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Optimization of shrimp shell waste deacetylation for chitosan production

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ABSTRACT

Chitosan, a versatile natural polymer is an amino polysaccharide prepared by processing shrimp shell waste which involved deacetylation of chitin. To obtain high degree of deacetylation (DDA), several parameters should be performed during alkaline treatment. The present study was undertaken at different time of soaking treatment (once and twice) and temperature (60°C and 80°C) to optimize the deacetylation process to produce chitosan with high solubility and degree of deacetylation. It was observed that the highest solubility and degree of deacetylation were obtained when deacetylation process was repeated twice and temperature of 80°C, with 99.48% and 97.63%, respectively. It can be concluded that by repeating the deacetylation twice with the support of heating treatment, a better quality of chitosan with higher solubility and degree of deacetylation can be produced.

1. Introduction

Shrimp is considered as one of the most important fisheries product in Sabah, Malaysia which mostly exported in frozen condition after separation of the head and shell. Shrimp industries in Sabah generally produces large scale of shrimp bio-waste during the processing which have only low economic value thus being dispose on landfill constantly. However, this bio-waste which includes head and shell can produce value-added products such as chitosan which is derived from chitin that generally found in marine invertebrates, insect, and fungi and chitosan is easily formed into various semi-solid and solid structures under mild conditions (Szymanska and Winnicka, 2015). According to Nouri et al. (2016) and Ning and Xi (2015), the shrimp waste contains protein (30-40%), calcium carbonate (30-50%) and chitin (20-30%).

Chitosan which is a modified natural carbohydrate is a polycationic copolymer which consisting of glucosamine and N-acetylglucosamine units possesses a free amino group (NH_2) which is reactive in many chemical reaction. The difference of group in the position C-2 of chitosan which is NH_2 instead of hydroxyl group (OH) for cellulose makes chitosan can easily form positive ionic charge which can increases the ability to chemically bind with negatively charged compounds such as fats, lipids, cholesterol, metal ions, proteins, and macromolecules (Hossain and Iqbal, 2014).

Due to its excellent properties, this has made chitosan attained increasing commercial interest worldwide such as agriculture, biochemistry, pharmaceuticals, biotechnology, biomedical, cosmetics, food, and paper industry (Rinaudo, 2006). Chitosan has been explored for its uses in agricultural and horticultural which primarily for plant defense and enhance production yield by promoting plant growth as well as in pharmaceutical and biomedical which includes prolonged or controlled release drug delivery systems, wound dressing, blood anticoagulants, cartilage and bone tissue engineering scaffolds, and also space filling implants.

Among several properties which make chitosan available in industries were the degree of deacetylation and solubility. In most commercial industry, it needs a soluble chitosan so that it can make it available in many chitosan based material processes.

The process of chitosan extraction from chitin usually is by using conventional method which comprises deproteination by alkaline solution, demineralization by acid solution and lastly deacetylation by concentrated alkaline solution. In this conventional method, generally the deacetylation process is done once at certain temperature to obtained high acetylated chitin. However, to achieve high degree of deacetylation,

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deacetylation process can be repeated twice at different temperature because heating treatment may increase the reaction. Therefore, the objectives of this study are to investigate the optimum condition during of soaking frequency and temperature to produce high degree of deacetylated of chitosan and to determine the characteristics of chitosan produced.

2. Materials and methods

2.1. Sample collection and preparation

The raw shrimp shell waste was collected from Kian Huat Seagull Sdn Bhd, Putatan which nearby Kota Kinabalu city and it is one of the factories which produced large quantities of shrimp shell waste in Sabah. The sample was collected is fresh conditions where it is collected during the peeled of process of the shrimp shell. The samples 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.

2.2. Deproteination

This process was performed at laboratory scale where 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°C (Kumari and Rath, 2014). 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 by filtration method.

2.3. Demineralization

The demineralization process were carried out by added with 1.0 M HCl in the ratio 1:16 (w/v) and allowed to stand for 24 hours (Puvvada et al., 2012) at room temperature (~25°C). The samples were then dried under the sun for 6-8 hours and the dried sample is now known as chitin.

3. Chitosan production

3.1. Deacetylation (DA)

3.1.1. Soaking frequency

The deacetylation process was done with a modification by Miya et al. (1985) and Wan et al. (2003) by soaking the dried chitin prepared with 48% NaOH for 48 hours at room temperature (~25°C). After soaking, the product is known as chitosan (Kumari and Rath, 2014). The chitosan was washed with tap water until neutral (pH6.5-8.0) and dried as described in demineralization process. The procedure was repeated to get different frequency of soaking process (deacetylation). Deacetylation process was carried out once (C1) and twice (C2) representing soaking once and soaking twice, respectively. The chitosan obtained from each treatment (C1 and C2) was further investigated for its properties.

3.1.2. Temperature

Treatment of deacetylation twice (C2) was further investigated at different temperature (60 and 80°C) to determine the optimum temperature in obtaining chitosan with high degree of deacetylation.

4. Characterization of prepared chitosan

The characterization of the extracted chitosan was performed in term of the solubility and degree of deacetylation (DDA) for all chitosan produced from each deacetylation treatments (C1 and C2).

4.1. Solubility in acid solution

1.0 g 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 as follows: (Puvvada et al., 2012).

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

$$\text{Insoluble}\ (\%) = \frac{\text{Insoluble},g}{Sample\ weight,g} \quad (1)$$

$$Solubility\ (\%) = 100 - \%\ insoluble \quad (2)$$

4.2. 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 Brugnerotto 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} which representing the peak for amide group and amine group, respectively. The DDA calculation was carried out as follows:

$$\%\ \text{DA} = \frac{(A_{1320}\ /\ A_{1420}) - 0.3822}{0.03133} \quad (3)$$

$$\%\ \text{DDA} = 100 - \%\text{DA} \quad (4)$$

where,

$DDA = degree\ of\ deacetylation\ (\%)$
$DA = degree\ of\ acetylation\ (\%)$

5. Results and discussion

5.1. The produced chitosan

The physical appearance of the C1 was slightly yellowish white, while for C2 it was white in color (Fig. 1). 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 which was slightly brownish to white and yellowish white and this indicates a good quality of chitosan was produced (Naznin, 2005; Nouri et al., 2016).

Fig. 1: Chitosan produced for C1 (a) and C2 (b)

5.2. Solubility

Among several characteristics, solubility of chitosan is one of the most important parameter for quality of chitosan, where higher solubility means better quality of chitosan. 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 (El-hefian et al., 2009). There are several main factors which may affecting the solubility of chitosan such as temperature and time treatment of deacetylation, concentration of alkaline solution, ratio of chitin to alkali solution and soaking frequency in NaOH solution for the deacetylation process. Fig. 1 shows the solubility of chitosan obtained from the effect of soaking frequency where for the deacetylation process once (C1) and twice (C2) which was conducted at room temperature (~25^{o}C). The solubility for C1 and C2 were 97.78% (±0.2450) and 98.32 (±0.0360), respectively. As seen it Fig. 1, the effect of soaking frequency increased the solubility of chitosan, where soaking twice in 48 % NaOH at room temperature for deacetylation process has higher solubility of 98.32% (±0.036) compared to one time soaking with 97.78 %. It shows that by repeating deacetylation twice, it can enhance the solubility of chitosan produced thus producing a better quality of chitosan (Figs. 2 and 3).

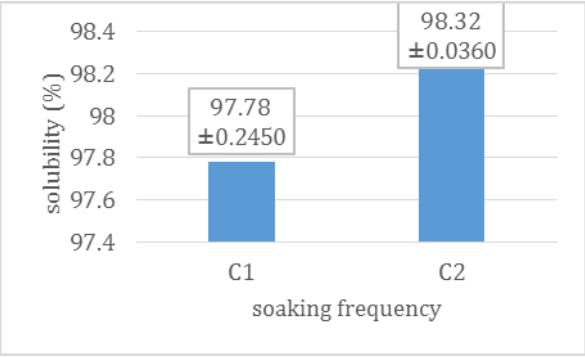

Fig. 2: Percentage of solubility of chitosan for C1 and C2 at room temperature (~25^{o}C)

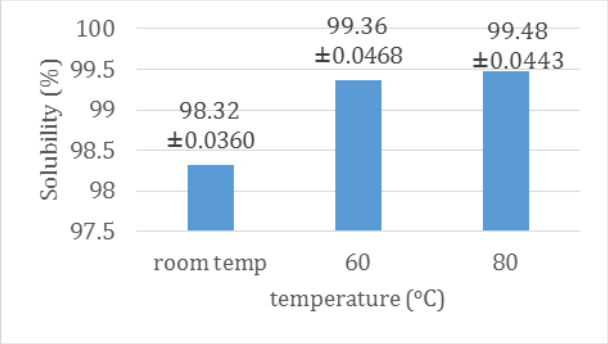

Fig. 3: Solubility of chitosan for deacetylation twice at different temperature

Meanwhile, when C2 treatment was performed under different temperature, the solubility of chitosan increased with average 99.36% (±0.0468) for temperature at 60^{o}C whereas at 80^{o}C the solubility can be achieved up to 99.48% (±0.0443), with the support of temperature (Fig. 2). Previous study which was done by Patria (2013) reported that the solubility achieved was 17.43 % to 95.29 % with average of 57.52 % at different deacetylation temperature 60, 70, 80 and 90^{0}C whereas another study also showed the solubility obtained was ranged from 48.30 % to 97.65 % by performing deacetylation once at temperature 65^{0}C but using different concentration of NaOH (30, 40, 50, 60 %) (Hossain and Iqbal, 2014). There is no literature reported on the solubility of deacetylated twice of chitosan yet, however by comparing the solubility of this study to previous studies which has been done, is shows that by repeating the deacetylation process twice, it increases the amount of amino groups (NH_2) in the chitosan polymer chain thus increasing the solubility of chitosan. When the deacetylation process was carried out at different temperature, higher percentage of solubility was found at higher temperature, i.e. 80^{o}C, with 99.48% solubility.

An increases in solubility is proportionally to the degree of deacetylation is due to the acetyl groups where in chitin deacetylation process, it will be removed and leaving only amine group. Chitosan will get protonated in the aqueous acid solution which leads to its solubility is due the presence of amino

group in its molecular structure (El-hefian et al., 2009) 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 as shown in Eq. 5 (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.

$$-NH_2 + H_2O \rightarrow -NH_2NH_3^+ + OH^- \quad (5)$$

5.2. Degree of Deacetylation (DDA)

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 value was determined by using FTIR as was explained in the experimental part using the ratio of the bands at 1320 and 1420 cm^{-1}. Fig. 3 shows the FTIR spectra for chitosan which produced in this study (Fig. 4).

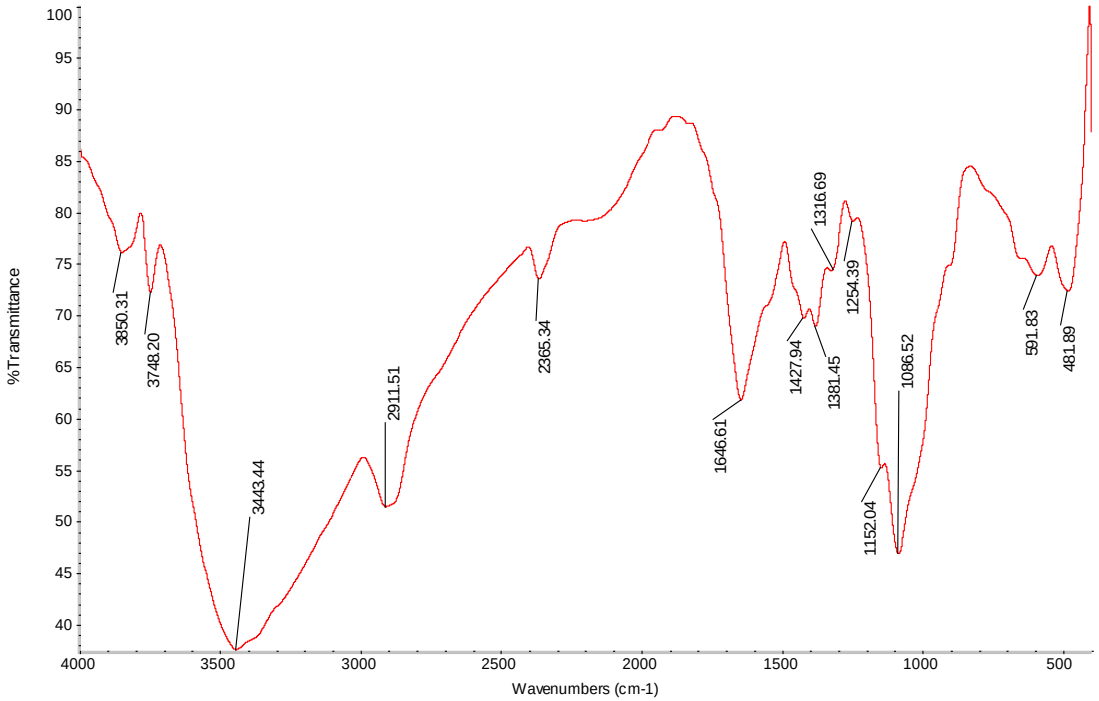

Fig. 4: FTIR spectra form chitosan obtained from deacetylated twice at 80^{0}C

Based on these spectra, the major absorption band is observed at 3443 which indicates the stretching vibration of OH and NH groups. Between 1250 and 1080 cm^{-1} which represents the free amino group (-NH_2) at C2 position of glucosamine indicates a major group that present in chitosan. FTIR spectra obtained for the chitosan produced showed the absorption bands for the free amino group between 1086 and 1254 cm^{-1} and also some bands which is also reported by Zhou et al. (2008) where same strong characteristic amino peaks of chitosan at 3420, 1655, and 1325 cm^{-1}. Some of the bands 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 intensity of the absorption band around 1595 for very short due to highly degree of deacetylation. Nevertheless, based on Coates (2000), it is not sufficient to characterize the functional group for the different classes of carbonyl compound overlap and the carbonyl frequency alone. The degree of deacetylation was calculated using the baseline equation as used by Brugnerotto et al. (2001), where the baseline used were 1320 and 1420 cm^{-1}. 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). It is also reported that the degree acetylation determined from this ratio did not change with humidity and hydrogens bonds (Kasaai, 2008) (Figs. 5 and 6).

By repeating deacetylation process, it does not as well affect the solubility but also the DDA value where a higher amino group content can be achieved. The DDA value of the chitosan produced from the deacetylation process once in this study was 75.24 %. Nessa et al. (2010) reported that the DDA value obtained ranged from 45 to 73 % although a longer time was applied for the deacetylation process performed once. Other study which is done by 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. (2016) 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 can be increased to 89.79% (Puvvada et al., 2012). The result shows in Fig. 4 indicates that the best condition to produce a high DDA value is by repeating the deacetylation process twice, where a higher DDA value can be increased to 88.57% compared to deacetylation once with 75.24 %. However, with the aid of heating treatment during deacetylaion twice, it enhanced the DDA value and the result is showed in Fig. 5.

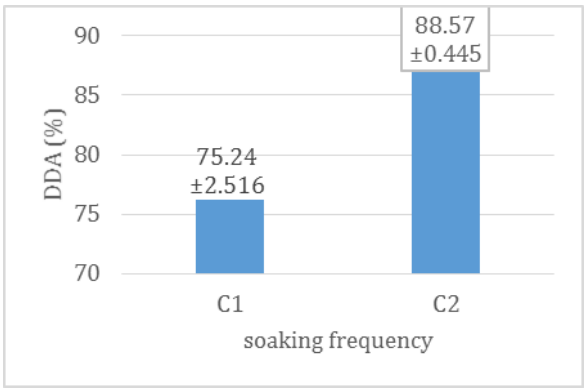

Fig. 5: Effect of soaking frequency at room temperature on DDA value

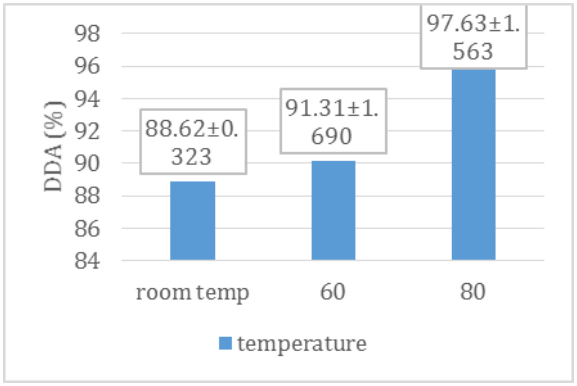

Fig. 6: DDA value for chitosan samples after deacetylated twice at different temperature

The degree of deacetylation (DDA) obtained from this study when deacetylation repeated twice was ranged from 88.62 to 97.63 %, where the highest value of DDA was obtained by deacetylation twice with the aid of heat treatment at 80 °C. These results showed that the heating treatment on the deacetylation process affects significantly the deacetylation of chitin to produce better quality of chitosan with higher DDA value. Previous study done by Wan et al. (2003) reported that the chitosan obtained highest DDA value 95.1% when performing deacetylation twice but the process was performed in a reactor at 100°C using 50 % NaOH for 1 hour treatment. Another study which also conducted deacetylation twice is done by Zhou et al. (2008) and managed to produce chitosan with DDA value 90.3 % by performing with 50% NaOH at 100°C for 30 minutes. This explains that the DDA values can be different due to series of parameters used or conditions during the deacetylation process. However, based on these several previous studies with this study, it shows that a higher DDA value can be obtained by repeating deacetylation process twice but with the aid of heat at 80°C and longer time treatment. The DDA value can be achieved up to 97.63 % compared to the other methods performed in the previous studies.

6. Conclusions

Based on the results obtained from this study, the traditional method of extracting chitosan can produces chitosan with solubility up to 97.78 % and DDA value 75.24 %. When the deacetylation process repeated twice with the aid of heating treatment, a better quality of chitosan can be obtained with the solubility of chitosan can be achieved up to 99.48 at temperature 80°C, while DDA value obtained was high up to 97.63%. Soaking frequency twice (C2) enhanced chitosan solubility but no significant effect observed when C2 performed at different temperature. Meanwhile, the DDA value increased for C2 and a higher DDA value was achieved at 80°C when performed twice. From these results, it can be concluded that by repeating the deacetylation twice and also with the support of heating treatment, it can be used to produce a better quality of chitosan with higher solubility and DDA value.

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