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EFFECT OF SYNTHESIS CONDITION ON DEGREE OF DEACETYLATION OF CHITOSAN FROM SHRIMP WASTE FOR SMART FILM APPLICATIONS

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Abstract

Shrimp is an Indonesian's export commodity with high economic value increasing every year. Usually, shrimps are exported in the form of frozen shrimp without shells, heads, tails, and entrails. It resulted in the accumulation of shrimp waste leading to the increasing environmental pollution. Shrimp waste contains high contents of chitin which can be processed to a chitosan owing several benefits. The purpose of this study is to determine the optimum condition of the synthesis of chitosan from chitin isolated by the autolysis method. The deproteination was carried out by soaking the shrimp waste in an acidic solution (pH 2 – 3) for 10 d. The demineralization process was done by reacting the deproteinated solids in a hydrochloric acid solution at pH 0 – 1 for 24 h. Parameters varied in this study time (1 – 3 h), chitin to NaOH ratio (1:10 – 1:30 (w/v)), and temperature (60 – 120℃). The higher all parameters used, the higher the obtained degree of deacetylation (DD) which is in the range of 18.35±1.13 to 48.6±0.51%. On the other hand, the obtained yield decreased from 50.66±1.98% to 47.78±0.81%. The optimum condition was obtained at a synthesis temperature of 120℃, chitin to NaOH ratio of 1:20 g/mL, and time of 3 h producing chitosan with DD of 54.25 ± 2.27%, and yield of 47.7 ± 0.65%. Chitosan synthesized using optimum conditions produced a relatively homogeneous thin film. Polyaniline was then introduced to the film to obtain a smart film prototype. This smart film was able to detect the pH changes proven by the change in its color. The smart film also could be potentially used as a "smart pack" for detecting product decay which releases ammonia gas.

INTRODUCTION

Shrimp are exported in the form of fresh frozen shrimp, which have undergone cold storage after the heads and shells have been separated. It reaches 142,000 tons with a total of unused shrimp shell and head waste reaching 60,000 tons [1]. Shrimp shell waste consists of three main components, such as protein (25-44%), calcium carbonate (45-50%), and chitin (15-20%). Chitin polymer is composed of monomers; 2-acetamide-2deoxy-D-glucose (N-acetyl glucosamine). The bond that occurs between chitin monomers is a glycosidic bond at the β-(1-4) position. The molecular structure of chitin is a long straight-chain [2]. Currently, shrimp waste has not been managed and utilized optimally. If shrimp waste is left alone, it can rot, cause an unpleasant odor, and pollute the environment. Chitin from shrimp waste is not widely used directly on an industrial scale. Alternatively, chitin can be converted into chitosan, a valuable product that can be utilized in various applications, especially bioplastics. Chitin is isolated by autolysis to reduce the use of chemicals and waste produced so this isolation by autolysis is environmentally friendly [3]. Isolation of chitin by autolysis is carried out using enzymes found in the shrimp itself, namely proteolytic enzymes, facilitated by strong acids [4]. The sample was conditioned at pH 2 to suppress bacterial growth during incubation which could affect protein hydrolysis in the sample. The filtrate resulting from autolysis incubation of shrimp waste can be used as a hydrolyzate product because it contains protein [5].

Chitosan [poly-(2-amino-2-deoxy-β-(1-4)-D-glucopyranose] is a linear biopolymer with 2000–5000 monomer units linked together by β-(1-

4) glycosidic bonds. It can be synthesized by removing part of the 2-acetyl group from chitin [6]. Chitin is the main component in shrimp shells. Chitin can be further processed to obtain chitosan through the deacetylation process. Chitosan has good bioactive, biodegradability, and biocompatibility properties, so it can be developed into a biodegradable plastic [7]. The deacetylation process of chitin is the key step in reducing the acetyl group content to produce chitosan having various degrees of deacetylation [8]. The degree of deacetylation (DD) of chitosan affects its physical and chemical properties, including solubility, stability, and mechanical strength. Therefore, determining the optimum degree of deacetylation of chitosan from shrimp waste is important in the development of chitosan-based bioplastics [9].

In this research, chitin was first isolated by using the autolysis method [4]. The obtained chitin was then reacted with sodium hydroxide (NaOH) solution to perform a deacetylation process producing chitosan. To date, the deacetylation process of chitin isolated from the autolysis method has never been reported before. Deacetylation of chitin into chitosan is carried out by varying the temperature, chitin to NaOH ratio, and time to achieve the optimum DD for film applications [10]. Chitosan having optimum DD was formed to a composite film with polyaniline (PANI). PANI is a conductive polymer that can improve the mechanical properties of chitosan film and can detect a change in pH by changing its color [11–14]. The chitosan/PANI composite film is expected to be a starting material for smart packaging that can detect product damage, especially food products release ammonia gas. This gas will increase the pH value and change the color of chitosan/PANI composite film.

EXPERIMENT

Material

The materials used are shrimp waste composed of shells, heads, tails, and entrails obtained from PT. Istana Cipta Sembada Seafood, Banyuwangi, Indonesia. Sulfuric acid (H_2SO_4), glycerol, and aniline were purchased from Smart Lab, while sodium hydroxide (NaOH), hydrochloric acid (HCl), acetic acid (CH_3COOH), ammonia (NH_3), ammonium persulfate (APS) were obtained from Merck. All reagents were of analytical grade and used without any further purification. However, aniline was distilled prior to polymerization to remove the presence of oligomers.

Instrumentation

The instrumentation used was Fourier Transform InfraRed (FTIR) Nicolet iS10 ATR Thermo Scientific.

Procedure

Isolation of Chitin

Shrimp waste was collected from the company and was directly stored in an ice box to maintain its freshness during the trip. Some parts of the shrimp waste were subjected to a water content analysis procedure by heating it in the oven at 105°C for 3h. Shrimp waste (6 kg) was transferred to a closed container and then incubated in a sulfuric acid solution having a pH of 2 for 10 d. The samples were stirred occasionally and kept at a constant pH of around 2 – 3. After 10 d, precipitates were filtered and washed with water until the pH of the solution was neutral [4]. The resulting product was demineralized by soaking in 1M solution of HCl (pH 0 – 1) for 24 h. The mixture was filtered, washed with water to achieve a neutral pH, and dried at 70°C for 24 h. The obtained solids were grounded and then sieved (50 mesh) to obtain the respecting chitin powder.

Synthesis of Chitosan

A NaOH solution of 65% (v/v) was introduced to the chitin powder in a beaker glass with various chitin to NaOH ratios ranging from 1:10 to 1:30 (w/v). The reaction mixture was kept at various temperatures (60 – 120°C) and time (1 – 3 h) under constant stirring. The mixture was vacuum filtered, and the obtained precipitate was washed with water to obtain a neutral pH and then dried at a temperature of 70°C to obtain chitosan powder. The obtained yield was calculated using the following formula:

$$\%\text{yield} = \left(\frac{(100-\%wc)\times a - b}{(100-\%wc)\times a}\right) \times 100\% \quad (1)$$

with %wc was the percentage of water content in shrimp waste, a was the mass of shrimp waste, and b was the obtained residue.

Determination of Chitosan's Degree of Deacetylation

The degree of deacetylation (DD) of all samples was determined semi-empirically from infrared spectra. Samples of chitin and chitosan were consecutively placed on the sample holder and were analyzed using Thermo Scientific FTIR Nicolet iS10 ATR at a wavenumber of 500 – 4000 cm^{-1}. The DD value was calculated using the following formula:

$$\%DD = 1 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{1}{1{,}33}\right) \times 100\% \quad (2)$$

with A_{1655} was absorbance value at 1655 cm^{-1}, A_{3450} was absorbance value at 3450 cm^{-1}, while 1,33 was the value of A_{1655}/A_{3450} of fully deacetylated chitosan [15].

Fabrication of Chitosan-polyaniline Composite Film

The chitosan used for film fabrication was the one synthesized by using optimum parameters. Firstly, chitosan solution was prepared by dissolving 1 g of chitosan powder in 60 mL of 1% (v/v) acetic acid solution. Then, 0.008 mg of glycerol was added to the mixture and stirred at 90°C until it thickened. On the other hand, the Polyaniline (PANI) solution was prepared separately by mixing two different solutions: 0.5 mL of solution A and 0.25 mL of 0.01 M ammonium persulfate solution. Solution A consists of 2.5 mL of 5% (v/v) HCl solution and 497 µL of aniline in 5 mL of H_2O [13]. The PANI solution was then introduced to the chitosan solution at 90°C under constant stirring. The solution mixture was evenly dispersed on the surface of the microscopic glass slide and left to dry. The obtained film underwent a re-doping process by soaking it in the previously prepared PANI solution for 1 min. The obtained chitosan/PANI composite film was dried at room temperature before it was used and characterized.

Response to Test of Ammonia Gas

The main purpose of the test is to verify the "smart" properties, i.e. detect pH changes, of chitosan/PANI composite film. The response test to ammonia gas was carried out by preparing 10 mL of ammonia solution (NH_3) in a transparent closed container. The chitosan-PANI film composite sample was cut into 5 × 5 cm^2 then attached to the lid of the container and closed into the container containing the ammonia solution for 2 h. The change of color was observed periodically [16].

RESULT AND DISCUSSION

Chitin Isolation

Initially, the shrimp waste was determined by its water content by heating the sample in the oven until completely dry. We obtained a relatively high percentage of water in the shrimp waste samples of 79.51%. It means that we only have 1.23 kg of solid raw material, i.e., shrimp shells and heads, used in this study from 6 kg of shrimp waste. Chitin isolation from shrimp waste is carried out in 2 processes, that is deproteination and demineralization. Deproteination is conducted to remove the protein bound to chitin. In this research, deproteinization is carried out enzymatically using protease enzymes found in the digestive system of shrimp waste, so that it is more environmentally friendly. Shrimp waste consisting of heads, shells, and digestive tracts, is incubated in a sulfuric acid solution for 10 d with pH monitoring. The incubation process uses a pH of 2-3 to suppress bacterial growth that could accelerate decay. It also provides the optimum pH for the pepsin enzyme, one of the protease-type enzymes found in stomach acid, to break peptide bonds from nitrogen and protein to produce amino acids [17]. The incubation process was declared successful through the absence of ammonia odor in the sample and a decrease in nitrogen levels [4]. Shrimp waste that has been incubated is separated and the obtained solids are washed until the pH is neutral. This treatment resulted in a color change in the sample to transparent and brittle orange. The obtained product is not pure chitin but requires a demineralization process to remove the mineral content in the shrimp waste [18].

Shrimp waste contains minerals in the form of calcium carbonate ($CaCO_3$) and calcium phosphate ($Ca_3(PO_4)_2$). The demineralization process is carried out by soaking the previous sample in HCl 1M for 24 h and maintaining pH at 0-1. The HCl solution reacts with minerals bound to chitin to form mineral salts according to equations (3) and (4).

$$CaCO_3\,(s) + 2HCl\,(aq) \rightarrow CaCl_2\,(aq) + H_2O\,(l) + CO_2\,(g) \quad (3)$$

$$Ca_3(PO_4)_2\,(s) + 4HCl\,(aq) \rightarrow 2CaCl_2\,(aq) + Ca(H_2PO_4)_2\,(aq) \quad (4)$$

During the demineralization process, bubbles i.e. CO_2 gases were produced indicating that reaction

(3) has occurred [19]. The mixture was separated and the obtained solids were washed until neutral. The obtained product, i.e. chitin, was more transparent and softer than before. The whole isolation process (deproteination and demineralization) produced orange-colored chitin powder with a mass of 389 g. By using equation (1), we obtained the calculated total yield of 31.64%. This value is acceptable and is in good agreement with other studies, which is approximately 15–40% of the dry weight of shrimp [6].

Effect of Temperature on Obtained Chitosan

The isolated chitin is deacetylated with NaOH to produce chitosan. The deacetylation process involves the removal of acetyl groups (–$COCH_3$) from chitin, converting them into amino groups (–NH_2) through the addition of an alkaline solution accompanied by heating. NaOH solution, as an alkaline solution, breaks the acetyl groups' bonds in chitin, transforming them into amino groups. The deacetylation reaction begins with the hydroxide ion (OH^-) from NaOH attacking the electropositive carbonyl carbon of chitin. The OH^- ion that enters the C carbonyl breaks the pi bond at C=O carbonyl. The O atom attracts electrons in H to form protons. The N atom with one free pair of electrons attracts a proton to form an ammonium ion. The C–N bonds are broken accompanied by the formation of C=O bonds and chitosan is formed [20].

The obtained chitosan at various deacetylation temperatures produces different colors, ranging from slight redness to yellowish-white, as the temperature increases. The physical appearance of chitosan can be influenced by temperature [15]. At higher deacetylation temperatures, chitosan appears yellowish-white. At 120°C, chitosan has a yellowish-white appearance and a fine powder texture. FTIR spectra of chitin and chitosan at various temperatures are shown in **Figure 1a**. In the chitin spectrum, the carbonyl group (C=O) appears at the wavenumber of 1622 cm^{-1} while the –NH group is found at the wavenumber of 3261 cm^{-1}. In the chitosan spectrum, there is an absorption band that appears at the wavenumber of 3364 cm^{-1}. This absorption band results from the stretching vibration of the –OH and –NH groups. The broadening of the absorption band is due to the overlap of the amino (-NH) groups on the –OH groups. The vibration of the C=O group is observed in the absorption band at the wavenumber of 1653 cm^{-1}. The N–H bending vibration of the primary amine is found at the wavenumber of 1589 cm^{-1}.

There is a decrease in the intensity of the C=O group absorption band in the spectra of chitosan compared to chitin. It indicates a higher amide group content in chitin compared to chitosan. In the chitosan absorption band, the wavenumber of the hydroxyl group has a higher intensity than the C=O from the amide group. Based on this, it can be inferred that the amide group in chitosan has decreased due to the loss of acetyl groups becoming amines. The difference between the three chitosan spectra lies in the intensity of the amide group absorption band at the wavenumber range of 1655-1589 cm^{-1} (**Figure 1b**). With the increase in deacetylation temperature, a decrease in absorption band intensity in the wavenumber range of 1655-1589 cm^{-1} is observed. The chitosan spectrum at 60°C has the highest absorption band intensity compared to other temperature variations. This indicates that the acetyl groups in chitosan at 60 °C are higher than in chitosan at 120°C. The degree of deacetylation (DD) is obtained by comparing the absorbance of the amide group with the absorbance of the hydroxyl group (equation 2). The results of this study show that the DD of chitosan at temperatures of 60, 90, and 120°C was 18.35 ± 1.13%; 31.48 ± 1.48%; and 48.6 ± 0.51 %, respectively (**Figure 1c**). It can be seen that the higher the temperature used in the deacetylation process, the obtained DD also increases. Despite the increase, the degree of deacetylation obtained in this study was quite low. A small value of the degree of deacetylation indicates that the cleavage of the acetyl group in chitin did not occur completely [21]. Based on the results obtained, it can be concluded that the best temperature variation for the highest degree of deacetylation is 120°C.

The temperature variations during the deacetylation process also affected the yield. **Figure 1c** presents a relationship curve between obtained yield and deacetylation temperature. The chitosan yield increases with increasing deacetylation temperature, but after reaching the maximum at 90°C, the yield decreases. The highest yield is obtained at the temperature of 90°C, with an average yield of 50.66 ± 1.98%, while the lowest yield is obtained at the temperature of 120°C, with an average yield of 47.7 ± 0.65%. If the deacetylation temperature is too high, it can degrade the polymer, causing it to have a low molecular weight. Degradation in chitosan involves the release of excess acetylation chains in chitin, forming lighter chitosan particles that dissolve in

the NaOH solution during the deacetylation process, leading to a decrease in chitosan yield. Despite the deacetylation temperature of 120°C producing a slightly lower yield than 90°C, it was chosen due to its DD value (48.6 ± 0.51%) being more acceptable for film applications than other temperatures (less than 35%).

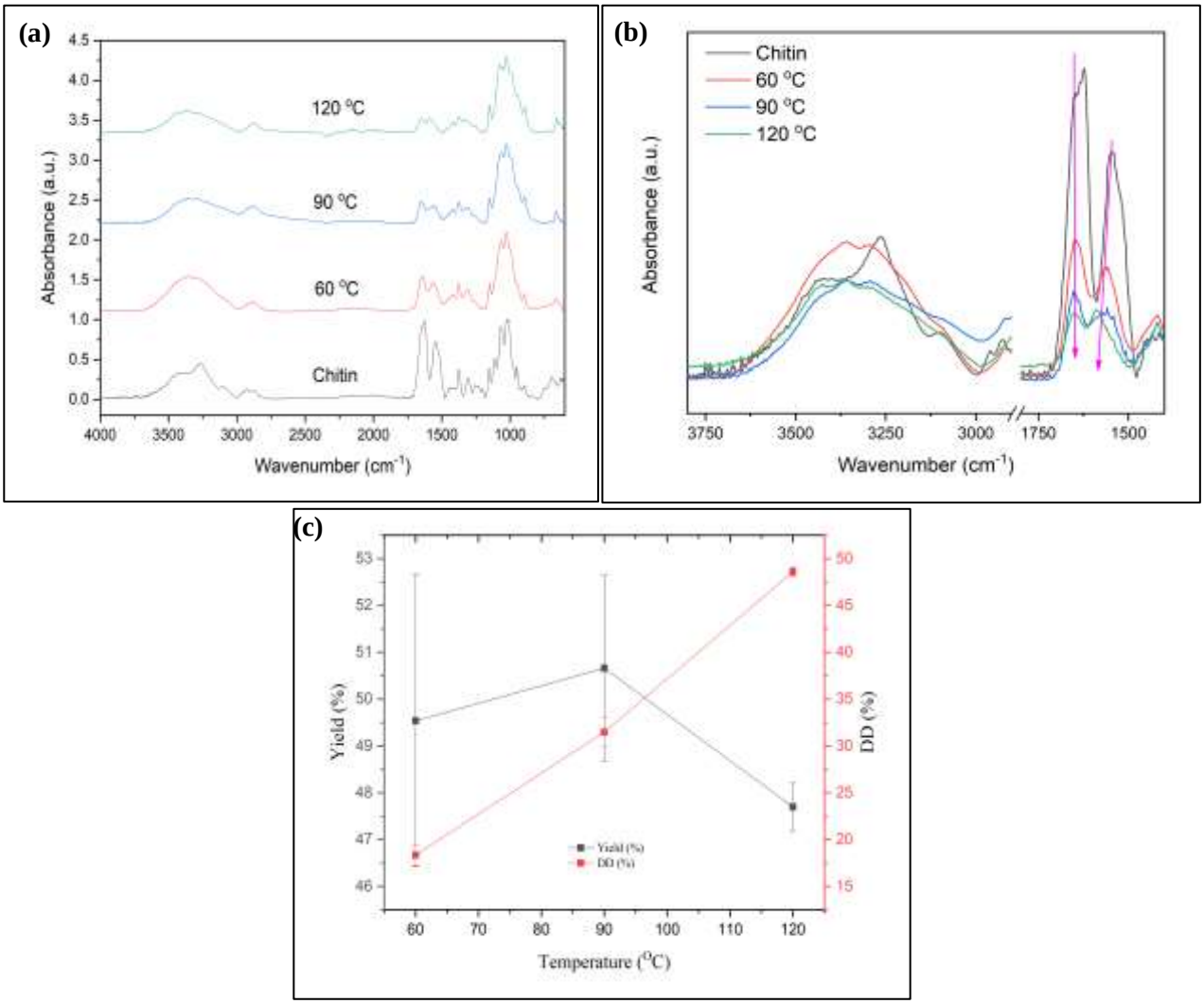

Figure 1. (a) FTIR spectra of chitin and chitosan synthesized at various temperatures. (b) The evolution of FTIR band at 1655-1589 and 3364 cm^{-1}. (c) Relationship curve between DD and %yield as a function of temperature.

Effect of Chitin to NaOH Ratio on Obtained Chitosan

The temperature of 120°C as the optimum temperature is used in the deacetylation process with variations in the chitin/NaOH ratio. The variations in the chitin/NaOH ratio resulted in the FTIR spectra presented in **Figure 2a**. Based on FTIR results, the spectrum of chitosan with chitin/NaOH ratio of 1:10, 1:20, and 1:30 (w/v) experiences changes in the intensity of several wavenumber peaks. The observed differences are evident in the intensity of the peaks at 3450 cm^{-1}, representing the vibration of hydroxyl (–OH) groups, and 1650 cm^{-1}, representing the C=O vibration of amide groups. The FTIR results showed a decrease in the absorption of both C=O from the amide group and –OH group (**Figure 2b**), representing the loss of the acetyl group from the main chain of chitin. Based on these two peaks, it can be observed that a higher NaOH ratio results in chitosan with a decreasing peak of C=O from amide groups and an increasing intensity of –OH. This is because, with more NaOH used, there is increased OH^- ion contact with chitin during the deacetylation process, so more acetyl groups (–$COCH_3$) are released from the chitin.

The FTIR spectra can also be used to calculate the obtained deacetylation degree/DD (equation 2). The obtained DD with chitin/NaOH ratios of 1:10, 1:20, and 1:30 (w/v) was 42.17 ± 1.77; 48.60 ± 0.51; and 51.38 ± 0.95%,

respectively. These results show that increasing the volume of NaOH used resulted in an increasing DD. In addition, the chitin to NaOH ratio also affected the obtained yield of chitosan. The curve above illustrates the relationship between the chitin/NaOH ratio, DD, and chitosan yield (**Figure 2c**). The increasing chitin/NaOH ratio results in an increasing DD value, but the yield of chitosan decreases. This is because the higher the amount of NaOH means it contains more OH^- ion which result in more acetyl groups ($–COCH_3$) being released from chitin so that chitosan is produced with a lower yield [22]. The yield of chitosan decreases with an increasing NaOH ratio, but at a ratio of 1:20 (w/v), there is an increase in yield. Therefore, it can be concluded that the optimum ratio for this treatment is 1:20 (w/v), with a degree of deacetylation of 48.60 ± 0.51% and a yield of 49.15 ± 0.47%. A ratio of 1:20 (w/v) as the optimum ratio is used in the deacetylation process with time variation treatment.

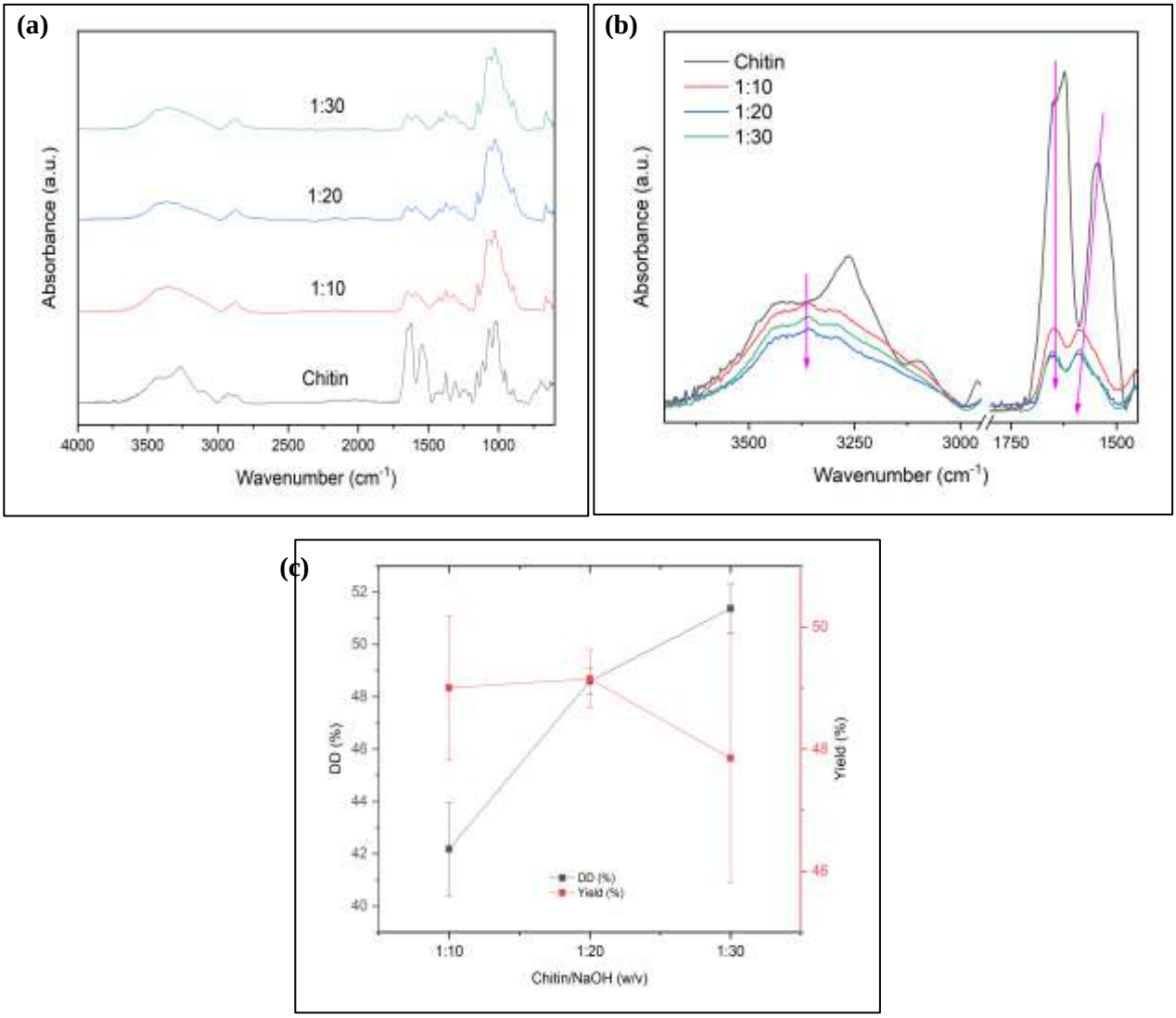

Figure 2. (a) FTIR spectra of chitin and chitosan synthesized at various chitin/NaOH ratios. (b) The evolution of FTIR band at 1655-1589 and 3364 cm^{-1}. (c) Relationship curve between deacetylation degree and %yield as a function of chitin/NaOH ratio.

E*ffect of Time on Obtained Chitosan*

Synthesis was carried out using the optimum parameters obtained previously (temperature of 120°C and chitin/NaOH ratio of 1:20). **Figure 3a** shows the FTIR spectra of chitin and synthesized chitosan with time variations of 1, 2, and 3 h. Changes in the peak intensity of the wavenumber are shown at the wavenumber 3450 cm^{-1} which is the vibration of the hydroxyl (–OH) group and 1650 cm^{-1} the C=O vibration of the amide group. There is a decrease in the intensity of the absorption of the C=O group from the amide and an increase in the intensity of the absorption of the –OH group

(**Figure 3b**). This is caused by the –OH addition reaction of NaOH which substitutes the acetyl group. These two wavenumber peaks indicate that the longer the deacetylation time, the C=O peak of the amide group will decrease and the OH group will increase. The FTIR results of chitosan at a time variation of 1 h show that the acetyl group is higher than chitosan at a time variation of 3 h which is caused by the longer the time used in the deacetylation process, the more acetyl groups (–$COCH_3$) will be released from the chitin. The degree of deacetylation value is calculated from the comparison between the absorbance of the amide group and the absorbance of the hydroxyl group. The DD value with time variations of 1, 2, and 3 h was 44.05 ± 1.61; 48.60 ± 0.51; and 54.25 ± 2.27%, respectively (**Figure 3c**). These results show that the longer the time used in the chitin deacetylation process to become chitosan, the obtained DD will increase.

The chitin deacetylation process with varying times not only affects the DD but also affects the yield of chitosan produced. **Figure 3c** shows the relationship between deacetylation time, DD, and chitosan yield. The longer the deacetylation time used, the greater the degree of deacetylation and the lower the yield value. The obtained yield tends to decrease with the highest value of 48.45 ± 2.69% obtained at a time variation of 1 h. The time variation of 2 and 3 h did not produce a significant difference in the obtained yield of around 47.7%. Meanwhile, the lowest degree of deacetylation value was obtained at a time variation of 1 hour with an average degree of deacetylation of 44.05 ± 1.61% and the highest degree of deacetylation was obtained at a time variation of 3 h with an average degree of deacetylation of 54.25 ± 2.27%. Based on these data, it can be concluded that the optimum time was obtained at 3 h with a deacetylation degree of 54.25 ± 2.27% and a yield of 47.78 ± 0.81%.

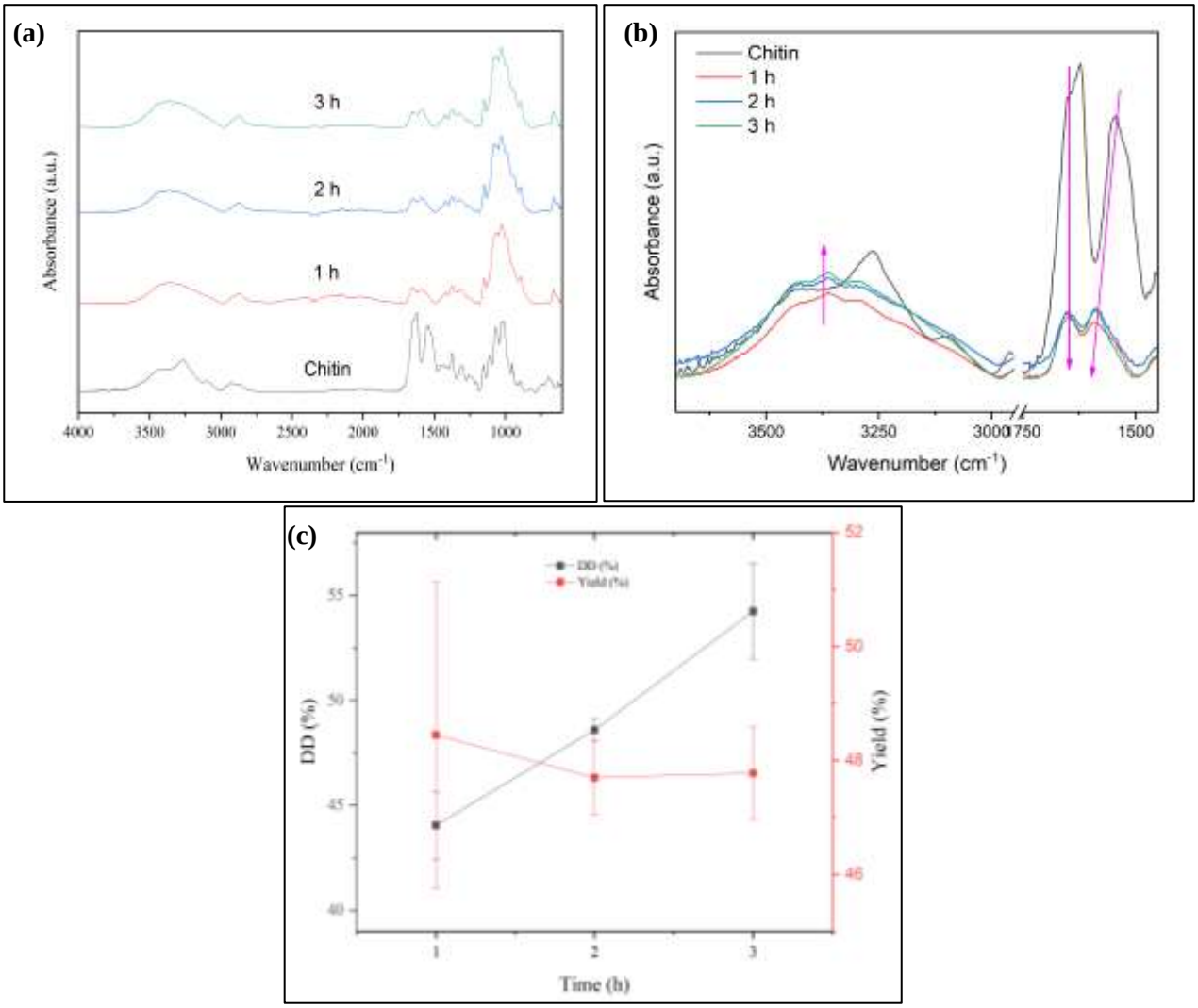

Figure 3. (a) FTIR spectra of chitin and chitosan synthesized at various time. (b) The evolution of FTIR band at 1655-1589 and 3364 cm^{-1}. (c) Relationship curve of deacetylation degree and %yield as a function of time.

Fabrication of Chitosan-PANI Film

Chitosan synthesized using optimum conditions (temperature of 120°C, chitin to NaOH ratio of 1:20 (w/v), and time of 3h) was used to fabricate a film. It is due to the obtained DD (54.25 ± 2.27%) is suitable for the film's application (47 – 59 %) [23]. The addition of glycerol as a plasticizer is used to increase the flexibility of film [24]. The resulting chitosan film is clear with a slightly yellowish color. Based on the color change obtained, the PANI contained in the chitosan film is PANI under leucoemeraldine oxidation conditions. PANI under leucoemeraldine oxidation conditions has a yellow or transparent color [25]. The results of the chitosan-PANI film are shown in **Figure 4**. The film that has been formed is then cut to a size of approximately 5 x 5 cm for immersion using PANI. Soaking using PANI aims to form a chitosan-PANI film in the oxidation state of emeraldine salt (redoping process). The film that has been soaked becomes greenish in color. The dried chitosan-PANI film changes color to dark green and is not clear. Based on the color change produced after immersion in PANI, it can be seen that the PANI contained in the film is emeraldine salt. The synthesized polymer is greenish black in the emeraldine salt state and bluish black in the emeraldine base state [25].

Figure 4. Chitosan-PANI film (left), chitosan-PANI film coating (middle), response test results (right).

Response Test to Ammonia Gas

The results obtained from this research were that there was a color change in the chitosan-PANI film which was exposed to ammonia gas during the first 1 h of observation. The color change that occurs is from a dark green film to blue and the film becomes clearer. The color change that occurs indicates the dedoping of PANI emeraldine salt into the emeraldine base. The dedoping process for emeraldine salt is a reduction reaction with ammonia as the reductant agent. The dedoping process occurs when emeraldine salt reacts with alkaline ammonia gas. Emeraldine salt will release protons (H^+) or deprotonation occurs followed by the binding of OH^- from ammonia gas. This causes the emeraldine salt to be reduced to a blue emeraldine base. The results of the response test to ammonia gas are shown in **Figure 4** and **Table 1**.

Table 1. Color change observation of chitosan/PANI film at different time.

Time (h)	Film color
0	green
1	Bluish-green
2	blue

The resulting film was then characterized using FTIR spectroscopy to see the absorption of functional groups contained in the film. The FTIR spectrum of the film obtained is shown in **Figure 5a**. The absorption band of the chitosan-PANI ammonia film (emeraldine base) can be seen at a wavenumber of 1633 cm^{-1} which indicates the presence of C=C stretching vibrations from the quinonoid ring and 1525 cm^{-1} indicates C=N stretching vibrations of the benzenoid. Meanwhile, in the chitosan-PANI (emeraldine salt) film absorption band, there is an absorption band at wavenumber 1620 cm^{-1} which indicates the C=C stretching vibration of the quinonoid ring and 1521 cm^{-1} indicates the C=N stretching vibration of the benzenoid. In the chitosan (leucoemeraldine) film absorption band at a wavenumber of 1634 cm^{-1} indicates the C=C stretching vibration of the quinonoid ring and 1539 cm^{-1} indicates the C=N stretching vibration of the benzenoid. The peak in the wavenumber region of 3250-3500 cm^{-1} in the three spectra is a typical absorption band for chitosan which indicates the presence of hydroxyl groups. The difference between leucoemeraldine, emeraldine salt, and emeraldine base lies in the area of the quinonoid and benzenoid regions. The wavenumbers are located in the area of 1550-1650 cm^{-1} and 1420-1490 cm^{-1}, respectively. Deprotonation of the emeraldine salt to become emeraldine base can be seen from the presence of absorption at the wavenumber 1435 cm^{-1} which indicates the presence of protonated quinonoid (**Figure 5b**). In the emeraldine base, absorption appears at a wavenumber of 1378 cm^{-1} which indicates C-N stretching vibrations. Absorption at wavenumber 3346 cm^{-1} which indicates stretching vibration =N-H. This indicates that the emeraldine base has been formed.

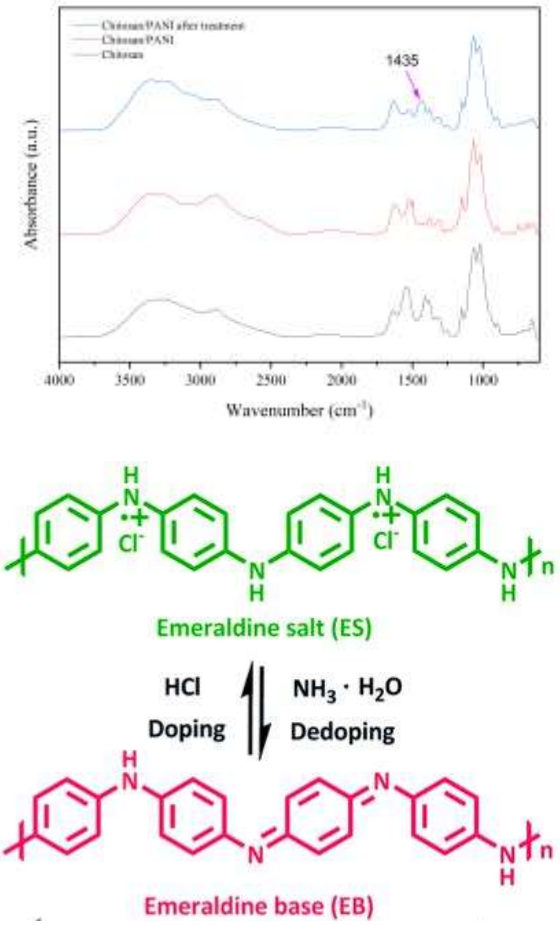

Figure 5. (a) FTIR spectra of chitosan/PANI film. (b) Doping and dedoping process of PANI.

CONCLUSION

The effect of several parameters such as temperature, chitin/NAOH ratio, and time on the degree of deacetylation (DD) and yield of synthesized chitosan was thoroughly studied. The increase of all parameters tends to increase DD ranging from 42 to 55%. On the other hand, the obtained yield decreases as the parameters increases which is due to the acetyl group removal from the main chain of chitosan. The obtained yield ranged from 47 to 51%. The obtained optimum parameters are a temperature of 120℃, a chitin/NaOH ratio of 1:20, and a time of 3 h. These parameters produced chitosan having DD of 54.25 ± 2.27%, and a yield of 47.7±0.65% which is suitable for film application. The response of the chitosan-PANI film to ammonia gas is that a color change occurs when ammonia gas is exposed to the chitosan-PANI film. The color change of the film that occurred was from green to blue. The obtained film not only has biodegradable properties but also smart properties due to its ability to detect pH changes.

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