TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Extraction and Characterization of Chitosan from Shrimp Shell Waste in Sabah

Flornica Alca Ahing* & Newati Wid
Faculty of Science and Natural Resources, Universiti Malaysia Sabah,
Jalan UMS, 88400, Kota Kinabalu, Sabah, MALAYSIA.
*Corresponding author. Email: ikalca88@gmail.com

Received: *19 March 2016*
Revised: *8 April 2016*
Accepted: *23 April 2016*
In Press: *1 May 2016*
Online: *30 June 2016*

Keywords:
Extraction; Chitosan; Moisture Content; Solubility; Degree of Deacetylation

Abstract
Chitin is the most widespread renewable natural sources following cellulose and the main source of chitin is crustacean waste. Chitosan which is a derivative of chitin after the process of deacetylation has multiple of commercial and possible medical uses based on its degree of deacetylation. This research aims to study the production of chitosan from shrimp shell waste in Sabah and characterize the chitosan quality which includes parameters including moisture content, solubility, and degree of deacetylation (DDA). The results obtained from this study show that moisture content ranged from 4-7%, while the solubility of chitosan achieved up to 90%. The DDA value obtained was high ranged from 70-85%. Based on these three characteristics, shrimp shell waste in Sabah can achieved chitosan standard quality for industrial application by performing traditional method of deproteination, demineralization and deacetylation.

Introduction

Shrimp is one of important fisheries product worldwide including Sabah, where this product is mostly exported as frozen food after undergone a process of head and shell removal. In Sabah, the shrimp processing industry has been rapidly increasing with the growing of cultured shrimp production at the local area thus increasing the bio-waste of shrimp shell. It is nearly about 45% waste of this processed seafood disposed on landfill, consequently leads to environmental pollution in terms of odor and aesthetic damage to the environment (Al-Sagheer *et al*., 2009). However, the fishery by product has an economical value for the chitin and chitosan industry (Patria, 2013). Generally, the shrimp waste contains protein (30-40%), calcium carbonate (30-50%) and chitin (20-30%) (Nouri *et al*., 2015).

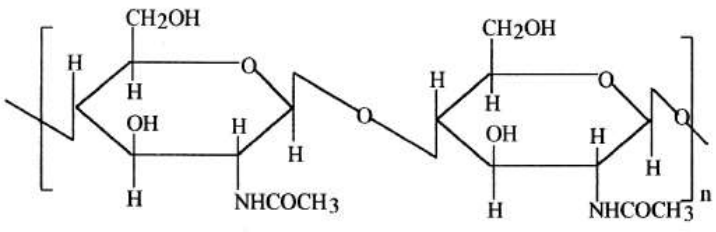


Figure 1. Structure of chitin

Chitin, a homopolymer of N-acetyl-D-glucosamine (Figure 1), is the most abundant renewable natural resources and the main source of it is crustacean waste. 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). Chitin and its derivatives have high economic value owing to their biological activities and agrochemical application.

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 can be obtained by performing a traditional method which consist of three main stages that is deproteination, demineralization and deacetylation process of shrimp shell waste (Abdulkarim *et al*., 2013). Deproteination is carried out by treating shell waste in an alkaline solution such as 1.0 M sodium hydroxide solution (NaOH) while demineralization is performed by treating shell waste in acidic solution such as 1.0 M hydrocholoric acid (HCl) solution. For the deacetylation process, chitin is treated with concentrated alkaline solution such as 45-50% sodium hydroxide (NaOH) to remove acetyl group from chitin polymer to obtain chitosan (Rinaudo, 2006).

Chitosan that derived from crustaceans that are soluble in organic acids as one of a feature of natural compounds is successfully used in maintaining the quality of harvested fruits and increase the growth of vegetables and other crops (Jitareerat *et al*., 2007). In previous studies, chitosan has been reported can maintain the quality of crops by reducing the respiration rates, ethylene production, and also transpiration as well as its fungi static or fungicidal properties against pathogens of various crops. Its solubility which depend on the pH as well as the percentage of degree of deacetylation value (DDA), is a bioadhesive and readily binds to a negatively charge compounds (Puvvada *et al*., 2012). As for its various potential, this make the chitosan as a promising candidate as pharmaceutical excipient.

However, the 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 by controlling several factors such as moisture content, deacetylation degree, and solubility in acidic solution (Szymanska & Winnicka, 2015). Degree of deacetylation (DDA) can be used to compare between chitin and chitosan because it determines the content of free amino groups in the polysaccharides. Besides, it also influence the physical, chemical, and biological properties of chitosan such as acid base and electrostatic characteristic, biodegradability, self-aggregation, sorption properties, and the ability to chelate metal ions (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 determination of the quality of chitosan because the dissolution of chitosan in diluted acid is a routine stage in many industrial applications (Szymanska & Winnicka, 2015). Therefore, the aim of this study is to extract and characterize the chitosan produced from the shrimp shell waste in Sabah, Malaysia.

Materials & methods

Sample Collection and Preparation

The raw shrimp shell waste was collected from Kian Huat Seagull Sdn Bhd, Putatan which nearby Kota Kinabalu city. It is one of the factories which produced large quantities of shrimp shell waste in Sabah. The sample was washed with tap water to remove any insoluble material on the shell then dried under the sun for 8 hours. The sample was stored in a closed container prior to use.

Extraction of chitin by chemical method

a. Deproteination (DP)

This study was performed at laboratory scale using 500 mL beaker. A total of 30 g samples of raw shrimp shell waste were added with 2.0 M NaOH in the ratio 1:16 (w/v) then left for 48 hours at room temperature, ~25^{o}C (Kumari & Rath, 2014) with pH ranged from 11-13. After that, the solution was filtered and the samples were washed with distilled water until neutral pH was achieved (pH6.5-8.0). Water from the samples was removed before performing the demineralization process.

b. Demineralization (dm)

Samples from deproteination process were added with 1.0 M HCl in the ratio 1:16 (w/v) and allowed to stand for 24 hours (Puvvada *et al*., 2012) with pH value ranged pH 1.0-2.5 at room temperature (~25^{o}C). After that, the solution was filtered and the samples were washed with distilled water until neutral pH was achieved (pH6.5-8.0). The samples were then dried under the sun for 6 hours and then the drying process was continued using an oven at 80^{o}C until constant weight were obtained. The dried sample is now known as chitin.

Chitosan production

a. Deacetylation (DA)

The deacetylation process was conducted by soaking dried chitin prepared from demineralization in a 48% NaOH for 48 hours at room temperature (~25^{o}C). After two days, the product is known as chitosan (Kumari & Rath, 2014). Chitosan was washed with tap water until neutral (pH6.5-8.0) and dried as described in deproteination and demineralization.

b. Analysis of Chitosan Yield

The chitosan yield (%) was calculated as the dry weight of the chitosan flakes relative to the wet weight of Sabah shrimp waste (Nouri *et al*., 2015).

$$\text{Chitosan extraction yield (\%)} = \frac{\text{Dried extracted chitosan weight (g)}}{\text{Sabah shrimp waste (g)}} \times 100\%$$

Characterization of prepared chitosan

The characterization of the extracted chitosan was performed in term of the moisture content, solubility and degree of deacetylation (DDA).

a. Moisture content

Loss on drying of the prepared chitin and chitosan was determined by a gravimetric method by performing three replicates. The water mass loss was determined by drying the sample to constant weight and the sample was measured before and after drying (Puvvada *et al*., 2012).

$$\text{Loss on drying (\%)} = \frac{\text{Wet weight (g)} - \text{dry weight (g)}}{\text{Sample weight (g)}} \text{ X 100 \%}$$

b. Solubility in acid solution

1.0g of chitosan obtained from the deacetylation process was dissolved in 100mL of 1% acetic acid solution and stirred by magnetic stirrer until a homogeneous solution was obtained. The chitosan acidic solution was then filtered using a vacuum pump. The procedure was repeated three times. The percentage of the solubility was calculated using the following (Puvvada *et al*., 2012).

Insoluble (g) = final weight of filter paper (g) - initial weight of filter paper (g)

$$\text{Insoluble (\%)} = \frac{\text{insolubleg}}{\text{sample weight,g}} \text{ X 100 \%}$$

Solubility (%) = 100 - % insoluble

c. Degree of Deacetylation

The samples of chitosan produced were characterized using Fourier Transform Infrared (FTIR) spectrophotometer in the range of 400 to 4000cm^{-1} and repeated for three replicates. The DDA of the sample were determined according to the method used by Brugnerotta *et al.,* (2001). The A_{1320} was the peak area of the band 1320 cm^{-1}, the A_{1420} was the peak area of 1420 cm^{-1} and A(1320) is peak for amide group and A(1420) is peak for amine group.

$$\text{\% DA} = \frac{(A_{1320} / A_{1420}) - 0.3822}{0.03133}$$

% DDA = 100 - % DA

where, DDA = degree of deacetylation (%)

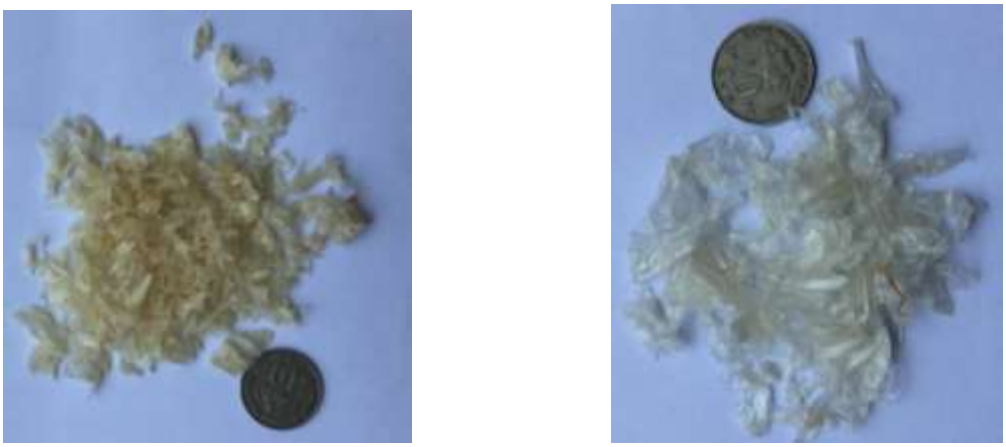
DA = degree of acetylation (%)

Results and discussion

The Produced Chitosan

This study has successfully produced about range 3.10 - 5.2 with average 4.09% of chitosan from wet weight of shrimp shell waste. Previous study done by Nouri *et al*. (2015) also shows the percentage

yield of extracted chitosan ranged from 5.6-13.5 % by using chemical method. The physical appearance of the chitin obtained was yellowish (Figure 2a), while for chitosan it was white in color (Figure 2b). It was also odorless and in a form of crystalline flakes. The characteristic of the chitosan produced from this study was similar to the chitosan obtained from previous studies (Naznin, 2005; Nouri *et al*. 2015). The white color of end product indicates a good quality of chitosan was produced which likely to be similar to previous studies as shown in Table 1.



a) Chitin b) chitosan

Figure 2. Chitin (a) and chitosan (b) obtained from this study

Table 1. Comparison of the physical appearance of chitosan produced with previous studies.

Region	Yield (%)	Color of Chitosan	Reference
Southeast Asia	15 - 30	Slight brownish to white	Naznin, 2005
Persian Gulf, Iran	5.6 - 13.5	Yellowish white	Nouri *et al*., 2015
North Iran	15.25	-	Alishahi *et al*., 2011
Sabah	4.09	white	This study

Characterization

Moisture content is one of the factors which can affect the quality of chitosan produced. In this study, moisture content for chitin and chitosan were measured and shown in Figure 3.

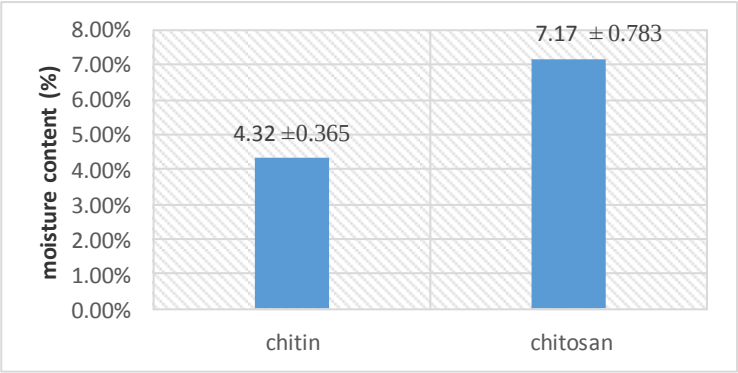


Figure 3. Moisture content of chitin and chitosan produced in this study

Normally, a good quality of chitin that produced after deproteination and demineralization processes has the moisture content less than 5%. Result in Figure 3 shows that the average moisture content of chitin obtained was 4.32% (±0.365) which indicates that the moisture content achieved the standard quality of chitin and suitable to proceed for the deacetylation process. The reason for the moisture content below 5% is because it may affect the concentration of concentrated NaOH used for the deacetylation process, thus may reduce the removal rate of acetyl group from chitin, consequently affect the degree of deacetylation of the final product. Figure 3 also shows the moisture content of the chitosan produced. It was found that the average percentage of moisture content was 7.17% (±0.783), slightly lower compared to the studies done by Puvvada *et al*. (2012), which was 9.34 %. A study done by Naznin (2005) also gives moisture content 6.62 to 8.01% by applying different concentration of alkaline solution for deacetylation process as well as previous study done by Alishahi *et al*. (2005) which reported that the moisture content of chitosan is 2.5% (Table 2). This may due to the difference in drying method used in this study, which was dried under the sun for 6 hours before drying in the oven. This method may further reduce the moisture content in chitin and chitosan samples, thus leads to a lower percentage of the moisture contents. The study performed by Szymanska and Winnicka (2015) also suggested that the moisture content of chitosan must be low which ranges from 6-10%, so that it has a greater capability to form hydrogen bonding. Furthermore, the higher the water content in the chitosan structure, the faster the damage of the polymer via hydrolysis reactions (Viljoen *et al*., 2014).

Table 2. Comparison of moisture content of chitosan produced with previous studies.

Region	Moisture Content (%)	Reference
India	9.34	Puvvada *et al*., 2012
Southeast Asia	6.62-8.01	Naznin, 2005
North Iran	2.5	Alishahi *et al*., 2005
Sabah	7.17	This study

Table 3. Comparison of solubility of chitosan produced with previous studies.

Region	Solubility (%)	Reference
Indonesia	17 - 95.29	Patria, 2013
Southeast Asia	Almost completely dissolved	Naznin, 2005
Sabah	97.92	This study

Solubility characterization is one of the most important parameter to determine the quality of chitosan, where higher solubility means better chitosan produced. This is because an increase in solubility is proportionally increased the deacetylation degree. In this study, the average solubility obtained was 97.92% (±0.07) (Table 3) compared to previous study done by Patria (2013), the solubility of chitosan obtained ranged from 17.43 to 95.29% with the average was 57.52%. The

dissolution of chitosan in 1% acetic acid also almost completely dissolved in the study done by Naznin (2005).

Chitosan is a compound which is very difficult to dissolve in water, alkaline solutions or most common organic solvents but it is soluble to some extent in dilute aqueous acid solutions (Esam *et al*., 2009). Chitosan will get protonated in the aqueous acid solution which leads to its solubility because of the presence of amino group in its molecular structure as shown in Figure 4 (Esam *et al*., 2009; Pillai *et al*., 2009).

$$-NH_2 + H_2O \longleftrightarrow NH_3 + OH^-$$

Figure 4. Chemical reaction for amine group in chitosan with water molecule

An increase in solubility is proportionally observed with increasing degree of deacetylation (Patria, 2013) due to the removal of acetyl group from chitin during deacetylation process leaving only amine group. Figure 5 shows the relationship of solubility and DDA of chitosan obtained from this study, where when the DDA increase, the percentage of the solubility of chitosan in aqueous acidic solution also increases. Chitosan consist of glucosamine and N-acetylglucosamine units which contributed to both hydrophilic and hydrophobic features in its structure. An aggregation of chitosan macromolecules in chitosan solution was due to the intra- and interchain interactions at lower pH value (Blagodatskikh *et al*., 2013). Amine group contains hydrogen ions which makes chitosan can easily interact with water through hydrogen bonding in addition with the presence of carboxyl group in acetic acid would facilitate the dissolution of chitosan due to the hydrogen interaction between the carboxyl group and the amine group of chitosan (Patria, 2013). Additionally, the solubility test of chitosan is important because it is a routine stage in most of the processing of chitosan for its application especially in pharmaceutical technology of chitosan based formulations.

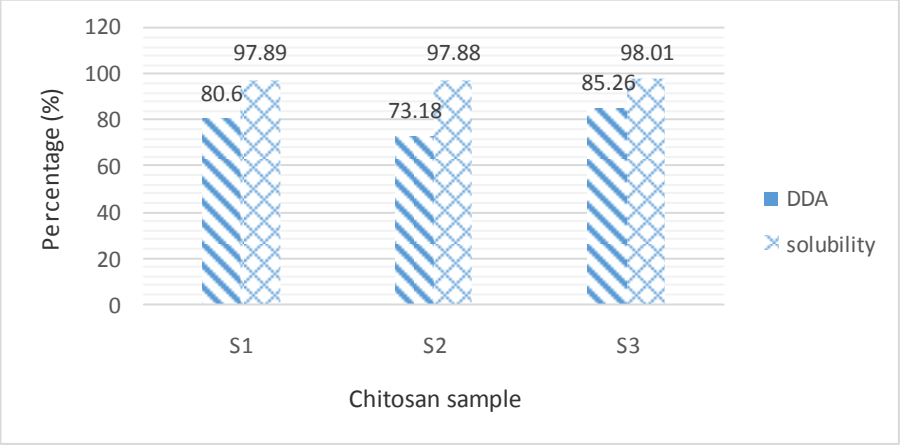


Figure 5. Relationship between DDA and solubility of chitosan.

Degree of deacetylation (DDA) of chitosan is the most important parameter in this study because DDA can be used to determine the quality of chitosan produced where it affects the chemical, physical and biological properties of the chitosan such as adsorption, covalent linking and encapsulation (Puvvada *et al*., 2012). The DDA of chitosan may affect the adsorption properties for application in water and wastewater treatment while the biological properties may be affected for application in antimicrobial based material. Figure 6 shows the comparison of FTIR spectra for one sample of chitosan obtained from the shrimp shell waste in Sabah (Figure 6a) with the chitosan obtained from Arabian region (Figure 6b).

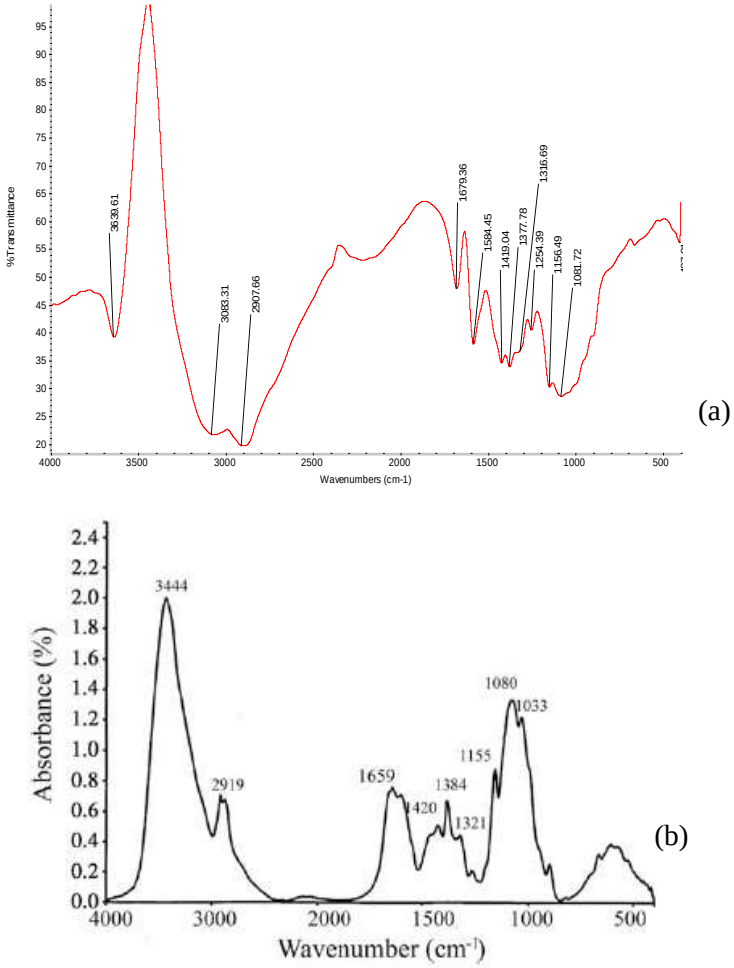


Figure 6. FTIR spectra of the chitosan from shrimp shell waste in Sabah (a) and Arabian Gulf (b).

Based on these spectra, the major absorption band is observed between 1220 and1020 cm^{-1} which represents the free amino group ($-NH_2$) at C2 position of glucosamine indicates a major group that present in chitosan. The results also showed the absorption bands for the free amino group is between 1086 and 1254 cm^{-1}. Similar results were also observed by Puvvada *et al*. (2002), 2911 (symmetric CH_3 and asymmetric CH_2 stretching), 1584 (-C=O secondary amide), and 1421 (-CN secondary amide). The same absorbance bands were observed at 3268, 2930, 1563, 1418, and 1020 cm^{-1} which indicates the structure of chitosan by previous studies (Puvvada *et al*., 2002). However, based on Coates (2000) and Abdulwadud *et al*., (2013), it is not sufficient to characterize the

functional group for the different classes of carbonyl compound overlap and the carbonyl frequency alone. According to Kumari and Rath (2014), the band at 1597 cm^{-1} has a larger intensity than at 1655 cm^{-1} which suggested that the deacetylation process was effective. The degree of deacetylation was calculated using the baseline equation as used by Brugnerotto *et al*., (2001), where the baseline used were 1320 represent amine while 1420 represent amide band. These two bands ratio A_{1320}/A_{1420} gives the narrower experimental error independent of the technique and state of material. As being only sensitive to the chemical composition of chitosan irrespectively of technique, state and secondary structure, this evidence supports the use of A_{1320}/A_{1420} (Brugnerotto *et al*., 2001). This equation also used by Abdou *et al*. (2008) and Al-Sagheer *et al*. (2009)as the IR spectra obtained also shows the major adsorption band at 1320 and 1420 cm^{-1}.

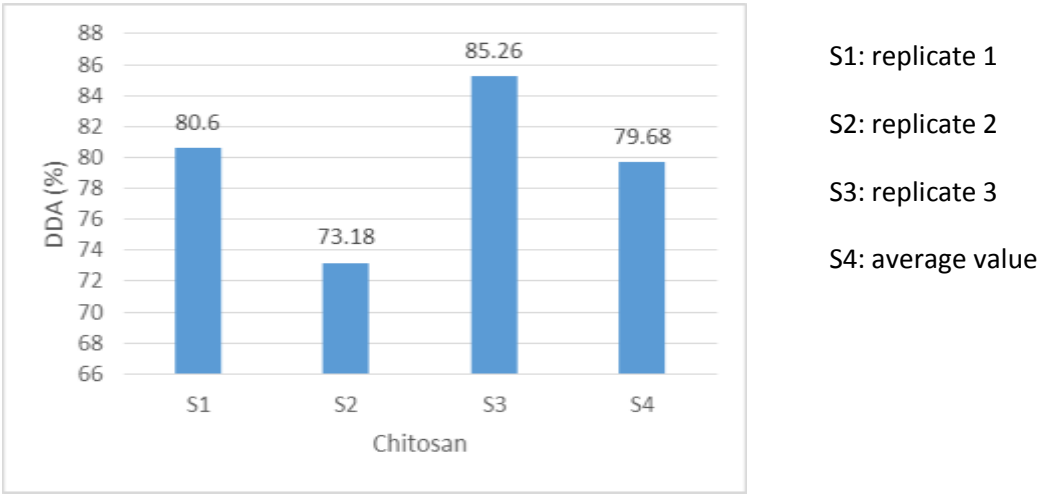


Figure 7. DDA values obtained from the shrimp shell waste in Sabah

The degree of deacetylation (DDA) obtained from shrimp shell waste in Sabah for this study was ranged from 73.18 to 85.26%. Figure 7 shows DDA value obtained for three replicates (S1, S2 and S3) of the shrimp shell waste, where Table 4 shows the comparison of DDA value obtained from the shrimp shell waste in Sabah with other regions. Sample S4 was the average DDA for all of the replicates and it shows that the shrimp shell waste has the potential to produce a good quality of chitosan based on the traditional method applied. By using the same equation to determine the DDA value, Abdou *et al*. (2008) reported that the DDA value of chitosan obtained was up to 90% by performing the deacetylation in an autoclave while Al Sagheer *et al* (2009) reported that the chitosan DDA obtained from shrimp shell waste in Arabian Gulf ranged from 88-94% by using traditional method. Nouri *et al*. 2015 also obtained DDA value ranged from 71.02-82.20% for deacetylation using traditional method while 79.01-88.60% for using microwave method. Besides, Alishahi *et al*. (2011) also performed deacetylation by using microwave and obtained chitosan with DDA value ranged from 87.5 - 93%. By using higher concentration of alkaline solution which was 50% NaOH for deacetylation process, DDA value obtained was high as well which was 89.79% (Puvvada *et al*., 2012). This explains that the DDA

values can be different due to different parameters used or conditions during the deacetylation process (Table 4).

Table 4. Comparison of DDA value obtained from shrimp shell waste in Sabah with other regions.

Region	DDA (%)	Method	Reference
Egypt	> 90	autoclave	Abdou *et al*., 2008
Arabian Gulf	88- 94	traditional method	Al Sagheer *et al.*, 2009
Persain Gulf, Iran	71.02 - 82.20	traditional method	Nouri *et al*., (2015)
	79.01 - 88.60	microwave method	
India	89.79	traditional method	Puvvada *et al*., 2012
North Iran	87.5 - 93	microwave	Alishahi *et al*., 2011
Sabah	73.18 - 85.26	traditional method	This study

Conclusion

Based on the results obtained from this study, the traditional method of extracting chitosan from shrimp shell waste can produce a whitish chitosan flakes with an average yield 4.09%. The moisture content of chitin and chitosan both were less than 10% by using method of drying under the sun for 6 hours and then proceed drying in the oven. The solubility of chitosan can be achieved up to 98.01%, while DDA value obtained was high up to 85.26%. From these results, it can be concluded that the shell waste in Sabah has the potential to be used in producing a good quality of chitosan that can be applied in different application such as agriculture and horticulture, water and wastewater treatment, food industry and other industrial uses.

Acknowledgements

The authors would like to acknowledge the Enviro Clean Energy (Sdn. Bhd) and Malaysian Nuclear Agency for the financial and technical supports (GL0129), as well as the lab assistants at the Faculty of Science and Natural Resources and Biology Tropical and Conservation Institute, Universiti Malaysia Sabah, for their assistance.

References

[1] Abdou, E. S., Nagy, K. S. A. & Elsabee, M. Z. (2008). Extraction and Characterization of Chitin and Chitosan from Local Sources. *Bioresource Technology*, **99**, 1359-1368.

[2] Abdulkarim, A., Isa, M. T., Abdulsalam, S., Muhammad, A. J. & Ameh, A. O. (2013). Extraction and Characterization of Chitin and Chitosan from Mussel Shell. *Civil and Environmental Research*, **3**(2), 108-114.

[3] Al-Sagheer, F. A., Al-Sughayer, M. A., Muslim, S. & Elsabee, M. Z. (2009). Extraction and Characterization of Chitin and Chitosan from Marine Sources in Arabian Gulf. *Carbohydrate Polymers*, **77**, 410-419.

[4] Alishahi, A., Mirvaghefi, A., Tehrani, M. R., Farahmunh, H., Shojaosadati, S. A., Dorkoosh, F. A. & Maher, Z. E. (2011). Enhancement and Characterization of Chitosan Extraction from the Wastes of Shrimp Packaging Plants. *Journal Polymer Environment*, **19**, 776-783

[5] Blagodatskikh, I. V., Kulikov, S. N., Vyshivannaya, O. V., Bezrodnykh, E. A., Yamskov, I. A. & Tikhonov, V. E. (2013). Influence of Glucosamine on Oligochitosan Solubility and Antibacterial Activity. *Carbohydrate Research*, **381**, 28-32.

[6] Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Arguelles-Monal, W., Desbrieres, J. & Rinaudo, M. (2001). An Infrared Invertigation in Relation with Chitin and Chitosan Characterization. *Polymer*, **42**, 3569-3580.
[7] Coates, J. (2000). Interpretation of Infrared Spectra, A Practical Approach. *In*: Meyers, R.A. (ed). *Encyclopedia of Analytical Chemistry*. John Wiley & Sons.
[8] Esam, A. E., Abdul, H. Y. & Misni, M. (2009). Characterisation of Chitosan Solubilised in Aqueous Formic and Acetic Acid. *Journal of Science and Technology*, **3**(03), 415-425.
[9] Prashanth, K. V. H. & Tharanathan, R. N. (2007). Chitin/chitosan: Modifications and Their Unlimited Application Potential- An Overview. *Trends in Food Science and Technology*, **18**, 117-131.
[10] Hussain, M. R., Iman, M. & Maji, T. K. (2013). Determination of Degree of Deacetylation of Chitosan And their Effect on the Release Behaviour Of essential Oil from Chitosan and Chitosan Gelatin Complex Microcapsules. *International Journal of Advanced Engineering Applications*, **6**(4), 4-12.
[11] Jitareerat, P., Paumchai, S., Kanlayanarat, S. & Sangchote, S. (2007). Effect Of Chitosan On Ripening, Enzymatic Activity, And Disease Development In Mango (Mangifera Indica) Fruit. *New Zealand Journal of Crop and Horticultural Science*, **35** (2), 211-218.
[12] Kumari, S. & Rath, P. K. (2014). Extraction and Characterization of Chitin and Chitosan from (Labeo Rohit) Fish Scales. *Procedia Material Science*, **6**, 482-489.
[13] Naznin, R. (2005). Extraction of Chitin and Chitosan from Shrimp (*Metapenaeus monoceros*) Shell by Chemical Method. *Pakistan Journal of Biological Science*, **8**(7), 1051-1054.
[14] Nouri, M. Khodaiyan, F., Razavi, S. H. & Mousavi, M. (2015). Improvement of Chitosan Production from Persian Gulf Shrimp Waste by Response Surface Technology. *Food Hydrocolloids*, **3**, 1-9.
[15] Pal, J., Verma, H. O., Munka, V. K., Maurya, S. K., Roy, D. & Kumar, J. (2014). Biological Method of Chitin Extraction from Shrimp Waste An eco-Friendly Low Cost Technology and Its Advanced Application. *International Journal of Fisheries and Aquatic Studies*, **1**(6), 104-107.
[16] Patria, A. (2013). Production and Characterization of Chitosan from Shrimp Shells Waste. *Bioflux*, **6**,4.
[17] Pillai, C. K. S., Paul, W. & Sharma, C. P. (2009). Chitin and Chitosan Polymers: Chemistry, Solubility and Fiber Formation. *Progress in Polymer Science*, **34**, 641-678.
[18] Puvvada, Y. S., Vankayalapati, S. & Sukhavasi, S. (2012). Extraction of Chitin from Chitosan Exoskeleton of Shrimp for Application in the Pharmaceutical Industry. *International Current Pharmaceutical Journal*, **1**(9), 258-263.
[19] Rinaudo, M. (2006). Chitin and Chitosan: Properties and Applications. *Progress Polymer Science*, **31**, 603-632.
[20] Szymanska, E. & Winnicka, K. (2015). Stability of Chitosan – A Challenge for Pharmaceutical and Biomedical Application. *Marine Drugs*, **13**, 1819-1864.
[21] Viljoen, J. M., Steekamp, J. H., Marais, A. F. & Kotze, A. F., (2014). Effect of moisture content, temperature and exposure time on the physical stability of chitosan powder and tablets. *Drug Development Industrial Pharmaceuticals*, **40**, 730-742.

ISSN 2289-8786. http://transectscience.org/