PRODUCTION AND CHARACTERIZATION OF
CHITOSAN FROM SHRIMP SHELLS

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ABSTRACT

Chitosan is versatile natural polysaccharides which produce from deacetylation of chitin. Chitin is the second most abundant natural biopolymer. It is a homo-biopolymer composed of β-1,4-N-acetylglucosamine and usually can be found in shell of crustaceans and the cell walls of fungi. Nowadays, seafood processing industry generates large amount of wastes, dumping these wastes into landfills and will create environmental problem such as water pollution and soil pollution. Current work is to produce and characterize chitosan from these shrimp shells wastes. Optimization of the chitosan production also was focused. Chitosan was produced through out several steps which were deproteination and demineralization of shrimp shells and lastly deacetylation. Different NaOH concentration and different reaction time in deproteination step were carried out for optimization. The optimization for reaction time for deproteination was carried out based on the optimum NaOH concentration determined. After that, the characterization of chitosan was done in aspect moisture content, ash content and the yield. Sample with 10% NaOH with 3 hours deproteination time gave highest yield of 13.10%. FTIR test was carried to was used to investigate the presented of amine and alcohol group in the chitosan. Most of the chitosan sample gave characteristic band of NH$_2$ at range 3450-3500 cm$^{-1}$ and hydroxyl group at range 1000-1100 cm$^{-1}$. Thermal tests like thermogravimetric analysis (TGA) test was carried out to evaluate the thermal stability of composites. It was found that the most thermal stability of chitosan was 10% NaOH with 3 hours. Synthesized chitosan was also used in treatment of waste water to remove the heavy metal. The highest efficiency of heavy metal removal subjected to chitosan with 10% NaOH with 3 hours which remove 97.39% of iron(Fe$^{3+}$), 92.16% of copper(Cu$^{2+}$), 72.21% of chromium (Cr$^{2+}$) and 52.72% of lead (Pb$^{2+}$) in 100ml of 10ppm solution. Thus, it could be conclude that 10% NaOH with 3 hours deproteination was optimum condition in production of chitosan.
KITOSAN DARI UDANG CENGKERANG

ABSTRAK

Kitosan adalah serba boleh polisakarida semula jadi yang dihasil daripada deacetylation chitin. Kitin adalah biopolimer kedua paling banyak semula jadi. Ia adalah homo-biopolimer yang terdiri daripada β-1,4-N-acetylglucosamine dan biasanya boleh didapati di shell krustasia dan dinding sel kulat. Kini, industri pemprosesan makanan laut menjana jumlah bahan buangan yang banyak, pembuangan sisa ini ke dalam tapak pelupusan dan akan menimbulkan masalah alam sekitar seperti pencemaran air dan pencemaran tanah. Kerja semasa ini adalah untuk menghasilkan dan mencirikan kitosan daripada cengkerang udang buangan ini. Pengoptimuman dalam penghasilan kitosan juga telah diberi tumpuan. Kitosan dihasilkan melalui beberapa langkah, iaitu deproteination dan demineralisasi cengkerang udang dan akhir sekali proses deacetylation. Kepekatan NaOH dan masa tindak balas yang berbeza dalam langkah deproteination telah dijalankan untuk pengoptimuman dalam penghasilan kitosan. Pengoptimuman untuk masa tindak balas bagi deproteination dijalankan berdasarkan kepekatan NaOH optimum yang telah ditentukan. Selepas itu, pencirian kitosan telah dilakukan dalam aspek kandungan lembapan, kandungan abu dan peratusan dalam penghasilan. Sampel kitosan yang NaOH 10% dengan masa 3jam deproteination memberikan hasil tertinggi 13.10%. FTIR ujian telah dijalankan untuk menyiaskan dibentangkan kumpulan amine dan kumpulan alkohol dalam kitosan. Kebanyakan sampel kitosan memberikan band ciri NH₂ pada julat 3450-3500cm⁻¹ dan kumpulan hidroksil pada julat 1000-1100cm⁻¹. Telah mendapati bahawa kitosan yang mempunyai kestabilan yang paling tinggi ialah 10% NaOH dengan 3jam. Kecekapan tertinggi penyingkiran logam berat adalah kitosan dengan NaOH 10% dengan 3jam yang mengeluarkan 97,39% besi (Fe³⁺), 92,16% tembaga (Cu²⁺), 72,21% kromium (Cr³⁺) dan 52,72% plumbum (Pb²⁺) dalam 100ml kepekatan 10ppm. Oleh itu, ia boleh dikatakan bahawa 10% NaOH 3jam dengan deproteination adalah keadaan optimum dalam penghasilan kitosan.
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CHAPTER 1

INTRODUCTION

1.1 Background of study

Polymers are substances whose molecules have high molar masses and are composed of a large number of repeating units. Generally, polymers can be classified into three groups which are natural polymers, synthetic polymers and polymers from microbial fermentation (Yu, Katherine, Li, 2006). Natural polymer such as polyamino acids, polysaccharides; synthetic polymers such as, polyamides, polyanhydrides, polyester and microbial fermentation, such as polyhydroxybutyrate (PHB). In recent years, biodegradable polymeric has gained importance in many field due to its raw material is easily obtained and its biodegradability that will not pollute the environment.

Polysaccharide-based polymers represent a major class of biomaterials, which includes agarose, alginate, carageenan, dextran, and chitosan. Nowadays, chitosan become more and more important and is widely used in many aspects
such as in pharmaceutical, cosmetic products, water treatment and food processing. The history of chitosan dates back to the 19th century, when the deacetylated form of the parent chitin natural polymer is discussed in 1859 (Dodane et.al, 1998). During the past 20 years, a substantial amount of work has been reported on chitosan and its potential use in various bioapplications (Pillai, 2001).

Chitosan are considerably versatile a promising biomaterial. Chitosan polymers are semi-synthetically derived amino polysaccharides that have unique structures, multidimensional properties, highly sophisticated functionality and a wide range of applications in biomedical and other industrial areas (Paul, 2000). Discussion of chitosan is always linked with chitin where chitin is always available from crustaceans such as crab shell, shrimp shell and many others seafood.

1.2 Problem Statement

Wastes are being produced everyday and the amount wastes produced keep rising. A lot of manpower and investment is used in processing and treatment of these wastes. Wastes are produced in all ways and they are required to be disposed of through approved routes.

Similar to most industries, seafood processing operations produce waste in a solid or liquid form which include viscera, skin, heads, shells, washing and
cleaning water discharge, blood water from drained fish storage tanks and brine (Subasinghe et al., 2010). These wastes must be stored so as to prevent the contamination to the processing environment, and should be disposed of in a manner that is not detrimental to the receiving environment. The magnitude of the problem of waste management in the fish industry depends on the waste volume, its polluting charge, rate of discharge and the assimilatory capacity of the receiving medium.

Nowadays, large amount of organic wastes including crab or shrimp shells are discharged from fish market and food processing. According to International Daily Newswire (2011), there are even a lot of unauthorized discharges of seafood processing waste to environment without proper treatment. These unauthorized discharges of seafood processing waste results in large seafood waste left on the seafloor and cause fostering oxygen-depleted conditions that result in unsuitable habitats for fish and other living creatures in Alaska. Besides, in east coast of United States, seafood industries are widely developed. Crabs and shrimps are important seafood product of United States. The cumulative amount of domestic landing for crabs in both 2003 and 2004, were more than 290,000 metric tons were reported from NOAA Fisheries (2004). In addition, more than 70% of seafood including crabs and shrimps shell is considered processed waste material (Brown, 1981).

The seafood processing industry generates a significant amount of waste. It is estimated that approximately 312,875 tonnes of seafood processing waste is produced each year in the UK. Approximately 80% (249,950 tonnes) of this is
finfish waste whereas 20% (62,925 tonnes) is shellfish (Archer et. al, 2005). It has not been possible to estimate the quantities of waste produced by other sectors of the industry. The residues from seafood processing plants were dumped into landfills and will create environmental problem such as water pollution and soil pollution. The seafood processing waste has produced economic strains for many processing plants. The disposal of seafood waste tends to attract pests such as flies and mosquitoes, pathogen, encourage growth of bacteria, produce offensive odor and create an eyesore to nearby residents (Burrows et, al., 2007).

It is a huge findings that chitin is found in the shell of crustaceans such as crab shells and shrimps shells. Chitin is the second abundant biopolymer in nature (Knorr, 1984). Chitin is always made from crustaceans and therefore; crab shell is a source of chitin and chitosan. A lot of study has been conducted show that chitin and its derivatives that extracted from crustaceans can be useful in environmental science, especially in waste water treatment and in agriculture.

1.3 Objectives of Study

The main objective of this study is to produce the chitosan from the shrimp shells wastes. The objective is divided into :

(i) To produce and characterize the properties of chitosan from shrimp shells wastes.
(ii) To optimize the maximum yield of formation of chitosan.

(iii) To test the efficiency of chitosan in wastewater treatment.

(iv) To enhance the utilization of seafood shells wastes and minimize the environmental pollution.

1.4 Scope of Study

There are two general aims of this research which are production and characterization chitosan. The shrimp shell is chosen as raw material to produce the chitosan through some steps as below:

- Deproteination using sodium hydroxide (NaOH)
- Demineralization using hydrochloric acid (HCl)
- Deacetylation using sodium hydroxide (NaOH)

Several of parameters are set to optimize the quality of product formed which is the concentration of alkaline for deproteination and the time of deproteination. Then the chitosan produced will be characterized by using:

- Chemical structure analysis by Fourier Transform Infrared Spectroscopy (FTIR)
- Thermogravimetric (TGA) for thermal analysis.
- Efficiency of chitosan on water treatment process by using Atomic Absorption Spectroscopy (AAS).
1.5 Significance of Study

In this study, by producing chitosan from shrimp shell waste plays an important role in waste water treatment process. Chitosan can act as coagulant in waste water treatment which is no harmful effect on human health.

Besides, producing chitosan is also one of an effective way to reduce the environmental pollution since large amount of organic wastes are discharged from seafood processing industry. Chitosan is a versatile biopolymer which can be used in many fields such as waste water treatment, cosmetic, agriculture, pharmaceutical and many more. Therefore, it is an effective treatment method or recycling technology of organic wastes as a renewable resource.
2.1 Seafood Processing Industry

In historical times, various preservation techniques were evolved, which included drying, salting and smoking of fish and shellfish, in large quantities in barrels. In all these methods, the intestines, head and shells were removed and thrown away or disposed of. As a result large catches of fish and shellfish are practiced from year to years due to the developed of seafood industry. Disposal of these wastes should be handling well to prevent environmental issues. Approximately, about 75% of the total weight of shellfish is discarded as waste, and in some cases, as in crustacean meat industries, the waste material can represent an amount greater than 80% of the landing (Simpson, 1978), from 20 - 30% of the dry weight of the waste is chitin, depending upon the processing method.
Currently methods of handling the waste of seafood processing industry include ocean dumping, incineration and land filling. But these methods will cause environmental problem when there are too large amount of waste. Ocean dumping is strictly banned worldwide because shellfish beds will be contaminated and infected by parasites, oxygen level decreased which can kill fish population (Collie and Russo, 2001). Incineration caused green house effect and land filling caused soil pollution.

Thus there are some ways that introduced to alleviating the waste disposal problem of the seafood industry. For examples: production of fishmeal, bioconversion of chitin wastes into single cell protein for animal feed and aquaculture fees and production of chitin and chitosan from crustacean wastes. Chitosan and chitin are biodegradable which obtained from crustacean wastes like crab or shrimp shells and are polymers classified under polysaccharides (Isabelle and Lan, 2009).

2.2 Chitin

Chitin is the second most abundant natural biopolymer. It is a homopolymer composed of β-1,4-N-acetylglucosamine (Khorrami et. al, 2012). Chitin has same chemical structure in fungi and animals, it usually can be found in the shell of crustaceans and the cell walls of fungi (Knorr, 1984). It could consider as cellulose because it functions naturally like polysaccharides. Besides, it is a highly insoluble material resembling cellulose in its solubility and
low chemical reactivity. Chitin is white, hard, inelastic, nitrogenous polysaccharide and major sources of surface pollution in coastal areas (Ravi, 2000).

Figure 2.1: Chitin Structure.

2.3 Chitosan

Generally, chitosan is modified natural polysaccharides derived from chitin. It derived from naturally occurring sources, which is the exoskeleton of insects, crustaceans and fungi that have been shown to be biocompatible and biodegradable. Chitosan is a natural biopolymer or also called as natural amino-polysaccharides (Hirano et. al, 1990). Chitosan is N-acetylglucosamine obtained after partial de-N-acetylation of chitin, which, in turn, is a major component of the shells of crustaceans and found commercially in the offal of seafood processing industry (Tharanathan and Kitture, 2003).

Chitosan is usually produced from deacetylation of chitin (Shepherd, 1997). A lot of study in chitin and chitosan were conducted and were reported that chitosan and its potential use in various bio-application. This is due to its
naturally occurring resources which are exoskeleton of crustaceans, insects and fungi and it is biocompatible and biodegradable.

![Chitosan Structure](image)

**Figure 2.2**: Chitosan Structure.

### 2.3.1 Properties of Chitosan

Chitosan properties include their solubility in various media, solution, viscosity, polyelectrolyte behavior, polyoxysalt formation, ability to form films, metal chelations, optical and structural characteristic. Since chitosan is taken from chitin, the main difference between chitin and chitosan is the percentage of the acetyl groups in their chemical structure (Viarsagh et al, 2009). The acetyl groups are taken off from chitin structure. If the percentage of acetyl glucosamine is more than 50%, we have chitin and if this percentage is less than half, the material is called chitosan.

Chitosan is essentially more soluble derivative of cellulose compared to chitin. It is soluble in most diluted acid such as acetic acid and formic acid whereas chitin is insoluble (Zohuriaan-Mehr, 2004). This is due to the amine groups that present in chitosan absorbs protons when pH of solution is less than 6.
Besides, chitosan have high efficiency in binding of metal ions and anionics dyes. Uptake may occur through chelation on free amino functions (at near-neutral pH) or by electrostatic attraction on protonated amino groups (in acidic solutions) (Guibal, 2004). In other words, its cationic properties in acidic solutions give it the ability to interact readily with negatively charged molecules such as fats, cholesterols, metal ions, and proteins.

Recently, there has been some researchers report that the gel forming ability of chitosan in N-methylmorpholine N-oxide with its application of controlled drugs releases formulation (Ravi, 2000). At room temperature, chitosan forms aldimines and kedimines with aldehydes and ketones respectively. Chitosan is also biocompatible and is not toxic for live cells. Chitosan offers another advantage by being able to form micro or nano-sphere formulations without the use of organic solvents, which maintains the immunogenicity of the antigens (Illum, 2001). The high chemical reactivity of chitosan, has also led to several chitosan-drug conjugates for cancer therapy (Onishi, 2001).
Table 2.1: Chemical and Biological Properties of Chitosan.

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<td></td>
<td>Binds to mammalian and microbial cell</td>
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<td>Regeneration effect on connective gum tissue</td>
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<td>Reactive amino groups</td>
<td>Hemostatic</td>
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<td></td>
<td>Fungistatic</td>
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<td></td>
<td>Spermicidal</td>
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<td>Reactive hydroxyl groups available</td>
<td>Antitumor</td>
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<td></td>
<td>Anticholesteremic</td>
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<tr>
<td></td>
<td>Accelerates bone formation</td>
</tr>
<tr>
<td>Chelates many transitional metal ions</td>
<td>Central nervous system depressant</td>
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<td></td>
<td>Immunoadjuvant</td>
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</table>

2.4 Application of Chitosan

Chitosan is a versatile environmental friendly material. A large increase in chitosan research due to its biocompatibility, biodegradability non-toxicity and others unique properties. Nowadays chitosan are widely used in many industries such as cosmetics, water engineering, paper industry, textile industry, food processing, biomedical, agriculture and many more. Many researchers interest in the study of chitosan due to its chemical physical properties. Different chitosan is needed for different field of uses such as the degree of deactylation and the molecular weight.
2.4.1 Chitosan in Water Engineering

In waste water treatment process, chitosan is researched for recovery of metal ion or organic compound in the industrial waste water. Chitosan is more environmental friendly and have high sorption capacities, easy degradation routes at the end of life cycle and relatively low cost compared to sophisticated resins (Dubois et. al, 1995). Chitosan can be used as flocculating agent. It can also act as chelating agent and heavy metals trapper because of its polycationic nature.

Chitosan can compete effectively with synthetic resins in the capture of heavy metals from processing water. Furthermore, chitosan molecules agglomerate largely anionic wastes in solution to form precipitates and floe. Thus, it can act as a flocculent for recycling of food processing waste (Sridhari, 2000). The chitosan is used as flocculent to investigate both the optimal pH and the dosage for turbidity, color, COD, suspended solid reducing and the economic effect. Besides, the amine (–NH₂) group is regarded as cationic electrolyte. A few study studies have shown the high efficiencies of using chitosan to coagulate the suspended particles in wastewater (Fung Hwa & Weng Po, 2006)

Chitosan was also used successfully as an anion exchanger in the purification of vinasse containing water (Ivo et. al, 2000). Further-more, chitosan is a substitute for carboxymethyl cellulose used for the treatment of
dairy wastewater to recovery proteins and fats prior to discharge to municipal sewers, and the recovered sludge can be used as a food additive. Therefore, using chitosan to treat the wastewater from milk processing plant to remove fat and protein in it is practical and feasible.

2.4.2 Other Application of Chitosan

In cosmetic industry, chitosan derivatives offer uses in three areas of cosmetics which are hair care, skin care and oral care. Usually acids are use as a good solvent for cosmetic application. Chitosan, a natural amino polysaccharide can replace these hydrocolloids which are polyanions. Chitosan is the only natural cationic gum that becomes viscous on being neutralized with acid. It facilitates its interaction with common integuments (skin covers) and hair (Kumar, 2004).

Besides, chitosan also use in paper industries because of its biodegradability. The biodegradability of chitosan can strengthen recycled paper and increase the environmental friendliness of packaging and other products. In textile industry, chitin can be used in printing and finishing preparation, while chitosan is able to remove dyes from dye processing effluent (Bhavani, 1999). Other applications of chitosan are in the production of toilet paper, wrapping paper and cardboard.

Chitosan also used in chromatographic support when there is the presence of free –NH₂, primary and secondary –OH groups. There is research
showed that the used chitin and chitosan as sorbent material to solid phase extraction of phenol and chlorophenols by using High-Performance Liquid Chromatography (HPLC) (Rhee et. al, 1998).

2.5 Characterization of Chitosan

2.5.1 Degree of Deacetylation of Chitosan

The quality and properties of chitosan product may vary widely because many factors in the production process can influence the characteristic of the final product. The degree of deacetylation in chitosan is greatly influences the polymer’s characteristic such as charge density, solubility and degradation rate (Aiba, 1989). Chitosan is commercially available from a lot of industries manufacturer in various grades of purity, molecular weight and degree of deacetylation. For instance, chitin with a degree of deacytaltion of 75% or above is generally known as chitosan. It is important that to determine the degree of deacetylation in chitosan since different degree of deacetylation in chitosan are used in different application.

The degree of deacetylation is the parameter that indicates the molar percentage of monomeric units that have amino groups and vary from 0, chitin to 100, fully deacetylated chitin (Kohler et. al, 2005). In addition, the degree of deacetylation can be employed to differentiate between chitin and