RECOVERY AND APPLICATION OF CHITOSAN FROM SABAH SHRIMP SHELL WASTE THROUGH DEACETYLATION OF CHITIN AT DIFFERENT PARAMETERS

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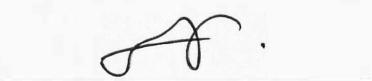

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ABSTRACT

The comparison between head shell and body shell was carried out to determine the best option to be used in this study. Besides that, the deacetylation of chitin at different parameters were also investigated which including soaking frequency, temperature and time treatment factor, to recover chitosan. This study was performed by soaking frequency once and twice, temperatures at 50°C, 60°C, 70°C and 80°C, and time treatment 12, 24, 36 and 48 hours while the characterization of chitosan was determined in terms of moisture content, ash value, solubility and degree of deacetylation. Besides that, a preliminary investigation was carried out to study the effect of chitosan in tilapia fish feed towards growth and survival rate by using 1.00%, 2.00% and 3.00% chitosan. The results obtained in this study shows that the body shell was a better option to produce chitosan as it produces higher chitosan yield compare to head which were 13.35% and 7.27%, respectively. In the meantime, deacetylation process at room temperature for twice soaking gave a higher degree of deacetylation value and solubility compare to once which were 75.24% and 88.57%, while the solubility were 97.78% and 98.32%, respectively. Furthermore, the moisture content and ash value of the chitosan produced in this study ranged from 4.27% to 9.19% and 0.22% to 0.90%, respectively. In addition, deacetylation process carried out at temperature 70°C and time treatment 48 hour gave the highest solubility and degree of deacetylation which was 97.00% and 90.96%, respectively. For the meantime, the application of chitosan-supplemented fish feed on tilapia suggests that 1.00% chitosan increased the highest fish weight with 142.80%, while the 3.00% chitosan increased the length with 48.74%. The survival rate of tilapia fish was also observed to be 100% when 1.00% and 2.00% chitosan were used as the feed, compared to control with 63.00% only. Thus, this study demonstrated temperature and time treatment factors play a crucial role in recovering a good solubility and degree of deacetylation quality of chitosan. Chitosan produced in this study was also high in quality with 90.96% of degree of deacetylation, which can be used in industry that need further investigation for different application.

ABSTRAK

Perolehan-semula dan Penggunaan Kitosan Daripada Sisa Kulit Udang Sabah Melalui Diasetilasi Kitin Pada Parameter Berbeza

Perbandingan di antara kulit kepala dan kulit badan sisa udang dilakukan untuk menentukan pilihan yang terbaik bagi diasetilasi dan keterlarutan untuk menjalankan kajian ini. Kajian ini juga dilakukan untuk mengenalpasti faktor yang mempengaruhi proses diasetilasi iaitu kekerapan rendaman sekali dan dua kali, suhu 50°C, 60°C, 70°C dan 80° C dan masa rawatan tindak balas iaitu 12, 24, 36 dan 48 jam. Pencirian untuk kitosan yang dihasilkan daripada kajian ini juga dianalisis dari segi nilai lembapan, nilau abu, keterlarutan, dan juga kadar diasetilasi. Selain itu, kajian awal juga dilakukan bagi mengkaji kesan penggunaan kitosan iaitu 1.00%, 2.00% dan 3.00% dalam makanan ikan tilapia terhadap tumbesaran ikan serta kadar hidup lebih lama. Kajian awal menunjukkan bahawa kulit badan dapat menghasilkan pengeluaran kitosan lebih tinggi berbanding dengan kulit kepala iaitu 13.35% dan 7.27%. Di samping itu, proses diasetilasi yang dilakukan rendaman dua kali menunjukkan peratusan kadar diasetilasi yang lebih tinggi iaitu 88.57% berbanding dengan sekali rendaman iaitu 75.24% manakala tahap kelarutan adalah 97.78% and 98.32%. Nilai lembapan dan nilai abu kitosan yang terhasil adalah dalam julat 4.26% ke 9.19% dan 0.22% ke 0.90%. Selain itu, proses diasetilasi pada suhu 70°C dengan masa rendaman 48 jam memberikan keputusan yang lebih baik iaitu kadar kelarutan 97% dan kadar diasetilasi 90.96%. Penggunaan kitosan dalam makanan ikan tilapia yang mengandungi 1.00% kitosan memberikan peratusan kadar peningkatan berat ikan yang tertinggi iaitu 142.80% manakala 3.00% kitosan pula memberikan peratusan kadar peningkatan panjang ikan yang tertinggi iaitu 48.74%. Kadar hidup lebih lama pula adalah 100% untuk makanan ikan yang mengandungi kitosan 1.00% dan 2.00% berbanding dengan diet kawalan iaitu 63.00% sahaja. Kajian ini menunjukkan kitosan yang dihasilkan dalam kajian ini mempunyai nilai diasetilasi 90.96% dengan kualiti yang baik dan boleh digunakan untuk industri dan harus lebih dilakukan kajian yang lebih mendalam bagi memanfaatkan semua orang.

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$CaCO_3$	-	Calcium carbonate
CH_3COOH	-	Acetic acid
CH_3CONa	-	Sodium acetate
$COCH_3$	-	Acetyl group
CO_2	-	Carbon dioxide
C1	-	Soaking frequency once
C2	-	Soaking frequency twice
D_1	-	1% chitosan diet
D_2	-	2% chitosan diet
D_3	-	3% chitosan diet
D_c	-	Control diet
DA	-	Degree of acetylation
DDA	-	Degree of deacetylation
DM	-	Demineralization
DP	-	Deproteination
DA	-	Deacetylation
DC	-	Decoloration
DO	-	Dissolved oxygen
HCl	-	Hydrochloric acid
H_2SO_4	-	Sulphuric acid
$KMnO_4$	-	Potassium permanganate
M	-	Molarity
M_W	-	Molecular weight

NaOH	-	Sodium hydroxide
KOH	-	Potassium hydroxide
$-NH_2$	-	Amine group
NH_3^+	-	Ammonium ion
-OH	-	Hydroxyl group
PET	-	Polyethelyne
PTO	-	Peeled tail on
PUD	-	Peeled undeveined
T	-	Temperature
t	-	Time treatment

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In Sabah, shrimp is one of the most important fisheries product where it is mostly exported as frozen food after undergone a process of head and shell removal (Hajjah *et al.*, 2010). In general, the removal of head and shell are treated as a waste as it is disposed on landfill for final disposal. The disposal of seafood processing waste has been considered as a worldwide problem due to the current tightening of regulations and guidelines surrounding the disposal of shrimp fisheries processing waste where it faces rising in cost and difficulty of its disposal. A potential impact such as physical, chemical, biological and socio-economic to the environment has raised a big concern to the authorities (Mazik *et al.*, 2005).

Usually seafood waste arises from trawling together with processing such as gutting, filleting, cooking, pickling, preserving, and packing of seafood products where it generates both solid waste and liquid effluent consist of both organic and inorganic materials. Interestingly, the fishery waste has an economical value for chitin and chitosan industries (Patria, 2013). For crustaceans processing waste, it is estimated around 40% shell (exoskeleton) and 60% offal and organic matter. The shrimp shell waste contains protein (30-40%), calcium carbonate (30-50%) and chitin (20-30%) which can be isolated to produce chitosan, glucosamine and other derivatives (Nouri *et al.*, 2015).

Chitin, a homopolymer of N-acetyl-D-glucosamine, is the most abundant renewable natural resources and the main source of it is crustacean waste (Limam *et al.*, 2011). It is often considered as cellulose derivative even though it does not occur in organism producing cellulose. Chitin is white, hard inelastic, nitrogenous

polysaccharides found in the exoskeleton as well as in the internal structure of invertebrates. It is a stable compound and generally biodegradable by some organism including humans (Pal *et al.*, 2014) but its slow biodegradation in crustacean shell waste, gathering of large quantities of processing crustacean shell such as shrimp shell waste is a major problem faced by seafood industry. Therefore, the isolation of chitin and chitosan from shrimp shell waste are carried out since chitin and its derivatives have high economic value owing to their biological activities and agrochemical application as well as in many other fields such as pharmaceutical, water engineering, food industry, and biomedical. Among the novel families of biological macromolecules, an increasingly evident for chitin and its main derivative, chitosan potential uses are estimated to be more than 200 (Abdulkarim *et al.*, 2013).

Chitosan, which is a natural polysaccharide consist of copolymers of glucosamine and *N*-acetylglucosamine has been widely used in various fields ranging from industrial to biomedical such as pharmaceutical and biotechnology. This derivatives of chitin becomes an interesting material due to its unique features which includes biodegradability, biocompatibility, as well as low toxicity. Moreover, chitosan also known to consist of amino groups to chemically react with anionic systems, which resulting in alteration of physicochemical characteristics such as binding, disintegrating and tablet coating properties (Puvvada *et al.*, 2012). As for its unique features, chitosan possesses numerous properties which includes chemical and biological aspects. Chemical properties of chitosan are enclosed with reactive amino group, reactive hydroxyl group, linear polyamine, and can chelates many transitional metal ions whereas the biological properties of chitosan comprise biocompatible, binds to mammalian and microbial cells, hemostatic, fungistatic, antimicrobial, spermicidal, antitumor, anticholesteremic, accelerates bond formation, immunoadjuvant, and depressant for central nervous system (Dutta *et al.*, 2004).

Chitosan can be obtained by a traditional extraction method that consist of three stages which are deproteination, demineralization and deacetylation (Abdulkarim *et al.*, 2013). By using this traditional method, deproteination process is carried out by immerse the shell sample in a diluted alkaline solution such as sodium hydroxide to remove proteins while for demineralization process the shell

sample is immersed in a diluted acidic solution such as hydrochloric acid to remove carbon and other salts that present in the crude form. For the last stage of the process which is deacetylation process, it is the most crucial process to obtained chitosan and also play an important role in determining the quality of the chitosan produced. The deacetylation process is carried out to remove acetyl group from chitin chain which leaving behind amino group. Early studies showed that some of the specific characteristics of chitosan can be affected by process condition. This process of converting chitin to chitosan can be affected by several factors such as temperature, concentration and volume of alkaline solution, time treatment, and size of chitin sample (Majeti and Ravi, 2000).

It is reported that poor stability of chitosan has restricted its potential application, thus it has become a challenge to many researchers to accomplish a good stability of chitosan such as strong intermolecular interactions between formed fragments of chitosan (interchain crosslinking) alter the polymer structure, thus leading to the irreversible loss of its physicochemical properties. Several factors which affecting chitosan stability is divided into two types that are internal and external which includes purity level, molecular weight, polydispersity index, deacetylation degree, pattern of deacetylation and moisture content while for external factors includes acidic dissolution, sterilization, thermal processing, and physical methods (Szymanska and Winnicka, 2015). The most crucial characteristic of chitosan is degree of deacetylation (DDA) which can be used to compare between chitin and chitosan because it determines the content of free amino groups in the polysaccharides as well as may influence the physical, chemical, and biological properties of chitosan (Hussain *et al.*, 2013). The DDA of chitosan usually ranges from 40-60% and the commercial chitosan samples have average DDAs of 70-90% (Hussain *et al.*, 2013). Chitosan is a hygroscopic compound which having a great ability to form hydrogen bonding with water compared to chitin, may affect its capability to absorb water. In addition, the solubility of chitosan in acidic solution also plays an important role in determining the quality of chitosan because the dissolution of chitosan in diluted acid is a routine stage in many industrial application (Szymanska and Winnicka, 2015). Its solubility in acidic solution where it is a positively charged and depends on the pH as well as the percentage of DDA value,

is a bioadhesive and readily binds to a negatively charge compounds (Puvvada *et al.*, 2012).

Chitosan has been successfully used in various field including agriculture specially to maintain the quality of harvested fruits and increase the growth of vegetables and other crops (Jitareerat *et al.*, 2007). In previous studies, chitosan also has been reported can increase the defense system in plants to maintain the quality of crops by reducing the respiration rates, ethylene production, and also transpiration as well as its fungistatic or fungicidal properties against pathogens of various crops (Bautista-Banos *et al.*, 2003). As reported by Rinaudo (2006) chitosan can serve as a promising candidate as pharmaceutical excipient as well as because of its several features such as immunologic, antitumor, hemostatic, anticoagulant, bacteriostatic and healing ability for wound. For water and wastewater treatment, chitosan has the ability as flocculant to clarify water, chelation of metal ions, reduce colors and turbidity, coagulation of suspended solids, dye removal, sludge treatment, and filtration (Renault *et al.*, 2009). Furthermore, in food and beverages industry, chitosan can be used as preservatives, dietary fiber, thickener and stabilizers, antibacterial coating, and bind lipids to reduce cholesterol intake.

1.2 Problem Statement

The disposal of shrimp shell waste in Sabah has significantly increasing and give rise to a big concern to the environmental issue because it is usually disposed by dumping on landfill. In the 1997 export market, frozen crustaceans leads the export value with a total of RM145,763,435 million worth of frozen shrimps, lobsters and crabs which along with 5837.67 tons of total crustaceans being exported. With this high value of total export frozen crustaceans, it may produce a very high amount of shell waste. By converting this shell waste into a valuable resource, it may reduce the disposal of shell waste on landfill. Chitosan can be extracted from shrimp shell waste by using chemical method which consists of deproteination, demineralization and deacetylation. However, the quality of chitosan which been extracted from shrimp shell waste can differ based from the parameter which is applied during the extraction process. The quality of chitosan can be determined by its degree of deacetylation, molecular weight, viscosity, water binding capacity, fat binding