of modification method is lower than traditional method with 78.7% and 85.8% respectively. Chromatography paper and HPLC analysis showed glucose and glucosamine present in sample of chitin and chitosan.

PENGHASILAN CHITOSAN DARIPADA KETAM LAUT, *Scylla serrata*

ABSTRAK

Kajian ini mengenai penghasilan kitosan daripada sampel kulit ketam, *Scylla serrata*. Kulit ketam ini perlu melalui proses dinyahmineral (DM), dinyahprotein (DP), dan dinyah kumpulan asetil (DA) sebelum mejadi kitosan. Tujuan kajian ini dilakukan untuk menghasilkan kitin dan kitosan dari kulit ketam ini. Kedua, untuk membezakan dan memasttikan cara yang terbaik untuk menghasilkan chitosan sama ada teknik tradisional (No dan Meyer, 1995) atau teknik modifikasi "reversing" (Fernandez, 2004). Terdapat kesan kimia fiziko dan bentuk terhadap sampel akibat perubahan proses untuk metodologi tradisional dan proses modifikasi di mana dimulakan dengan proses dinyahmineral bukan dengan proses dinyahprotein seperti metod tradisional. Dalam kajian ini, kitosan yang dihasilkan oleh metod modifikasi (DMPA) lebih banyak dengan 53.7% berbanding 53.0% daripada metod tradisional (DPMA). Keputusan analisis kimia fiziko (kelembapan dan sampel abu) menunjukkan kadar kelembapan metod tradisional lebih rendah berbanding modifikasi 1.3% dan 1.7%. Kadar kelembapan kedua-dua sampel adalah diterima. Bagi sampel abu, juga menunjukkan peratusan yang rendah bagi metod modifikasi berbanding metod modifikasi dengan 78.7% dan 85.8%. Bagi analisis kertas kromatografi dan juga HPLC, menunjukkan sampel kitin dan kitosan mengandungi gula glukosa dan glukosamin.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Market demand for the aquatic livestock is high. In Quebec, there are several crustacean species that are commercially harvested. The industry produces large volumes of waste material that cause a disposal problem for the shellfish industry. Approximately 9587 tons of crustacean wastes were produced in 1989 with approximately 3300 tons were put to better economic use. About 6041 tons were buried and the remaining 228 tons were dumped in the sea (Alibhay *et al.*, 1990). Recently, the level of concern to environmental pollution and health safety among people increases and caused the waste management became costly. Many options were explored to overcome the waste material by converting them to value-added forms such as nutrient (proteins, minerals) and other useful biochemical (chitin, pigments, flavours, etc.), which are all present in an appreciable quantities in crustacean wastes.

Chitin, the precursor to chitosan was first discovered in mushrooms by the French Professor Henri Braconnot (1811). Improvisation of chitin isolation technique commenced in the 1820's when chitin was succeedly isolated from insects (Odote *et al.*, 1823). Chitin is a component structure that contains long chain of N-acetyl-D-glucosamine units. Chitin is the most abundant natural fiber next to cellulose and respectively similar many aspects. Crab and shrimp are the most abundant source of

chitin with the carapace being the main site of extraction source. The global shellfish harvest was estimated to be able to supply 50,000 tons of chitin annually (Johnson and Peniston, 1982) and recent study by Knorr (1991), had successfully recorded an increase of production to 120,000 tons of chitin. In the United States alone, the harvest could give, up to 15,000 tons of chitin each year (Shahram, 1992).

After chitin was discovered in 1811, chitosan was discovered in 1859 by Professor C. Rouget (1859). It was made by cooking chitin in alkaline, much similar to the process of making natural soaps. Heat from cooking process break down chitosan chain links into glucosamine units. Each glucosamine unit contains of free amino group. These groups can take a positive charge which gives chitosan its valuable properties with high level of usage. Research on the usage of chitin and chitosan flourished in 1930s and early 1940s but the high interest on synthetic fibers, such as synthetic medicines, overshadowed the interest in natural products. Interest in natural products, including chitin and chitosan, gained resurgence in 1970s and has continued to expand. Lately, Odote *et al.,* (2005) proved the earlier studies from Bough *et al., (*1978) on effects of different species method used for production of chitosan, differ the physicochemical characteristics and functional properties. Furthermore chitosan, the deacetylated form of chitin, had founded greater use than chitin due to its relatively high polyelectrolyte nature. Recently, ability to form semi permeable films, antimicrobial properties and ability to preserve fruits and vegetables have been explored (El Ghaouth *et al.,* 1991a, b, Lidster, 1987a, b).

1.2 Importance of Study

Chitosan application in areas of cosmetics, pharmaceuticals and agriculture is quite broad. It is use as a component of toothpaste, hand and body creams, shampoo, cosmetics and toiletries. In pharmaceuticals, it is used for lowering the serum cholesterol. Chitosan also served as a drug carrier in enzyme and cell immobilization as according to Synowiecki (1986). The chelating ability of chitosan makes it an excellent adsorbent for removing pollutants (Swapna *et al.*, 2005). As the material for production of contact lenses, or eye bandages, as well as seed coats and etcetera (Muzzarelli, 1989; Sandford, 1989). Chitin and chitosan is usually isolated from crustacean shell and more particularly from the shrimps and crabs shells. The production of chitosan from crustacean shells obtained as a food waste is economically feasible. The study conducted is done to explore the technique that can be applied in the effort to change food waste into useable elements and to provide the general public with information regarding chitosan and the related applications. Thus, the local mud crab, *Scylla serrata* is chosen as the object of study for its availability and affordable qualities.

1.3 Objectives

The study is moving towards this scope;

- To produce chitin and chitosan from crab shell
- To differentiate and confirm the better way to produce chitosan yield either from reversing method or the traditional method.

CHAPTER 2

LITERATURE REVIEW

2.1 Chitin and Chitosan

Chitin, (ß-(1.4)-2-acetamido-2-deoxy-D-glucan), is the most abundant natural occurring amino polysaccharide. It also second, most plentiful natural polymer next to cellulose. Chitosan is a chain of (1, 4)-[2-amino-2-deoxy-ß-D-glucan], naturally derivation of chitin and it is a collective name of a partially group. Fig. 2.1 showing the chemical structured differ between cellulose, chitin and chitosan are the alcohol group for cellulose, amide group and amine group. Chitosan is obtained by chitin partial deacetylation. It is a nontoxic, biodegradable and highly biocompatible with a basic polysaccharide structure. Chitosan usually isolated from the exoskeletons of crustaceans and more particularly from shrimps and crabs where α-chitin is produced (Blackwell and Minke, 1978, Austin *et al.*, 1989).

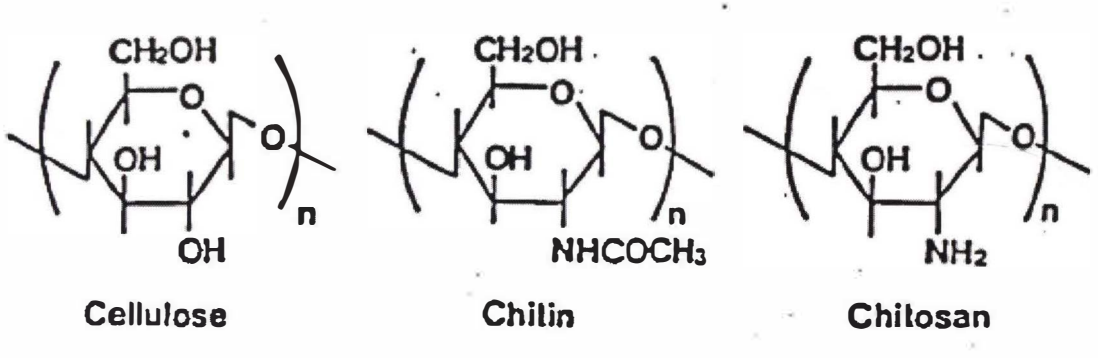


Figure 2.1 Chemical structures of cellulose, chitin and chitosan (Gagne, 1993)

2.2 Usage of Chitosan

There is numerous usage of chitosan in the different side of application. Tables 2.1 below showing several applications that need chitosan as the essential component.

Table 2.1 The usage of chitosan (Hennen, 1996)

Industrial uses
- Waste water purification - Stabilizing fats in food preparation - Flavour stabilizer - Ion exchange media - Cosmetic and shampoo additive - Absorbent for heavy metal remover - Stabilizing oil spills - Antibacterial perishable fruits/vegetables - Bacterial immobilizer - Tableting excipient
Health and Nutrition Uses
- Absorb and binds fat - Reduce LDL cholesterol - Promotes wound healing - Acts as antacids - Helps control blood pressure - Helps to speed bone repair - Promotes weight loss - Boosts HDL cholesterol - Antibacterial/ anticandida/ antiviral - Inhibit the formation of plaque/ tooth decay - Help dental restoration/ recovery - Improves calcium absorption

2.2.1 Chitosan as Fertilizer

Chitosan used primarily as a plant growth enhancer and as a substance to boost the ability of plants to defend against fungal infections (antimicrobial). It is approved for use outdoors and indoors on most plants grown commercially or home-grown. The active ingredient found in the shells of crustaceans, such as lobsters, crabs, and shrimps and in other organisms such as calcium content that can react as liming agent in agricultural land with additional magnesium to be included (Hood and Zall, 1979). Low potential for toxicity and its abundance in the natural environment, chitosan is not expected to harm people, pets, wildlife, or the environment when used according to direction.

2.2.2 Suspended and Hard Metal Remover

Chitosan also can be used in water processing engineering as a part of filtration process. Chitosan causes the fine sediment particles to bind together and its subsequently removed with the sediment during sand filtration. Chitosan also removes phosphorus, heavy metals and oils from water. Sand filtration apparently can remove up to 50% of the turbidity alone while the chitosan with sand filtration removes up to 99% turbidity (Woodmansey, 2002).

2.2.3 Agricultural use

Storing food is important in agricultural production, it is essential to ensure the quality of the product is high and period of uses is longer. Chitosan has a potential for use as food packaging, especially as edible films and because of its film-forming properties and unique property of increased viscosity upon hydration. A number of functional properties including antioxidant, antimicrobial, and oxygen barrier properties have been reported for chitosan film. The oxygen permeability of edible film is one of the key factors that limit the shelf life of packaged or coated products. During the frozen storage, oxidation occurs and changes colour, and flavour of the fish. It is very important to limit oxygen transport from the storage environment to the fish. Research on shelf life extension of fish by edible coating has so far been limited.

2.2.4 Biomedical use

Ability of chitosan to rapidly clot blood impress many scientist and have recently gained approval in the United State of America (USA) for use in bandages and other homeostatic agents. Chitosan purified from shrimp shells is used in a granular homeostatic product, Celox, made by Medtrade Biopolymers Inc. of Crewe, England and for dressings made by HemCon Medical Technologies Inc. of Portland, OR, USA. The Celox product rapidly reduces blood loss from life threatening injuries such as severe trauma and arterial bleeding even if the patient is using anticoagulants such as Heparin. The Hemcon product reduces blood loss in comparison to gauze dressings and increases patient survival. Chitosan is hypoallergenic, and has natural anti-bacterial properties, further supporting its usage in bandaging.

According to Stanford (2004), he states that he properties of chitosan that have long chain of glucosamine were demand for the treatment of arthritis and osteoarthritis is huge with worldwide sales of $392 million making glucosamine the ultimate nutraceutical sold in the United States. The procedure was taken by the National Institutes of Health (NIH), which is enrolling patients in a major, long term, nation wide clinical trail [Glucosamine/ Chondroitin Arthritis Intervention Trial (GAIT)] to review glucosamine effectiveness and safety.

2.2.5 Claimed benefits

Chitosan is frequently sold in the market and health stores as tablet form as a 'fat attractor'. It is supposed to have the ability to attract fat from the digestive system and expelling it from the body so that users can lose weight without eating less or excessive exercise. However, some scientific research suggests that these claims are likely without substance. At best, unmodified chitosan would remove roughly 30 calories per day from a person's diet but it has no taste. Modified chitosan is claimed to absorb up to three to six times its weight in fat and oils. Detractors claim that using chitosan may have the deleterious effect of rendering ineffective certain minerals found in foodstuffs and required by the body in order to remain healthy.

2.3 Mud Crab

The mud crabs, *Scylla* sp are large commercially-important portunids that inhabit coastal waters throughout the Indo-Pacific. Given the domestic name "Ketam Nipah" it is widely known as edible mud crab founded in most of the mangrove areas, in other regions *Scylla* sp is better known as the green crab. In the study of Le Vay (2001), high demand and ease of capture have lead to overexploitation. The crabs became the food source of local people, play important role in food chain and the use of the shell, in many areas such as cosmetics, biotechnology and pharmaceutical. This reduction of the crabs caused by maximum yield of capture and also effect of mangrove exploitation (natural habitat). Some of the country or regions took safeguard measurements of protecting the stock by imposing minimum landing sizes.

Nowadays, restoration of mangrove habitat is widely practiced in south-east Asia, but little is known about the recovery of the mud crabs. In Philippine, more than 70% of the mangroves have been lost in the last century due to overexploitation for timber and charcoal and agriculture, aquaculture and settlement (Primavera, 2000). Primavera *et al.,* (2004) state a number of mangrove reforestation projects have set about reversing this decline of which the study site, Buswang, is one of the more successful.

Mud crabs taxonomy has presently been described to four species, *Scylla serrata*, *Scylla paramamosain*, *Scylla tranquebarica* and *Scylla olivacea* (Keenan *et al.*, 1998). Although a lot of literature on *Scylla* sp exists, prior 1998 it is difficult to certain species which is reported, with the exception of studies in Africa to Australia, where the range of *S. serrata* extends beyond of others (Keenan *et al.*, 1998). It is likely that

there are some important differences in behavior, ecology and physiology that need to be identified in order to understand the differences in the geographic range of species and niche separation in sympatric species. The suggested place is both of the habitats when mature and size at maturation may be important in the prevention of cross breeding between species as well as courtship or behavioural differences.

Figure 2.2 Mud crab

Phylum Arthropoda

Class Malacostraca

Family Portunidae

Genus Scylla

Species *Scylla serrata*

This mud crab known scientifically as *Scylla serrata,* belonging to genus *Scylla.* It can be found in tropical, subtropical and warm areas. The habitat areas of this crab include brackish and salt water estuaries or mangrove forest. The common behavior particular for the crab; it like to burrow itself into soft muddy bottom (Shokita *et al.*, Keenan *et al.*, 1998). Generally, mud crab found in muddy mangrove habitats during adult. Like the blue crab, female mud crabs are thought to migrate to the coastal shelf to spawn (Hill, 1994). Their zoea cannot survive at low salinities (Hill, 1974) and their megalopae have been caught in offshore waters but rarely at estuaries (Arriola, 1940). Despite a substantial research effort, difficulties in finding significant numbers of megalopae and early-stage crabs (30 mm carapace width (CW)) have hampered investigations into the mechanisms of their recruitment into estuaries (Hill *et al.*, 1982, Moser and Macintosh, 2001).

Usually, mud crabs are tougher than brackish water fish and it does not need specific water quality to survive. Physically, mud crab has high, bluntly pointed frontal lobes spine, where pairs of large spine obvious on carpus and prosodies and the polygonal patterning present on all appendages.

2.3.1 Life Habit

Scylla serrata is a euryhaline animal where it is live in the area with water salinity is in range 5 ppt to 33.2 ppt. The optimum salinity is between 13.7 ppt to 26.9 ppt. The adaptation behaviour occured when the salinity of the water area decreased below than 7 ppt, thus resulting mud crab to dig deeper hole to survive (Hill, 1974).

2.3.2 Temperature

Mud crab lives in the temperature area between 18°C till 32°C. It also can tolerate the surrounding temperature between 12°C to 35°C. The feeding rate of this species will decrease when the temperature depleted below 18°C, and this will become dormant when the temperature continues to drop to 7°C.

CHAPTER 3

METHODOLOGY

3.1 Sampling

The crabs were collected at the Setiu market. Samples were kept in labelled plastic bag and stored below -20 °C in laboratory.

3.1.1 Raw Material Preparation

Only the carapace was used prevent the excess protein. A separation effort is needed. A separation of carapace from tissues is needed, and then the carapace was stored in -20°C until analysis.

Carapace was cleaned and washed with warm tap water to remove the shell from protein, organic soluble and particle, then followed by drying. The carapace was dried between 60°C for 24 hours (Fernandez, 2004). Then, carapace was homogenizer and sieved with 500μm mesh size to uniform the powder size. The powder were kept in clean plastic bottle and stored at room temperature.

3.1.2 Isolation of Chitosan

The sample powder or the homogenizer carapace undergoes several processes as below (Figure 3.1).

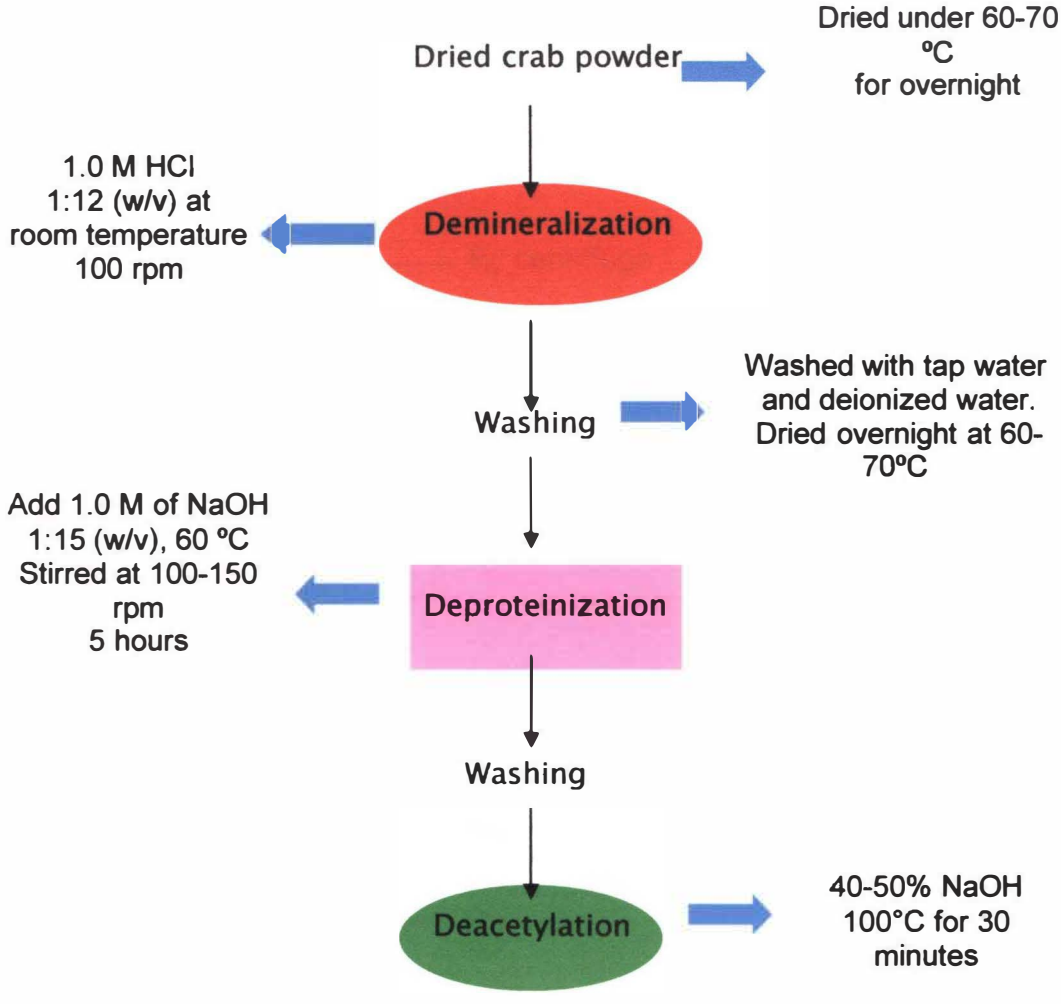


Figure 3.1 Isolation of chitosan process

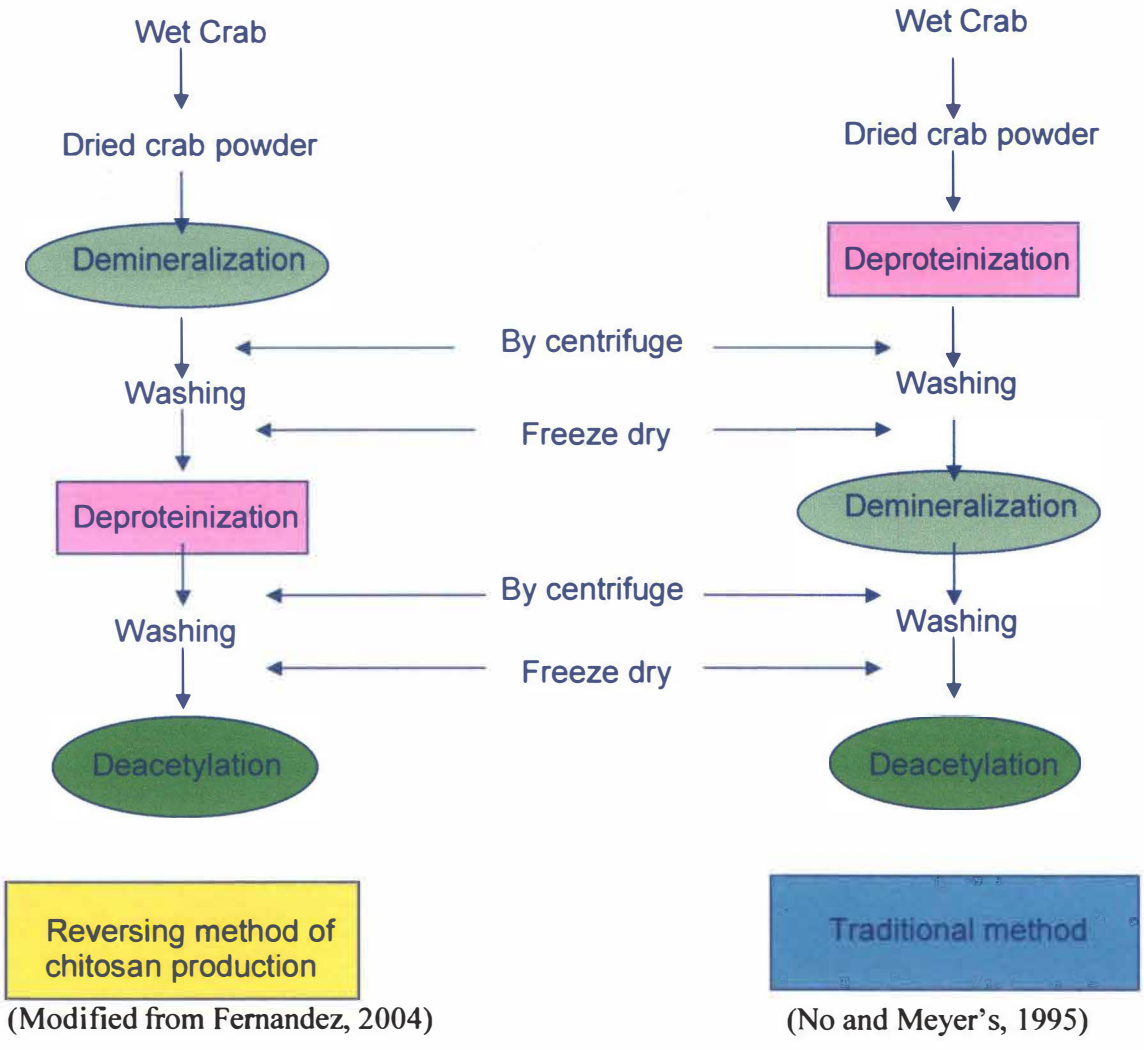


Figure 3.2 Flow chart of the reversing method and traditional method.

Traditional method was introduced by No and Meyers (1995) with implementation of Diproteinization-Dimineralizatin-Decoloration-Deacetylation (DPMCA) procedures (Fernandez, 2004). The reversing method is modified by Fernandez in her research to study if the sequence of the method changes gave effects to the chitosan production.

a) Deproteinization (DP)

Deprotienization was performed using alkaline treatments. The number of treatments depends on clarity of the solution; absence of protein was indicated by medium colour. The color is highly pink (Entsar *et al.,* 2007).

Carapace powder slowly adding powder into 1.0 M of sodium hydroxide solution (NaOH) to obtain 1:15 (w/v) (modified method from No *et al.*, 1989). The temperature of the reaction mixture was maintained at 60°C with constant stirring at 100-150 rpm for 5 hours. Next the sample was washed under tap water and deionized water before dried overnight, in the oven with temperature 60°C.

b) Demineralization (DM)

Demineralization was carried out at room temperature using 1 M hydrochloric acid treatment. The emission of carbon dioxide gas was more or less an important indicator according to the studied species. This case of crabs is strong compared to shrimp and crayfish which weak. (Entsar *et al.,* 2007).

The demineralized shell powder was added into 1.0 M hydrochloric acid solution at room temperature with constant slow stirred at 100 rpm for one to two hours. The stirred was setup at 100 rpm. The ratio solid to acids solution for the mixture are 1:12 (w/v) (modified method from No *et. al.*, 1989). Next, sample was put under run off tap water and then deionized water to neutralize before left it drying in the oven at 60°C for overnight.

d) Deacetylation (DA)

Dried chitin powder poured into boiling flask containing of 50 % (w/v) sodium hydroxide to obtain 1:15 (w/v) ratio of solid to alkaline solution. Then, the samples washed with tap water until the foaming is gone and rinsed with the ionized water. Samples dried at 60°C for overnight in oven.

3.2 Physicochemical and Functional Properties Measurement

3.2.1 Moisture Content

The crab chitosan moisture content was determined using gravimetric method by Black (1965).

$$\textbf{\% of moisture content} = \frac{\text{(The water mass, g)}}{\text{(Wet weight, g)}} \times 100$$

Exact 1.0g of chitosan were weighed and dried for 24 hours, at 60°C for 24 hours. Then, the samples were put inside dessicator to cool and prevent outside moisture disturbance. To get constant weight, water mass of the raw material were measured before and after drying session.

The water mass (or weight) = wet sample weight, g – dry sample

3.2.2 Ash Content

The crucible was weighed. After the weight is constant, 2.0 g of chitosan were filled crucible (triplicate). The samples were heated in a muffle furnace at 600ºC for 6 hours. The crucibles then, left in the furnace to cool until less than 200ºC and place into desiccators with a vented top for cooling. The crucibles and ash weight had been recorded.

$$\% \textbf{ ash} = \frac{\text{(Weight of residue, g) x 100}}{\text{(Sample weight, g)}}$$

3.2.3 Hydrolysis of Chitosan

A analysis of chemical compound of the chitosan was hydrolyzed with 4 M Hydrochloric Acid (HCl) for 12 hours at 100 ºC in a scaled test tube. After evaporated, the hydrolyzite was analyzed by paper chromatoghraphy (PC) and High Performance Liquid Chromatography (HPLC).

3.2.4 Paper Chromatography (PC)

Paper chromatography (PC) was carried out by the descending method on Whatman No. 1 filter paper with ethyl acetate-acetic acid-formic acid-water (18:3:1:4, v/v) as the solvent. The hydrolyzate of the chitosan was drop on the paper three times. After the suitable development time (15 hours), the paper was removed from the chamber, dried and slipped in the alkaline silver nitrite reagent. The spot colour were recorded,

R_f value (retardation factor or ratio-to-front) and the number of spots separated were calculated. The Rf value is useful to identify compound.

$$\mathbf{R_f} = \frac{\text{Distance from Origin Spot Travel}}{\text{Distance from Origin Standard Travels}}$$

3.2.5 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a form of liquid chromatography. It used to separate compound such as sugar, amino sugar and acid sugar that has dissolved in solution. HPLC consisted of universal evaporation light scattering detector (ELSD). In a mobile phase, a solution of acetonitrile and water (ratio 70/30) was filtered. The column used was the carbohydrate 5 μL column (ZORBAX® Carbohydrate Analysis Column) at the temperature of 40^0C. The working flow rate is 1.0 ml/min with temperature 80^0C. Nitrogen gas flow is set at 3.2 ml/min and injection volume is 10 μL using water as solvent.

CHAPTER 4

RESULTS

The result of the chitin and chitosan production and their physicochemical properties are shown in the Table 4.1.

4.1 Yield of chitin and chitosan

The yield of chitin and chitosan production is calculated from 30 g of *Scylla serrata* dried and powderized.

Table 4.1 Chitin and chitosan yield production, moisture content and ash content

Method	Chitin Yield (%)	Chitosan Yield (%)	Moisture Content (%)	Ash (%)
Traditional (DPMA)	69.6	53.0	1.3	78.7
Reversing (DMPA)	70.9	53.7	1.7	85.8

DPMA= deproteinization-deminerilazation- deacetylation, DMPA= demineralization-deproteinization- deacetylation

Chitins production from crab are 69.6 % and 70.9 % after treated with 1M hydrochloride acid and 1 M sodium hydroxide for both traditional and reversing method. Then, after the chitin was undergone deacetylation, the yield of chitosan gained was 53.0% to 53.7%, higher than what No and Meyers (1989) has reported for

approximately 23% of chitin. The reversing method gave the higher yield with range of 0.7 % different to traditional method.

For moisture content, both methods gave reversing method had 1.3% while traditional method had 1.7%. DMPA (reversing method) sample shown it contains more moisture than DPMA (traditional method). According to KFDA (1995), the moisture content of chitosan should be below 10 %.

The result of the ash production is higher than that suggested by No and Meyers (1995) for high quality of chitosan. The high quality of chitosan should not exceed 1% of ash production.

4.2 Paper Chromatography (PC)

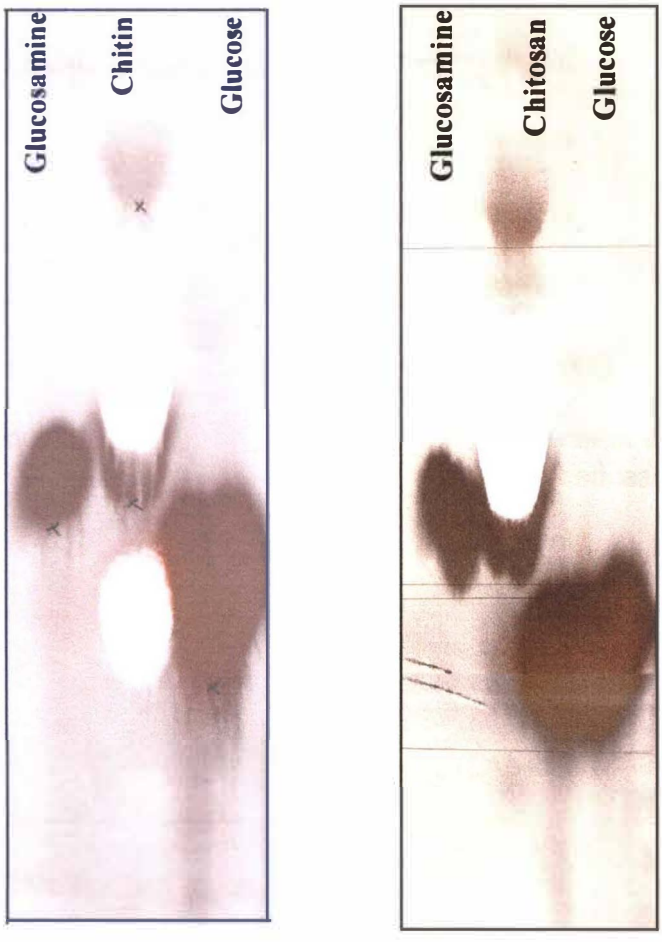


Figure 4.1 Paper chromatography samples result

Figure 4.1, shown result the chitin and chitosan sample contents that were dissolved are glucose and glucosamine. Glucosamine was used as standard sugar.

Table 4.2 Result of paper chromatography analysis

No.	Type of Sugar	Retardation Factor (R_{glc})
1.	Rhamnose	Absent
2.	Glucuronic Acid	Absent
3.	Xylose	Absent
4.	Arabinose	Absent
5.	Mannose	Absent
6.	Glucose	1.0 (both)
7.	Glucosamine	0.78 (chitin sample) 0.84 (chitosan sample)
8.	Maltose	Absent
9.	Lactose	Absent
10.	Trehalose	Absent
11.	Raffinose	Absent

4.3 High Performance Liquid Chromatography (HPLC)

The figure 4.2 is shown the standard sugar chromatogram for sample reference. The next figure 4.3 - 4.6 are shown the result HPLC run for the component sugar that content in the samples (C1, TMR1, TMR2 and RMR1) sample.

Table 4.3 High Performance Liquid Chromatography result for standard sugar and samples.

Sample	Retention Time Glucose (min)	Retention Time Glucosamine (min)
Standard sugar	1.766	2.167
Chitin (C2)	1.416	2.007
TMR1	1.833	2.703
TMR2	1.915	2.719
RMR1	1.865	2.266

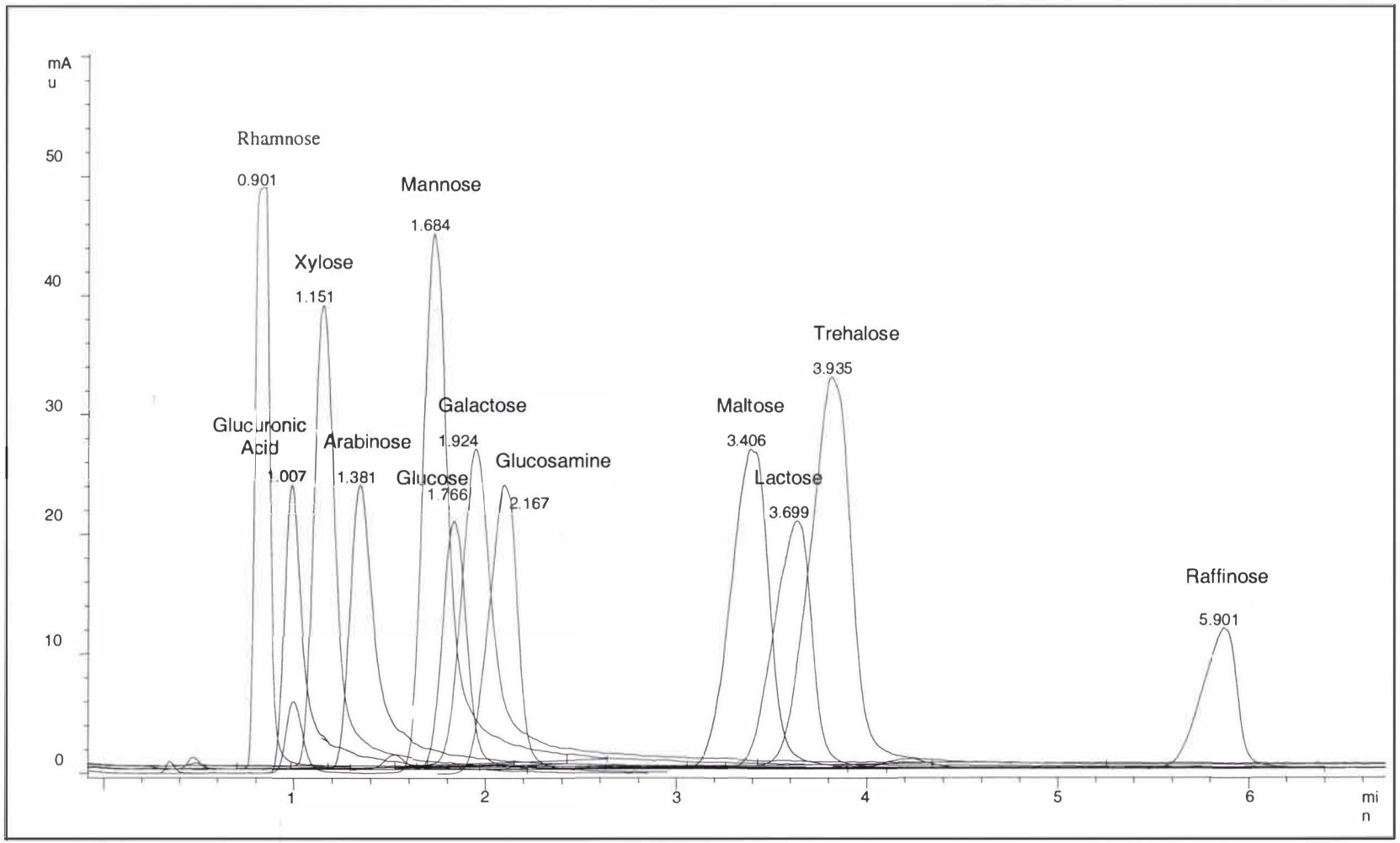


Figure 4.2 Standard Sugar Chromatogram

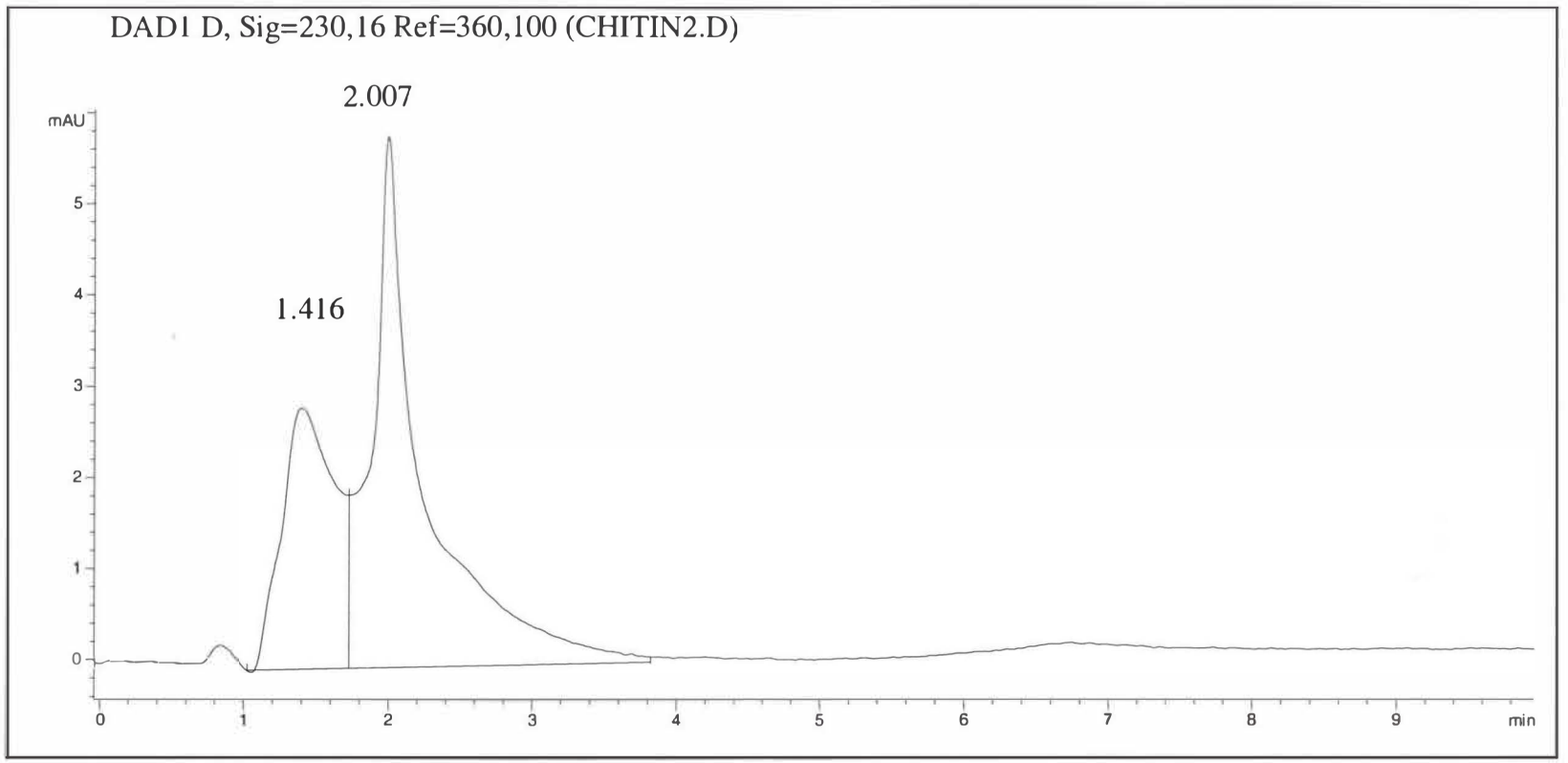


Figure 4.3 Chitin Sample Chromatogram

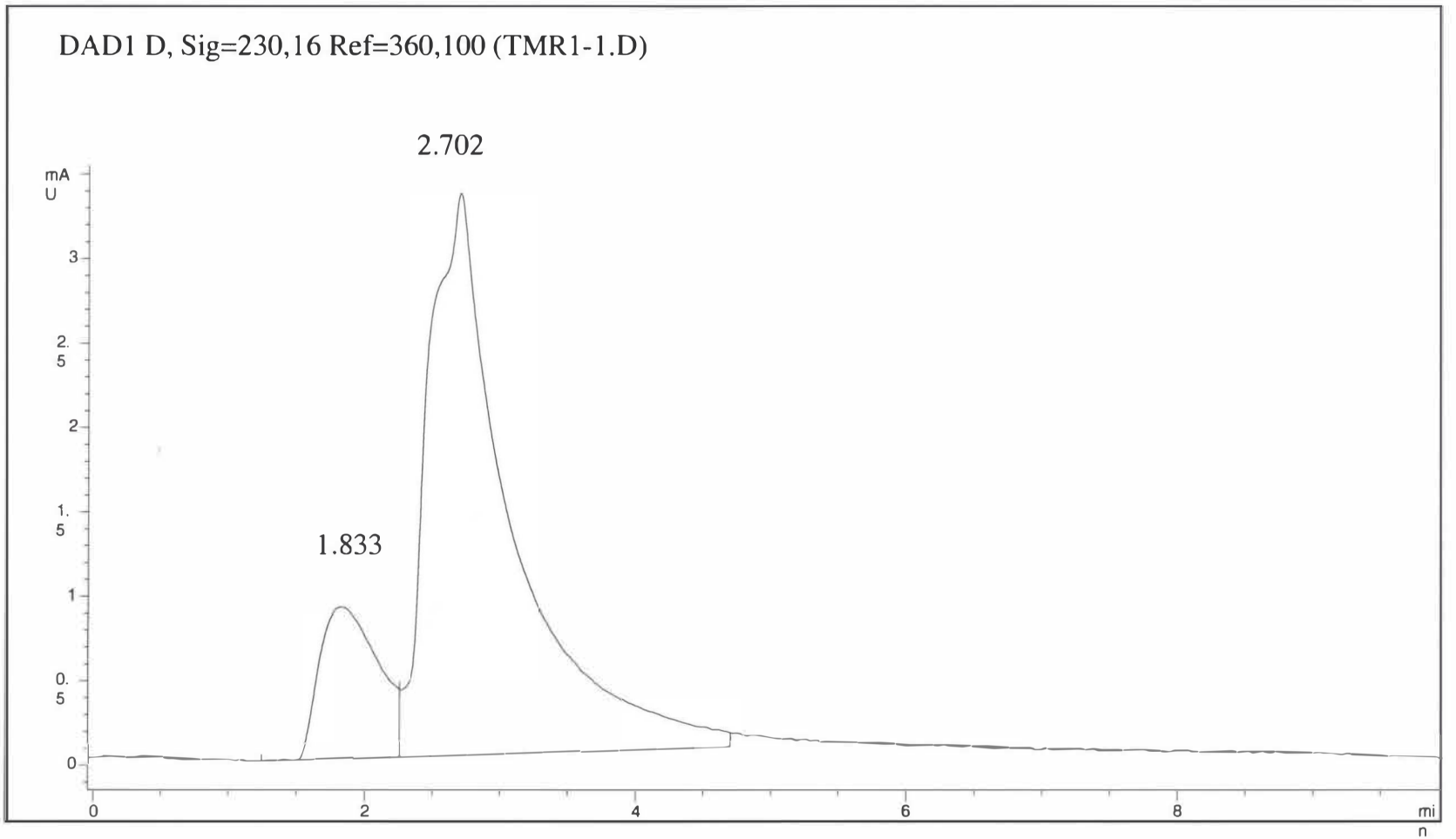


Figure 4.4 Chitosan Sample (TMR1) Chromatogram

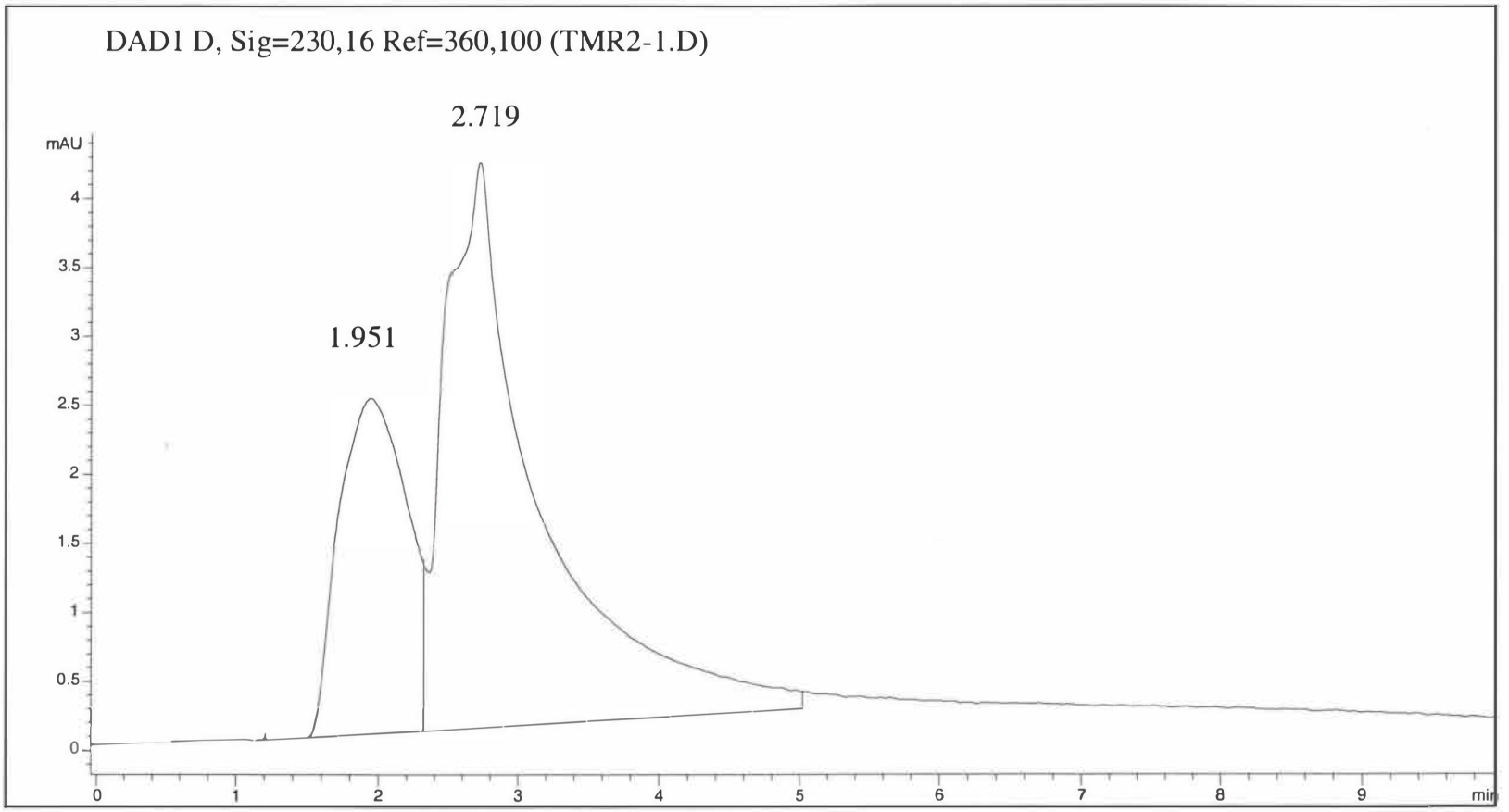


Figure 4.5 Chitosan Sample (TMR2) Chromatogram

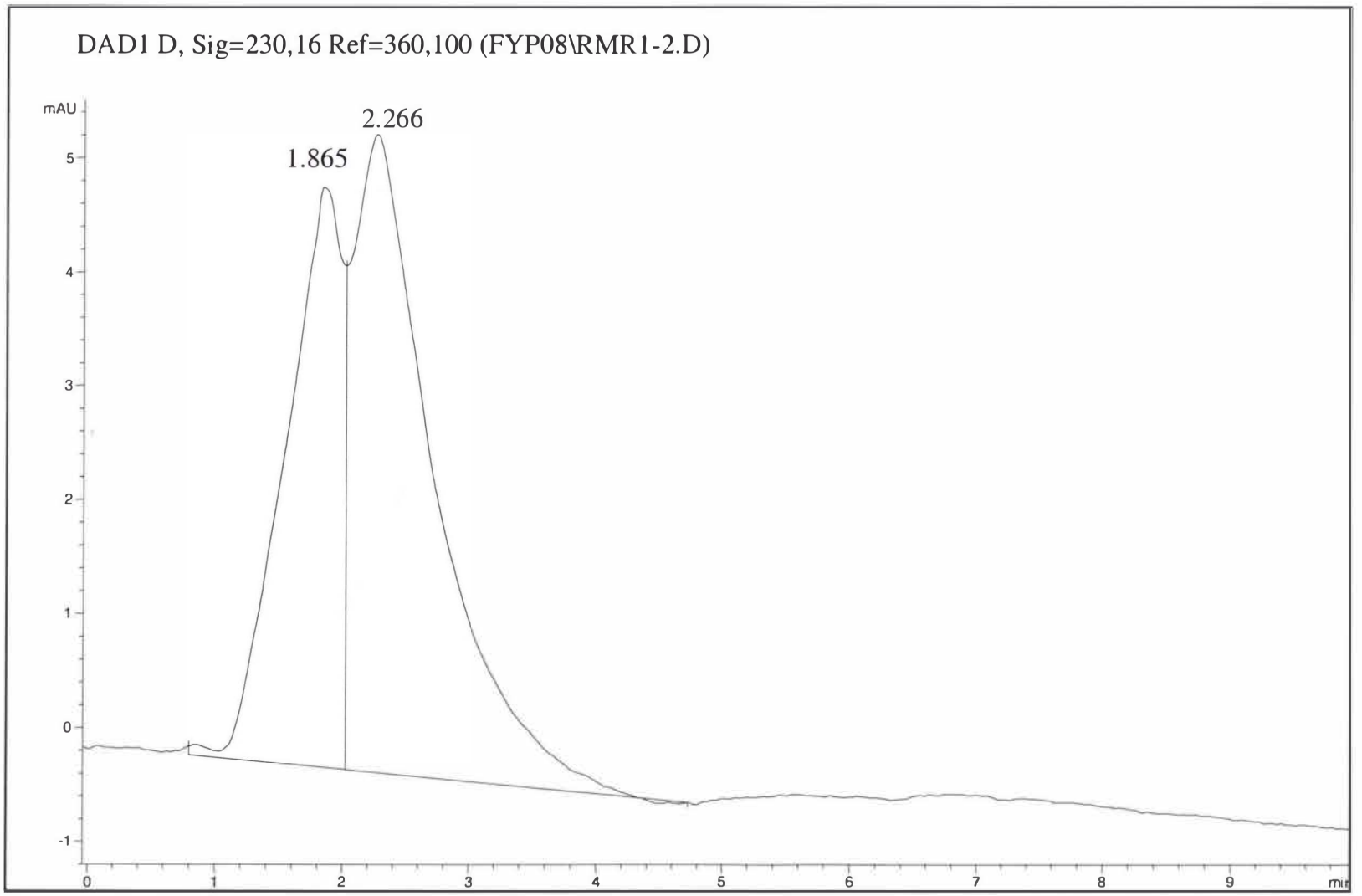


Figure 4.6 Chitosan Sample (RMR1) Chromatogram

CHAPTER 5

DISCUSSION

This study is initiated by dividing the sample into two groups of study which undergo two different methods. The first sample was done based on the traditional method (DPMA) and the other, reversing method. The period and the concentration of the solution were standardized and the study were revised according to the method sequences by No and Meyers (1995).

Yield that has been gained were calculated from 30 g of dried and powderized crab shell. Chitosan yield is 53.0 % and 53.7 %, respectively with small difference which is 0.7 %. The DMPA gave the higher yield of 53.7 %.

From the research by Odote *et al.,* (2005), the yield of chitosan from prawns, crabs, lobsters and insect larvae was 75.1 %, 74.6 %, 74.3 % and 66.7 % respectively and it was higher than the yield that has been gained in this study. Although this study revealed lower production of chitosan, but consist higher than what been predict by production of chitin (23%). Many researches revealed that chitin and chitosan yields differ between species. Chitin yields of 14-27% been reported by Ashford *et al.*, (1977) for shrimp and No and Meyers (1995) reported yields of 13-26% from crab. The results are vary were the yield is higher with 69.6-70.9%. For chitosan yield, Brzeski (1982) and Anderson *et al.*, (1978) reported yields of 70% and 90% respectively from krill. While Anderson *et al.*, (1978) reported 60% yield from crab chitin and Aluminiar and Zainuddin (1992) have reported 80% yield from prawn

shells. This higher yield compared to this study due to the different size of the powder used, where in this study size of powder which was sieved with 500 μm mesh size are used. This may relate to the concentration of the solution that was used, as in the Odote *et al.,* (2005), the demineralization was treated with 2 M hydrochloric acid but in this study used 1 M hydrochloric acid only.

Moisture content of the crab chitosan shown the have good quality and can be preserve longer because of the percentage of their moisture content is below than 10 % according to KFDA (1995) with 1.3 % and 1.7 %. The reversing method shown it higher content of moisture compared to traditional method. The result were differ with the previous study by Azeyanti (2007), where moisture content in reversing method is lower than traditional method.

Ash content is one of procedures that can predict to determine the chitosan production is in high quality or lower. Usually ash content for lobsters and crabs are normally higher than in shrimps. Thus, for ensuring good quality chitosan the reduction their level to below 1 % production of ash is important. According to the research that has been done by No *et al.*, (1989), ash contents as high as 63% have been reported in crayfish, lobsters *Linuparus trigonus* 54.7% (Ahn and Lee, 1992), crab *Callinectes sapidus* 50% (Muralidhara and Maggin, 1985), prawn *Paenaeus monodon* 29% (Benjankul and Sophanodora, 1993). Ash content for *Sylla cerrata,* the lobster *Panulirus ornatus* and prawn *Paenaeus indicus are* 45%, 35% and 27% respectively (Odote *et al.*, 2005). The result of ash content that had been yield for crab is 78.7-85.8 % respectively. The reduction in ash production should be done by increasing the acid concentration in demineralization step. Other worker that has been proven was from

No *et al.* (1989) that have been using crayfish, had reduced ash content with 1N HCl from 63% to 0.3 %. The purpose using higher concentrations of hydrochloric acid were to remove excess protein, organic soluble and particle during the demineralization process. On the other hand, the problem may affect when using wild crab which possibilities they are contain heavy metal that concentrated from their environment.

Paper chromatography and HPLC result, both shows the sample content glucose and glucosamine sugar. In paper chromatography result, white spots were observed on the paper.

High performance liquid chromatography analysis (HPLC) shows the result on chitin and chitosan substances containing glucosamine. The retention time peak for the four figures is not well developing precisely. The curve is too big in the result than the actual peak is sharp and thin. The reading may contain other sugar particle that cannot be separated accurately even several time repeated. This problem may need the column to be serviced.

CHAPTER 6

CONCLUSION

The production of chitosan from traditional method is average 53% and reversing method is average 53.7%. There is no major difference between both methods in production of chitosan.

From the paper chromatography and HPLC analysis, glucose and glucosamine present in both chitin and chitosan samples.

For further study is suggested to p-Anisidine Hydrochloride (Rondle and Morgan, 1955) method to confirm the presence glucose and glucosamine (Appendix 8).

REFERENCES

Alibhay, R. Saito, T.I. and Shaw, D.N.R. 1990. Un exemple de la valorisation de la biomasse manne par la biotechnologie. Proceedinçs of "Atelier sur la Biotechnologie Marine", Université du Québec à Rimouski. 9pp. In Gagne, N. 1993. Production of Chitin and Chitosan from Crustacean Waste and Their Use as a Food Processing Aid. Department of Food Science and Agticultural Chemistry, McGill University, Montreal.

Ahn, C.B and Lee E.H. 1992. Utilization of Chitin Prepared from the shellfish Crust. 1. Functional properties of chitin, chitosan and microcrystalline chitin. *Bulletin of the Korean Fisheries Society.***25:** 45 -48

Alimuniar, A., & Zainuddin, R. 1992 An Economical Technique for Producing chitosan. *Advances in Chitin and chitosan.* Brine, C.J.; Sandford, P.A; Zikakis, J.P. (Ed) . Elsevier Applied Science.Essex, UK, p 627-632.

Anderson, C.G., De Pablo N. & Romo, C.R., 1978. Antarctic Krill (*Euphausia superba*) as a Source of Chitin and Chitosan. In *Proceedings of the First International Conference on chitin and Chitosan.*

Austin, P.E., Castle, J.E., Albisetti, C.J., 1989. In: Skjak-Braek, G., Anthonsen, T., Sandford, P. (Eds.), Chitin and Chitosan. Elsevier, Essex, p. 749.

Arriola, F.J., 1940. A preliminary study of the life history of Scylla serrata (Forskal). Philipp. J. Sci. 73, 437–455

Ashford, N.A., Hattis, D. & Murray, A.E. 1977. *Industrial Prospects For Chitin and Protein From Shellfish Wastes.* MIT Sea Grant Report. MISG 77-3: MIT Cambridge, MA 1977.

Azeyanti N., 2007. Production of chitosan from exoskeleton of prawn. B. Sc. Thesis. University Terengganu Malaysia. 34 pp

Benjakul, S. & Sophanodora, P. (1993). "Chitosan Production from Carapace and Shell of Black tiger Shrimp. *ASEAN Food Journal.* **8:** 145-148

Black C. A. 1965. Methods of Soil Analysis: Part I physical and mineralogical properties. *American Society of Agronomy*, 1965. Madison, Wisconsin.

Blackwell, J. and Minke, R. 1978. The structure of a-chitin. Journal of Molecular Biology 120, 167.

Braconnot, H. 1811, Sue la natrue ces champignons. *Ann Chim Phys*; 79:265 in Hennen, W. J. 1996. Chitosan, Woodland Publishing Inc. Pp 31.

Bough, W.A., Salter, W.L., Wu, A.C.M., & Perkins, B.E. 1978. Influence of manufacturing Variables on the Characteristics and Effectiveness of Chitosan Products. I Chemical Composition, Viscosity and Molecular Weight Distribution of Chitosan Products. *Biotechnology Bioengineering.* 20: 1931-1942

Brzeski, M.M., 1982. Concept of Chitin and Chitosan Isolation from Antarctic Krill (*Euphasia superba*) Shells on a Technical Scale. In *Proceedings of the Second International Conference on Chitin and Chitosan* Hirano S., Tokura, S. (Ed). The Japan Society of Chitin and Chitosan, Sapporo, Japan, p 15-29.

EI Ghaouth, A., Arul, J. and Ponnampalam, R. 1991 b. Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. J. Food Process. Preserv., 15:359-368.

Entsar S. Abdou, Khaled S.A. Nagy, Maher Z. Elsabee, 2007. Extraction and characterization of chitin and chitosan from local sources. Food Technology Research Institute, Agriculture Research Center, Department of Chemistry, Cairo University. [Online] http___www.sciencedirect.com_science__ob=MImg&_imagekey=B6V24-4NBRFPB-1-11&_cdi=5692&_user=5674159&_orig=search&_coverDate=03%2F26%2F2007&_sk=999999999&view=c&wchp=dGLzVlz-zSkWA&md5=ce2ad5cd27e24c34f442df97220b2764&ie=_sdarticle.pdf. [Access 14th August 2007].

Fernandez,.K., 2004. Physicochemical and functional properties of crawfish chitosan as affected by different processing protocol. M. Sc. Thesis. Seoul National University.

Gagne, N. 1993. Production of Chitin and Chitosan from Crustacean Waste and Their Use as a Food Processing Aid. Department of Food Science and Agticultural Chemistry, McGill University, Montreal.

Hennen, W. J. 1996. Chitosan, Woodland Publishing Inc, Pleasant Grove. Pp 31.

Hill, B.J., 1974. Salinity and temperature tolerance of zoeae of the portunid crab Scylla serrata. Mar. Biol. 25, 21–24.

Hill, B.J., 1994. Offshore spawning by the portunid crab Scylla serrata (Crustacea: Decapoda). Mar. Biol. 120, 379–384.

Hill, B.J., Williams, M.J., Dutton, P., 1982. Distribution of juvenile, subadult and adult Scylla serrata (Crustacea: Portunidae) on tidal flats in Australia. Mar. Biol. 69, 117–120.

Hood L.F. and R.R. Zall. Recovery, Utilization and Treatment of Seafood Processing Wastes. Advances in Fish Science and Tech., Jubilee Conference of the Terry Research Station, Aberdeen, Scotland, 1979. Fishing News Books Ltd.

Johnson, E. L. and Peniston, Q. P.1982. Utilization of shellfish waste for chitin and Chitosan production. Chp 19 In Martin, R. E., Flick, G. J., Hebard, C. E. and Ward, D. R. (eds.).: Chemistry and Biochemistry of Marine Food Products. p.415-. AVI Publishing Co., Westport, CT.

Keenan, C. P. Mann, D. L., Lavery, S. and Davie, P. 1998. Genetic relationships, morphological identification and taxonomy of mangrove crabs, genus *Scylla,* from through out the Indo-Pacific. ACIAR Project Report, QDPI, Brisbane.

KFDA, 1995. Food Additive Code. Seoul: Korea Food and Drug Administration. Pp 449-451

Knorr, D. 1991. Recovery and Utilization of Chitin and Chitosan in Food Processing Waste Management. Food Technology. P 114-122

Lidster, P.D. 1987a. Storage of selected vegetable crops in modified atmospheres using a chitin-based differentially permeable coatinç. Agriculture Canada, Agi-Food Development Agreement, Technology Development Program, AFDA TDP85-106.

Le Vay, 2001 L. Le Vay, Ecology and management of mud crabs, *Scylla* spp., *Asian Fisheries Science* 14 (2001), pp. 101–111.

Moser, S. M., and Macintosh, D. J., 2001. Diurnal and lunar patterns of larval recruitment of Brachyura into a mangrove estuary system in Ranong Province. Thailand Mar. Biol. 138, 827–841.

Muralidhara, H.S. & Maggin B. (1985). In Preparation of Chitin and Chitosan (No, H.K & Meyers, S.P. Chitin Handbook. Muzarelli, R.A.A. & Peter M.G. (Eds.). Chitin handbook. p 475- 489 Muzzarelli, R.A.A., (1977). *Chitin,* Pergamon Press, Oxford.309p

Muzzarelli, R. A. A., Weeks, M. & Filipini, 0. 1989. Removal of trace metal ions from industrial waters, unclear effluents and drinking waters, with the aid of cross-linked N-carboxymethyl chitosan. *Carbohydr. Polym.,* 11,293-297.

No, H. K. and Meyers, S. P. 1989. Crawfish of Chitosan as Coagulant in Recovery of Organic Compound from Seafood Processing Stream. J Agric Food Chem. 37(3): 580-583.

No, H. K. and Meyers, S. P. 1995. Preparation and Characterization of Chitin and Chitosan-A Review.*Journal of Aquatic Food Product Technology.* 4(2). P.27-52.

Odier, A. 1823. Memoire sur la composition chemique des parties cornees desinsectes. *Mem Soc Hist Nat Paris* 1:29. In Gagne, N. 1993. Production of Chitin and Chitosan from Crustacean Waste and Their Use as a Food Processing Aid. Department of Food Science and Agticultural Chemistry, McGill University, Montreal.

Odote, P. M. O., Marcin, H., Struszczyk and Peter, M. G. 2005. Characterisation of Chitosan from Blowfly Larvae and Some Crustacean Species from Kenyan Marine Waters Prepared Under Different Conditions.Western Indian Ocean J. Mar. Sci. Vol. 4, No. 1, pp. 99–107.

Primavera, J. H. 2000. Development and conservation of Philippine mangroves: institutional issues, *Ecological Economics* **35** (2000), pp. 91–106.

Primavera et al., 2004 J.H. Primavera, R.B. Sadaba, M.J.H.L. Lebata and J.P. Altamirano, Handbook of Mangroves in the Philippines, South East Asian Fisheries Development Centre (SEAFDEC), Aquaculture Department, Iloilo, Panay, Phillippines. 106 pp.

Rondle, C. J. M. and Morgan, W.T. J. 1955. The Determination of Glucosamine and Galactosamine, London: The Lister Institute of Preventive Medicine. 4 pp.

Rouget, C. 1859. Des substances amylacees dans le tissue des animux, specialement les Articules (Chitine). *Compt Rend* 48:792. In Gagne, N. 1993. Production of Chitin and Chitosan from Crustacean Waste and Their Use as a Food Processing Aid. Department of Food Science and Agticultural Chemistry, McGill University, Montreal.

Shahram, H. 1992. Seafood waste: the potential for industrial use. *Kem Kemi.* 19(3),256-8. In Gagne, N. 1993. Production of Chitin and Chitosan from Crustacean Waste and Their Use as a Food Processing Aid. Department of Food Science and Agticultural Chemistry, McGill University, Montreal.

Sandford, P. A. 1989. Chitosan: commercial uses and potential applications. In *Chitin and Chitosan,* ed. G. Skjak-Braek, T. Anthonsen P. Sandford. Elsevier Appl. Sci. London-New York, pp. 5169.

Sandford. P. A. 2004. Wound Healing: Methods and Protocols, L.A. DiPietro, A.L. Burns (Eds.); Humana Press, Inc., Totowa, NJ, USA, 2003, xv þ 467 pages, ISBN 0-89603-999-4

Shokita, S., K. Kakazu, A. Tomori and T. Tome, 1991. Aquaculture in tropical areas. Midori Shobo Co. Ltd.Japan (English edition by M. Yamaguchi). Pp 360

Swapna Pradhan, Shyam S. Shukla, Kenneth L. Dorris,2005. Removal of nickel from aqueous solutions using crab shells. Department of Chemistry, Lamar University, Beaumont,USA. [Online] http___www.sciencedirect.com_science__ob=MImg&_imagekey=B6TGF-4GJK8H9-4-C&_cdi=5253&_user=5674159&_orig=search&_coverDate=10%2F17%2F2005&_sk=998749998&view=c&wchp=dGLbVtz-zSkWA&md5=a18379788d50cd7582ac96d433dbbc20&ie=_sdarticle.pdf. Access [14th August 2007].

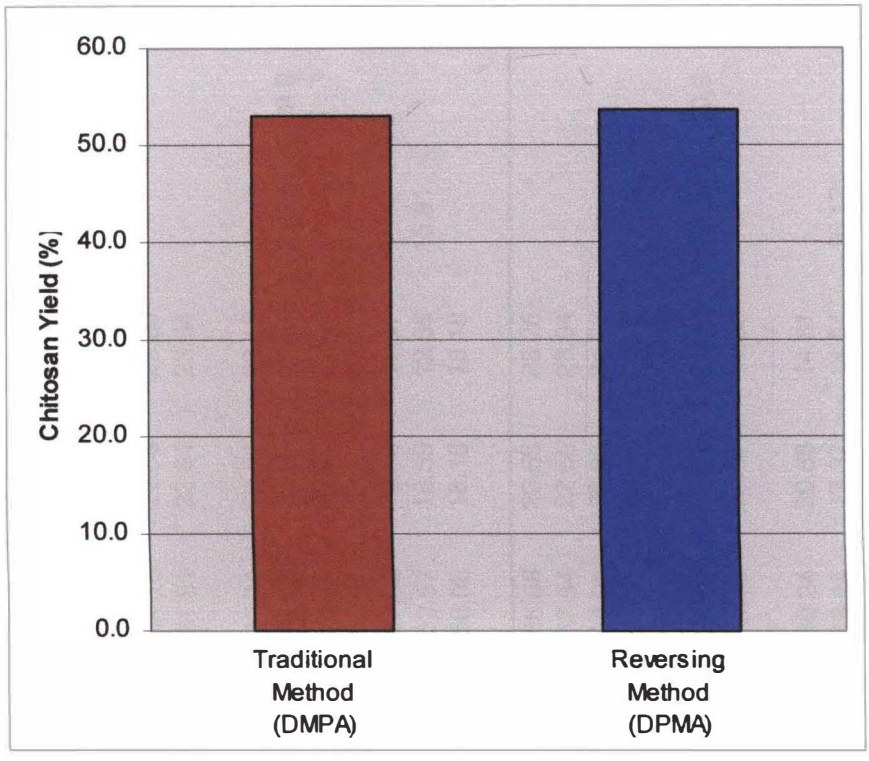
Synowiecki, J. 1986. Use of krill chitin as an enzyme support. In *Chitin in Nature and Technology,* ed. R. A. A. Muzzarelli,C. Jeuniaux & G. Gooday. Plenum Press, New York,pp. *4* I *7420.*

Woodmansey, A. (Highway Engineer) (2002). Chitosan Treatment of Sediment Laden Water - Washington State I-90 Issaquah Project. *Federal Highway Administration.* U.S. Department of Transportation. [Online] http://en.wikipedia.org/wiki/Chitosan._[Access 10th July 2007].

APPENDICES

Appendix 1: High Performance Liquid Chromatography (HPLC)

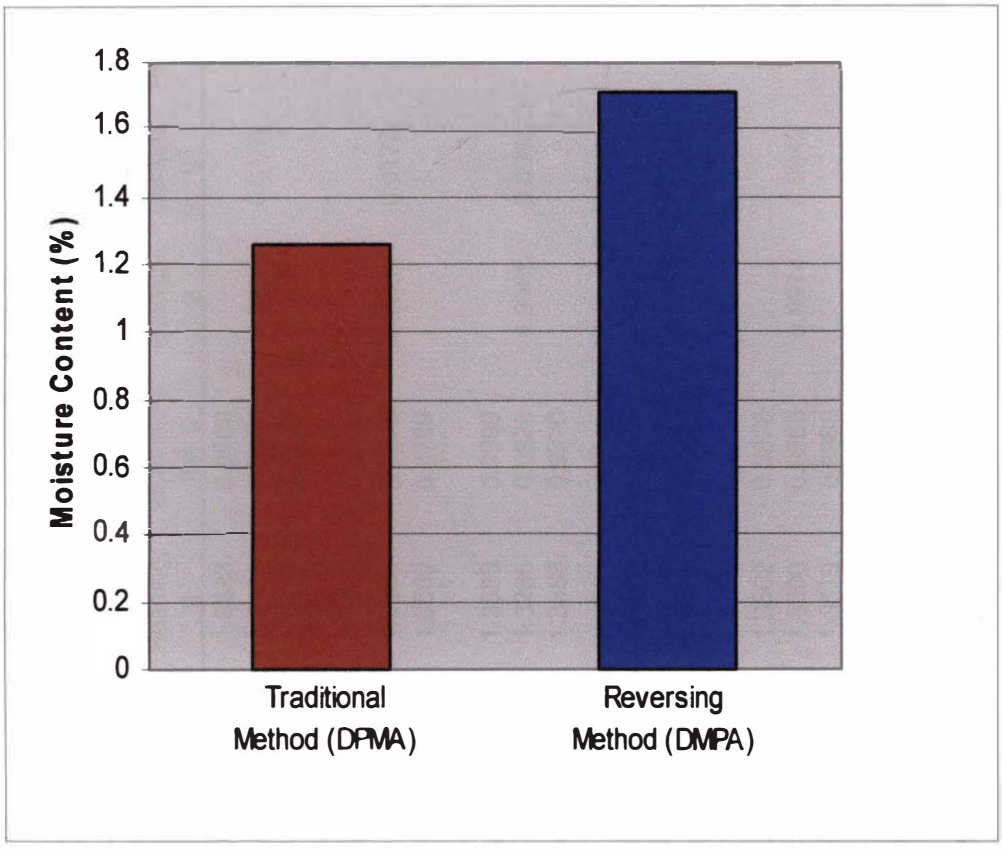
Appendix 2: Chitosan yield percentage



Appendix 3: Calculation for chitosan yield.

Method	Process	Replicate	Average of powder, g	Petridish weight, g	Wet weight, g	Dry weight, g	Total water loss, g	Dried powder, g	Average	Chitin %	Chitosan %
Traditional	DM	r1		49.86	96.89	73.26	23.63	23.40			
		r2	30.03	40.20	86.72	64.16	22.56	23.96			
		r3		37.87	84.15	61.51	22.64	23.64			
	DP	r1			90.55	70.04	20.51	20.18			
		r2			83.26	61.88	21.38	21.68	20.91	69.6	
		r3			79.27	58.74	20.53	20.86			
	DA	r1			85.22	67.02	18.20	17.17			
		r2			71.16	52.57	18.59	12.38	15.91		53.0
		r3			74.24	56.06	18.18	18.18			
Reversing	DP	r1		35.43	81.33	58.68	22.65	23.25			
		r2	30.03	34.50	80.04	57.54	22.50	23.04			
		r3		37.39	88.36	62.01	26.35	24.62			
	DM	r1			75.18	56.65	18.53	21.22			
		r2			74.81	55.33	19.48	20.83	21.28	70.9	
		r3			80.16	59.19	20.97	21.80			
	DA	r1			70.93	50.24	20.68	14.81			
		r2			66.81	49.44	17.37	14.94	16.12		53.7
		r3			73.20	55.99	17.21	18.60			

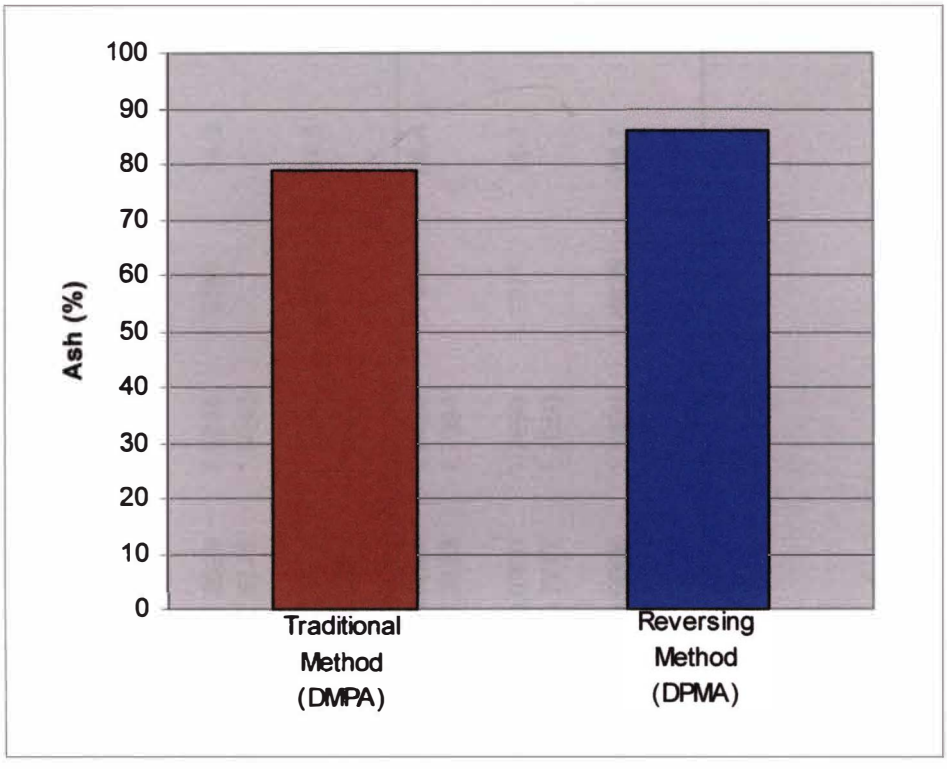
Appendix 4: Moisture content percentage



Appendix 5: Calculation for moisture content

Method	Sample	Aluminium foil weight, g	Initial Reading, g	Chitosan weight, g	Average, g	Reading, g	Chitosan weight w/o aluminium foil, g	Average, g	moisture content, g	Percentage, %
Traditional	TMR1	0.3835	1.3885	1.0050		1.3523	0.9688			
		0.3448	1.3466	1.0018	1.0033	1.3402	0.9954	0.9880	0.0138	1.3755
		0.3891	1.3921	1.0030		1.3889	0.9998			
	TMR2	0.331	1.3339	1.0029		1.3540	1.0230			
		0.3725	1.3740	1.0015	1.0016	1.3300	0.9575	0.9838	0.0178	1.7772
		0.3956	1.3960	1.0004		1.3665	0.9709			
	TMR3	0.3684	1.3716	1.0032		1.3683	0.9999			
		0.3339	1.3340	1.0001	1.0028	1.3265	0.9926	0.9965	0.0063	0.6249
		0.3413	1.3463	1.0050		1.3383	0.9970			
Reversing	RMR1	0.3514	1.3516	1.0002		1.3459	0.9945			
		0.3414	1.3499	1.0085	1.0032	1.2467	0.9053	0.9644	0.0388	3.8644
		0.2986	1.2994	1.0008		1.2920	0.9934			
	RMR2	0.3526	1.3548	1.0022		1.3502	0.9976			
		0.3335	1.3350	1.0015	1.0015	1.3300	0.9965	0.9974	0.0042	0.4160
		0.3599	1.3608	1.0009		1.3579	0.9980			
	RMR3	0.3815	1.3844	1.0029		1.3774	0.9959			
		0.3359	1.3363	1.0004	1.0014	1.3347	0.9988	0.9929	0.0084	0.8422
		0.3348	1.3356	1.0008		1.3189	0.9841			

Appendix 6: Ash content percentage



Appendix 7: Calculation for ash content

Method	Sample	Crucible weight, g	Initial reading, g	weight of chitosan, g	After reading, g	Ash weight, g	Average, g	Percentage, %	Average
Traditional	TMR1	19.2171	21.2284	2.0113	20.8558	1.6387	1.6078	79.9	
		19.2920	21.2998	2.0078	20.8688	1.5768			
	TMR2	21.7293	23.7537	2.0244	23.2529	1.5236	1.5141	74.8	78.7
		21.1070	23.1209	2.0139	22.6115	1.5045			
	TMR3	23.2184	25.2185	2.0001	24.8441	1.6257	1.6302	81.5	
		24.7880	26.7581	1.9701	26.4226	1.6346			
Reversing	RMR1	16.6818	18.6969	2.0151	18.4244	1.7426	1.7410	86.4	
		20.4729	22.5342	2.0613	22.2123	1.7394			
	RMR2	19.1602	21.1636	2.0034	20.9300	1.7698	1.7374	86.7	85.8
		19.0727	21.0744	2.0017	20.7777	1.7050			
	RMR3	16.3066	18.3063	1.9997	18.0066	1.7000	1.6826	84.1	
		18.8519	20.8521	2.0002	20.5171	1.6652			

Appendix 8: p-Anisidine Hydrochloride (Rondle and Morgan, 1955) method

This study was focusing to the glucosamine content in the chitosan. Based on observation by Pauly and Ludwig (1992), they stated that in alkaline solution in 100 °C, the amino sugar react to the acetyl acetone to form chromogenic material. Then the chromotophore occur on treatment in acid solution with ethanolic *p*-di-methylamino-benzaldehyde.

The procedure began with 1 drop of phenolphthalein initially added into 1 ml of crude chitosan (hydrolizite) that contained in the test tube. Then, 1 ml of acetyl acetone reagent was added and gently mixed. The wall of test tube was washed with 1 ml of distilled water. Then, minimum quantity of N-NaOH was run in to give a full pink colour. Dilute HCl (approximately 0.3 N) was added until the indicator colour discharge.

The tube are closed with long necked (2-3cm) sealed glass ampoules (2-3 ml volume) each containing 1-2 ml of water. After that, the tube was heated vigorously in boiling water bath for 20 minutes. The tube was cooled to room temperature after heated (in cold water bath) and step continually by added ethanol (approximately 5.0 ml). 1 ml of Ehrlich's reagent and ethanol were also added until reach 10 ml mark. The contents was mixed thoroughly but gentle and warmed for 10 minutes in water bath at 65-70 °C to accelerate liberation of carbon dioxide (CO_2). The tube was cooled to 18 °C and the mixture was mixed again and the colour of mixture was measured. Below is the figure of this method result;

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PRODUCTION OF CHITOSAN FROM MARINE CRAB, SCYLLA SERRATA- SITI ASMAA' BINTI BAHARUDIN