Effect of acetic acid hydrolysis on the characteristics of water soluble chitosan

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Abstract. Chitosan is a derivative product of chitin deacetylation and has many functions in food industry. However, it has limitation on application caused by low solubility in water. In order to increase the solubility in water, the hydrolysis using acetic acid was performed to reduce the molecular weight. The concentration of acetic acid, temperature of hydrolysis and hydrolysis time related to the solubility were investigated. Hydrolysis using 5% acetic acid was able to produce water soluble chitosan (WSC) with a molecular weight of 198.64 kDa and 46.10% solubility in water. WSC that was obtained at 60 oC for 90 min of hydrolysis process in 5% acetic acid had characteristics of viscosity, molecular weight, solubility in water and deacetylation degree were 0.85 cP, 166.34 kDa, 53.66% and 92.92%, respectively.

Keywords: chitosan; solubility; molecular weight.

1. Introduction

The shrimp processing industry produces shrimp waste mainly in the form of exoskeleton (carapace), reaching 35% of the total production. The exoskeleton contains 27.7-29.8% chitin, 22.8-26.20% protein, 28.89-31.80% minerals, and 79.80-83.60 mg/kg carotenoids [1]. The high amount of chitin in exoskeleton, so it has the potential to be used in making chitosan. Chitosan is a copolimer consisting of β-(1,4)-2-acetamido-D-glucose and β(1,4)-2-amino-D-glucose unit, derived from chitin through deacetylation with alkali [2, 3].

Chitosan has been extensively studied, including as an antibacterial, edible film and forming gels properties. However, it has limiting factor for application in food industry due to the low solubility in the water. Large molecular weight is one of the factor that affect water solubility. The production of water soluble chitosan (WSC) from chitosan is of interest due to benefits related to achieve water solubility. There are two main methods on producing WSC namely chemical hydrolysis mainly in acid condition [4-7] and enzymatic hydrolysis by chitosanase [8-10].

WSC is widely applied in various fields due to high solubility in water. Chouljenko et al. [9] (2016) studied on application WSC to keep quality of shrimp, whereas Jiang et al. [11] and Bonilla et al. [12]
used WSC to extend shelf life of tuna slices and catfish fillet, respectively. Furthermore, application WSC as an antimicrobial was reported by Qin et al. [13] and Alfaro et al. [8].

In this study, hydrolysis using acetic acid was chosen because it was able to produce WSC with low molecular weight, high deacetylation degree and high yield [1]. The objective of this study is to determine the concentration of acetic acid, hydrolysis time and temperature needed to produce WSC and evaluate its characteristics.

2. Material and Method

2.1. Materials
The fresh exoskeleton (carapace) as a raw material on making chitosan was obtained from PT Wirontono Baru (Jakarta, Indonesia). Chemicals i.e. acetic acid, sodium hydroxide, hydrochloric acid, hydrogen peroxide were purchased from Merck Chemical and Life Sciences Group Co. Ltd. (Jakarta, Indonesia). All chemicals were of reagent grade.

2.2. Preparing of Chitosan
Chitosan was made referring to the method of Varun et al. [14] with slight modification. The procedures consisted of demineralization (HCl 2N, 25 °C for 2 h), deproteination (NaOH 2 N, 50 °C for 2 h), and deacetylation (NaOH 2 N, 60 °C for 8 h).

2.3. Preparing of Water Soluble Chitosan (WSC)
Water soluble chitosan (WSC) was prepared by acetic acid hydrolysis according to the method of Kamala et al. [15]. Different concentrations of acetic acid (1, 3 and 5%) at 40 °C for 30 min for hydrolysis were performed (first experiment). The resulting WSC were analyzed for the solubility and molecular weight. The best concentration of acetic acid on producing WSC with the lowest molecular weight and the highest solubility was chosen to prepare the WSC by treating the different temperatures (30, 40, 50 and 60 °C) and times (30, 60 and 90 min) (second experiment). The resulting WSC in each combination treatments were analyzed the molecular weight, solubility, deacetylation degree and viscosity properties.

2.4. Analytical methods
Solubility of WSC
Solubility of WSC was measured based on the method conducted by Kanpairo et al. [16]. WSC sample was dissolved in water (proportion sample and water 1:10), stirred and then filtered using filter paper. The filter paper then is dried and weighed. The solubility was calculated by comparing the weight of soluble fraction with initial sample weight.

Deacetylation Degree of WSC
Deacetylation degree was measured by the acid base titration method of Domard & Rinaudo [17] with modifications. In brief, chitosan (0.1 g) was dissolved in 30 ml HCl aqueous solution (0.1 mol/l) at room temperature with 5-6 drops of methyl orange added. The red chitosan solution was titrated with 0.1 mol/l NaOH solution until it turned orange. The deacetylation degree was calculated by the formula:

$$DD (%) = \frac{(C1V1 - C2V2)}{M \times 0.0994} \times 0.016$$

Where: $C1$ = concentration of standard HCl aqueous solution (mol/l), $C2$ = standard NaOH solution (mol/l), $V1$ = volume of the standard HCl aqueous solution used to dissolve chitosan (ml), $V2$ = volume of standard NaOH solution consumed during titration (ml), and $M$ = weight of chitosan (g). The number 0.016 (g) is the equivalent weight of NH2 group in 1 ml of standard 1 mol/l HCl aqueous solution and 0.0994 is the proportion of NH2 group by weight in chitosan.
Viscosity of WSC

Intrinsic viscosity was measured using Gilmont Falling Ball Viscometer according to the method conducted by Siregar et al. [18] with modification. WSC in various concentrations were prepared and their viscosity measured using equation as follows:

\[ \eta = \eta_{water} \times \left( \frac{\rho_{ball} - \rho_{fluid}}{\rho_{water} - \rho_{water}} \right) \times t_{fluid} \]

where:
- \( \eta_{water} = 1 \) cp,
- \( \rho_{ball} = 8267.76 \) kg m\(^{-3}\),
- \( \rho_{water} = 1000 \) kg m\(^{-3}\),
- \( \rho_{fluid} = 1049 \) (kg m\(^{-3}\)),
- \( t_{water} = 1 \) s,
- and \( t_{fluid} = \) viscometer ball flow time in s.

The viscosity of the solution is obtained by plotting a graph between viscosity and concentration. In order to get the value of viscosity of WSC, extrapolating of y value to zero concentration is needed.

Molecular Weight of WSC

The determination of viscosity-average molecular weight (Dalton), the intrinsic viscosity (\( \eta \)) of the polymer was used. From the intrinsic viscosity, the molecular weight was determined employing the Mark-Houwink equation [19] as follows:

\[ [\eta] = K M^a \]

Where: M = viscosity average molecular weight; K and a = constants, whose values depend on the polymer type and the chosen solvent. For chitosan and the solvent (0.5 M AcOH - 0.2 M NaOAc), these constants are 3.5 \( \times 10^{-4} \) and 0.76, respectively and they do not depend on the deacetylation degree.

2.5. Statistical Analysis

Single and factorial complete random designs were used to analyze the data among various treatments in the first and second experiments, respectively. Data were analyzed using analysis of variance and Tukey's range test at a significance level of \( \alpha = 0.05 \). Results are expressed as mean value ± standard deviation.

3. Result

3.1. Molecular Weight and Solubility of WSC Resulting of Acetic Acid Hydrolysis at Various Concentrations

As shown in Figure 1, molecular weight and solubility of WSC were significantly affected by acetic acid concentration on hydrolysis process (\( p < 0.05 \)). Hydrolysis using 5% of acetic acid produced the lowest molecular weight and the highest solubility of WSC with values were 198.64 kDa and 46.10%, respectively. Those values were significantly different compared to others. Therefore, hydrolysis using 5% acetic acid was chosen for the next experiment (second experiment) on preparing WSC through assessing the temperature and hydrolysis time.

3.2. Molecular Weight of WSC Resulting of 5% Acetic Acid Hydrolysis at Various Temperature and Time

Temperatures, time and both interaction gave significantly effect on decreasing the molecular weight of WSC (\( p < 0.05 \)) (Figure 2). Molecular weight ranges were between 166.34 kDa and 379.59 kDa. In general, increasing the temperature and time hydrolysis produced the WSC in low molecular weight. Hydrolysis at 60 °C for 90 min resulted the lowest molecular weight of WSC and was significantly different from other treatments.
Figure 1. Molecular weight (A) and solubility (B) of WSC at different concentration of acetic acid on hydrolysis. The following numbers with different superscripts (a, b, c) indicate significant differences (p < 0.05).

3.3. Solubility of WSC Resulting of 5% Acetic Acid Hydrolysis at Various Temperature and Time
There is a tendency to improve solubility of WSC by increasing the temperature and hydrolysis time (Figure 3). However, only the temperature had significantly effect on increasing solubility of WSC, while the time and interaction between temperature and time did not give significant effect. Hydrolysis at 60 °C and 30 °C obtained the highest and the lowest solubility of WSC with average values were 52.99% and 44.18%, respectively.
3.4. Deacetylation Degree of WSC Resulting of 5% Acetic Acid Hydrolysis at Various Temperature and Time

The deacetylation degree of WSC had a short range values between 83.92% to 94.01%, as presented at Figure 4. The deacetylation degree of WSC was only affected significantly by the time ($p < 0.05$), while the hydrolysis temperature and both interaction did not give significant effect ($p > 0.05$).
Hydrolysis for 60 min, produced the WSC with average value of deacetylation degree was 90.67% and significantly different compared to 30 and 90 min with average values were 87.71% and 88.54%, respectively.

3.5. Viscosity of WSC Resulting of 5% Acetic Acid Hydrolysis at Various Temperature and Time

The viscosity value of WSC is grouped in very low with the range values was 0.76 - 0.93 cP (Figure 5). Temperature, hydrolysis time and interaction between temperature and time did not have significant effect on the viscosity value of WSC ($p > 0.05$).

![Figure 5. Viscosity of WSC at different temperature and time hydrolysis of 5% acetic acid.](image)

### 4. Discussions

Increasing concentration of acetic acid for hydrolysis process, causes the low molecular weight of WSC, while the solubility of WSC increases. This is due to the cutting off glycosidic and N-acetyl bounds [20]. Qin et al. [21] reported, when the molecular weight of chitosan decreased, the solubility of chitosan in water increased due to its shorter chain of polymer chitosan with more free amino groups in the D-glucosamine unit. Research conducted by Kasaai et al. [20] reported that hydrochloric acid (0.1 - 1 M) was able to decrease the molecular weight of WSC from 1616 kDa to 296.9 kDa. Jia & Shen [22] hydrolyzed chitosan using phosphoric acid for 4 h, resulted that the molecular weight decreased from 214 kDa became 74 kDa.

Based on the molecular weight, chitosan can be grouped into low molecular weight (< 100 kDa), medium molecular weight (100 - 1000 kDa), and high molecular weight (> 1000 kDa) [23]. In our research, we succeeded on making WSC in medium molecular weight (198.64 - 592.89 first experiment) and (166.34 - 379.95 kDa second experiment). High molecular weight chitosan is difficult to dissolve in water due to its long chain, therefore needs to be decreased of molecular weight to be soluble in water [23]. The degrees of polymerization of WSC are usually 2 - 20 [24].

Chitosan is generally insoluble in water, however it is soluble in acidic conditions. Several types of acid commonly are used such as acetic acid, formic acid, and ascorbic acid can dissolve chitosan well at concentrations of 1 - 5%, including inorganic acid [25]. The use of 5% acetic acid was able to cut off the long chitosan chain (mainly glycosidic bounds) and reduced the molecular weight, as a result the solubility in water increased. The reducing of molecular weight and improving of WSC solubility were strengthened by increasing the temperature and hydrolysis time.

Increasing the deacetylation degree is thought to also contribute to the reducing of the molecular weight of chitosan. The use of high temperatures for a long time in the hydrolysis process causes
bounds between molecules to be weaker, so that it will remove the N-acetyl chitosan group into amines and also cause lower molecular weight [26]. The deacetylation degree chitosan increased with increasing hydrolysis time and temperature [27, 28]. The effect of hydrolysis by removing of N-acetyl groups did not have a strong influence on deacetylation degree. This shows that acid hydrolysis was more appropriate in breaking of glycosidic bounds of glucose group than hydrogen bounds of the N-acetyl group. The deacetylation process involves the removal of N-acetyl groups from the biopolymer and alkali treatment using NaOH is the best method [2, 3, 29].

The viscosity of WSC did not show significant differences, although molecular weight and solubility were significant different. This indicates that besides molecular weight there are still other factors that influence the value of the WSC viscosity. Factors that are thought to influence such as the presence of disturbing ions, bond type and pressure [30].

5. Conclusions
In this study, the best hydrolysis on producing WSC was obtained from the treatment of 5% acetic acid at 60 °C for 90 min. The resulting WSC had lowest molecular weight of 166.34 kDa, highest solubility of 53.66% and deacetylation degree of 92.92%.

Acknowledgements
Authors thank to Directorate International Program, IPB University (Bogor Agricultural University) and Center for Coastal and Marine Resources Studies, IPB University (Bogor Agricultural University) for their financial support on attending the World Seafood Congress 2019 (Penang Malaysia, 9 - 11 September 2019) and publishing the article. Help of PT Wirontono Baru (Jakarta, Indonesia) for providing fresh exoskeleton (carapace) as a raw material on making chitosan is highly appreciated.

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