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Effect Of Hydrogen Peroxide Concentration on The Deacetylation Degree Of Chitosan Extracted From Maggot Waste

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ABSTRACT

Pupae shell of Black Soldier Fly (BSF) potentially serve as a green and new alternative source of chitosan. Deacetylation by using H2O2 is the most important step for the quality characteristic of chitosan. The objective of this study was to determine the effect of hydrogen peroxide concentration on the deacetylation degree of chitosan extracted from maggot waste. Different hydrogen peroxide concentration (0%, 13%, 15%, and 20%) was studied. The degree of deacetylation of chitosan, determined using FTIR, was found to be 75.3%, 77.6%, 78%, and 78.6% for H_2O_2 concentrations of 0%, 13%, 15%, and 20%, respectively. The quality of chitosan extracted from the pupae shell of maggot waste meets the requirements of SNI 7949-2013. Higher H_2O_2 concentrations increased the deacetylation degree of chitosan extracted from maggot waste

Keywords: *Chitosan; Deacetylation Degree; Hydrogen Peroxide; Maggot Waste*

INTRODUCTION

Black Soldier Fly (*Hermetia illucens*) is an insect belonging to the Stratiomyidae family. Black Soldier Fly (BSF) undergoes various life stages, including maggot larvae, prepupa, pupae, and fly stages. Maggot larvae serve as organic decomposers, playing an essential role in returning nutrients to the soil (Gold et al., 2018). Maggot larvae grow and digest biowaste which can be harvested as nutrient-rich animal feed production (Surendra et al., 2020) and potentially used as a food source food for humans (Bessa et al., 2020). Through maggot larvae activities, organic matter was converted into protein and lipids, contributing to microbial and chemical product safety (Gold et al., 2018), and offering potential economic benefits (Gold et al., 2018). Through these activities, maggot larvae produce waste in the form of pupae shells
which could potentially serve as a new alternative source of chitosan (Pintowantoro et al., 2021). This environmentally friendly, land-originating source, often referred to as green technology, holds significant promise in the pharmaceutical (Vaz et al., 2018), food, and chemical industries (Morin-Crini et al., 2019).

Chitosan [poly-(2-amino-2-deoxy-β-(1-4)-D-glucopyranose)] is a Poly-amino-saccharide compound synthesized by partial removal of the 2-acetyl group from chitin [poly(2-acetamido-2-deoxy-β-(1-4)-D-glucopyranose)], a linear biopolymer with 2000-5000 monomer units, mutually bound by -(1-4) glycosidic bonds (Shahid-ul-Islam & Butola, 2019). Chitosan (C_6H_{11}-NO_4) is a compound in the form of a yellowish-white amorphous solid, which is a polyelectrolyte. Generally soluble in organic acids, pH about 4-6.5, insoluble at lower or higher pH. Solubility of chitosan is affected by molecular weight and degree of deacetylation. Chitosan can be produced by hydrolysis of chitin using a strong base (Firnanelty et al., 2021). Chitin and chitosan compounds are easily decomposed and do not possess toxic properties, and are environmentally friendly (Shahid-ul-Islam & Butola, 2019).

The deacetylation degree (DD) is the most important quality characteristic of chitosan (Annu et al., 2017). The concentration of H_2O_2 immersion of chitin will affect the degree of deacetylation which refers to the removal of the acetyl group in the chitin molecule which

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then becomes chitosan (Tian et al., 2004)**.** The concentration of H_2O_2 immersion in the process of chitosan extraction from maggot waste is currently limited. Thus, this study aims to determine the effect of hydrogen peroxide concentration on the deacetylation degree of chitosan extracted from maggot waste.

METHODS

Material

Pupae shells from maggot waste were obtained from a local farmer. These were sorted, and naturally dried. The shells were then ground to a 50-mesh size and stored in powder form in a dark bottle.

Tool

Fourier Transform Infrared Spectroscopy (FTIR, Thermo Scientific FTIR Nicolet iS10 ATR, USA), hot plate, oven, pH meter, vapodest, furnace, water bath, analytical balance, micropipette, laboratory glassware.

Chitin extraction

The extraction process of chitin from the grinding of the pupae shell of maggot is carried out through a 3-step process involving of deproteination, demineralization, and depigmentation. The deproteination process was carried out with 3.5% NaOH, at a ratio of 1:10 at 70°C for 2 hours. It was washed by using aquadest until pH 7 followed by the demineralization process. This was carried out by immersing in 1.5 N HCl, at a ratio of 1:15, at a temperature of 40°C for 30 minutes, followed by washing with aquadest until pH 7 and drying at 60 ^oC for 24 hours. The yield was immersed in 1% $KMnO_4$ and 1% acetic acid for depigmentation.

Chitosan extraction

Chitin is first washed to reach a pH of 7 and then by drying. The final stage involves is deacetylation using 60% NaOH at room temperature for 6 days followed by the immersion in H_2O_2 with different concentrations (0%, 13%, 15%, and 20%) at 40 ^oC for 4 hours to obtain chitosan with a certain deacetylation degree. The deacetylation degree is measured using FTIR (Heidari *et al*., 2018). Chitosan quality is assessed based on SNI (BSN, 2013) which depends on deacetylation degree, nitrogen content, water content, and ash content. The chitosan extraction was done triplicates. Quantitative analysis is conducted using infrared spectrophotometry based on transmittance (%) or absorbance values. The deacetylation degree is calculated using the following formula:

$$\%\,DD = 1 - \left[\frac{A1655}{A3450} - \frac{1}{1{,}33}\right] \times 100\%$$

Note:
A1665 = Absorbance at a wavelength of 1665 cm^{-1}
A3450 = Absorbance at a wavelength of 3450 cm^{-1}
1,33 = Constanta obtained from the ratio of A1665/A3450 for chitosan with full acetylation

Statistical analysis

The differences in the concentration of H2O2 were determined by using one-way ANOVA (SPSS version 13.0) at a significance level of $P<0.05$.

RESULT AND DISCUSSIONS

The chitin transformation process, with variations of concentration of H_2O_2, was used to determine the deacetylation degree of the chitosan formation process aiming to achieve the highest degree of deacetylation. The immersion time of chitin in H_2O_2 affects the breakdown of the chitin molecular chain (Hahn et al., 2020). Samples of chitin in powder form, with a mesh size of 50, were subjected to soaking in various concentrations of H_2O_2. This process enhanced the contact between particles and the alkaline solution during the deacetylation process. Chitosan is a product of the deacetylation of chitin using hot concentrated NaOH. Annu et al. (2017) revealed that the concentration of NaOH used for deacetylation aimed to break the hydrogen bonds between oxygen in the carboxyl group and hydrogen in the amine group between 40–50% at 80–150 ^{o}C. Furthermore, the converted chitosan was characterized by using the FTIR spectrophotometric method. The spectrum results for chitosan extracted from pupae shell waste are shown in Figures 1 to 4, while Figure 5 displays the spectrum for commercial chitosan. The spectrum of converted chitosan extracted from the pupae shell of maggot waste exhibited typical absorption bands, namely 3212.02 (-OH), 2850.36, and 2918.63 (stretching C-H and -C=O of CONH-R group), 1633.27 (Amide), 1537.86 ($-NH_2$), 1316.59 (-CO) and 1061.64 (stretching C-O-C) as shown in Table 1. The presence of noise in Figures 1-4 suggests a potential cause in the form of residual NaOH resulting from an imperfect washing process. The spectrum of chitosan converted from commercial chitin has characteristic absorption bands, namely 3260.79 (-OH), 2878.09 (stretching C-H and -C=O of CONH-R group), 1588.47 (Amide), 1412.82 ($-NH_2$), 1322.79 (-CO) and 1022.74 (stretching C-O-C) as shown in Table 1. These were similar to the previous study by Ssekatawa *et al.*(2021).

Table 1. The wavelength and groups in chitosan extracted from pupae shell of maggot waste and commercial chitosan

Group	Wavelength in chitosan extracted from pupae shell of maggot waste (cm^{-1})	Wavelength in commercial chitosan (cm^{-1})
-OH	3212.02	3260.79
Stretching C-H and -C=O of CONH-R group	2850.36 and 2918.63	2878.09
Amide	1633.27	1588.47
$-NH_2$	1537.86	1412.82
-CO	1316.59	1322.79
Stretching C-O-C	1061.64	1022.74

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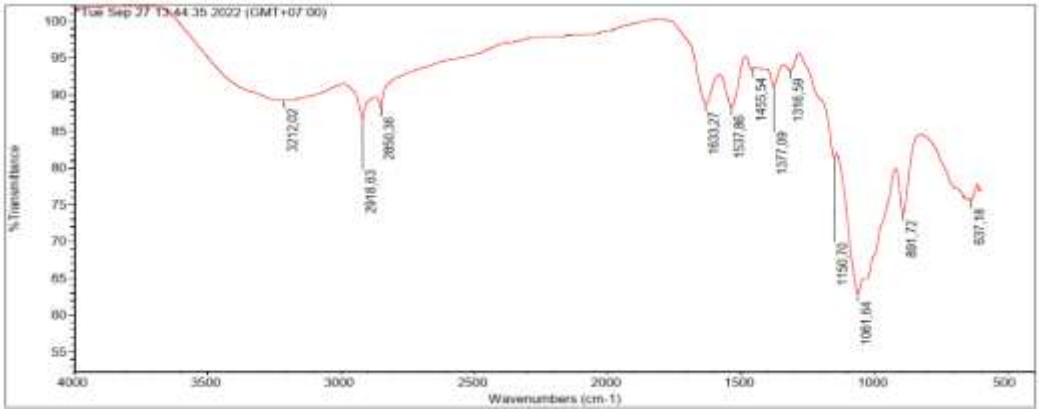

Figure 1. FTIR Spectrum of chitosan extracted from pupae shell of maggot waste without H_2O_2 immersion with DD 75.3 %

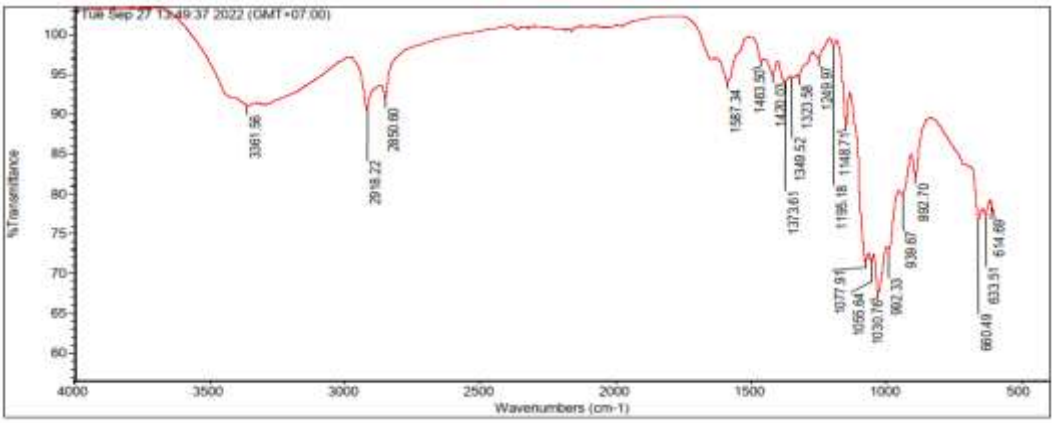

Figure 2. FTIR Spectrum of chitosan extracted from pupae shell of maggot waste of H_2O_2 13% with DD 77.6 %

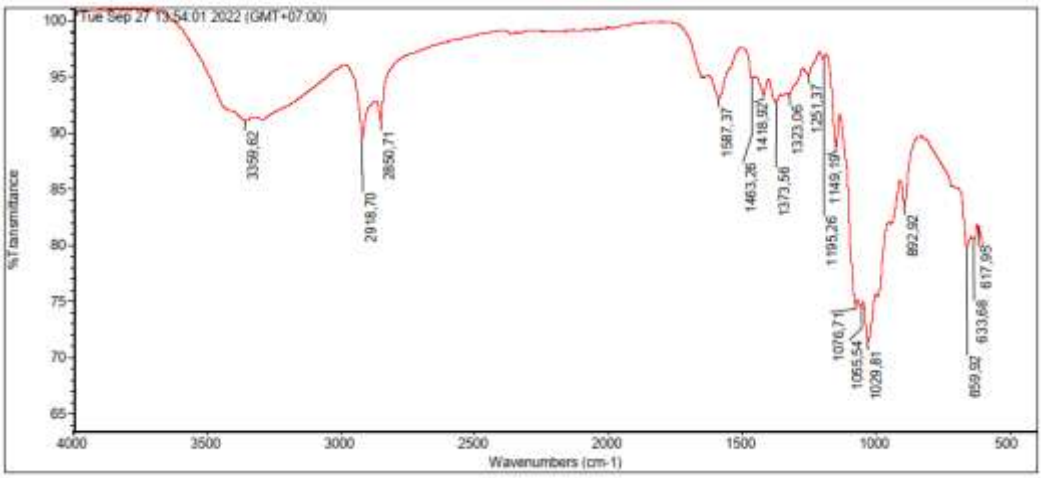

Figure 3. FTIR Spectrum of chitosan extracted from pupae shell of maggot waste with H_2O_2 15% with DD 78 %

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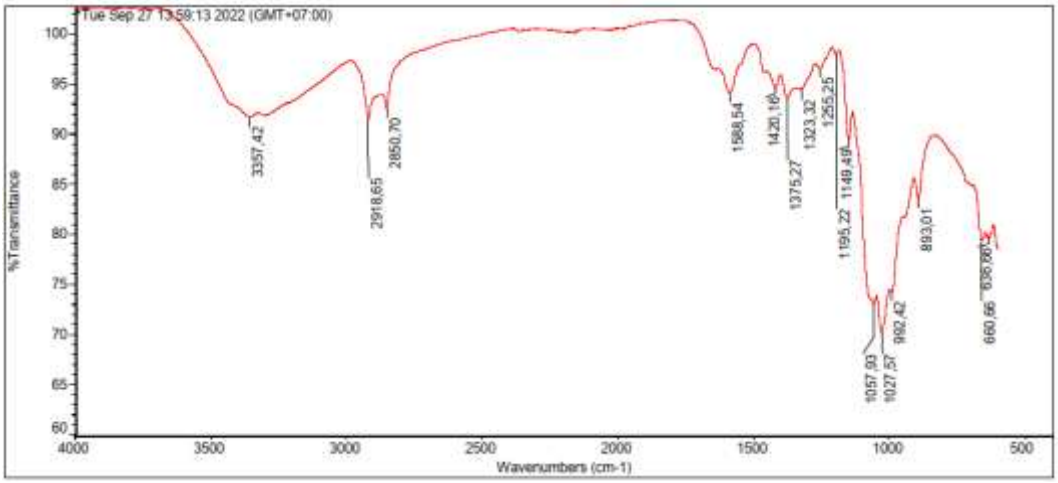

Figure 4. FTIR Spectrum of chitosan extracted from pupae shell of maggot waste with H_2O_2 20% with DD 78.6 %

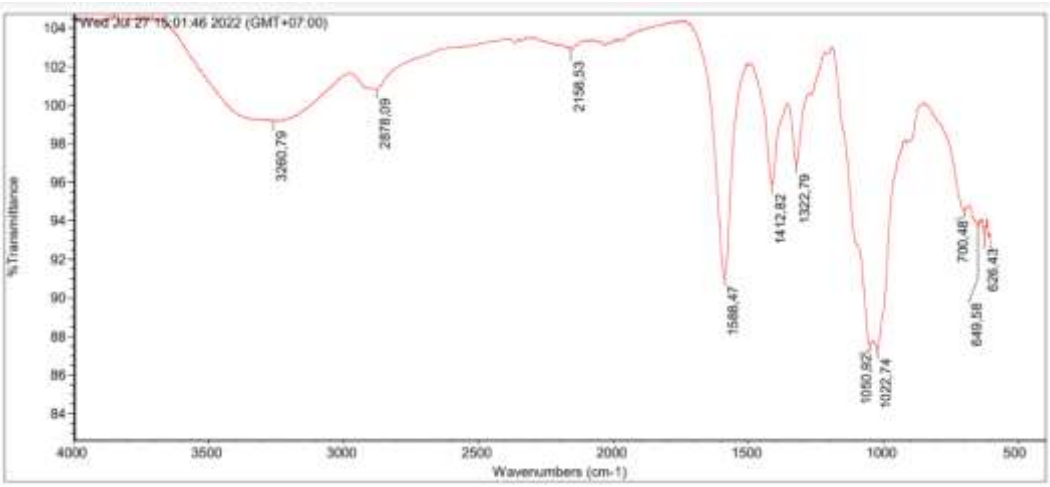

Figure 5. FTIR Spectrum of commercial chitosan at DD 84%

Table 2. Characteristic of chitosan extracted from pupae shell of maggot waste

Parameter	Value	SNI 7949-2013
Yield (%)	22 – 23	-
Acidity (pH)	7	7
Water content (%)	5.13 - 5.58	$\leq$ 12
Ash content (%)	0.41 – 0.73	$\leq$ 5
Nitrogen content (%)	6.71 - 6.98	$\geq$ 5
Deacetylation degree (%)	75.3 - 79	$\geq$75

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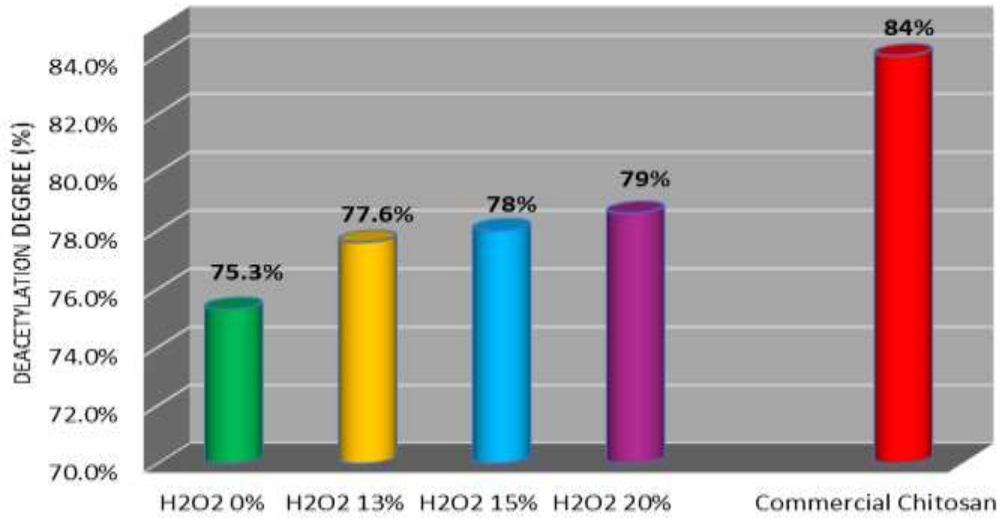

Figure 6. Deacetylation degree of chitosan extracted from pupae shell of maggot waste and commercial chitosan

By increasing the concentration of H_2O_2, the bond between carbon in the acetyl group and the nitrogen in the amine group was broken and, resulting in the increase of deacetylation degree. Statistical analysis showed significant difference ($P < 0.05$) between different concentrations of H_2O_2. This study is similar to those of Tanasale et al. (2016). Tanasale et al. (2016) found that chitosan conformation can be broken by H_2O_2 solution. There did not show significant difference ($P > 0.05$) between H_2O_2 13% and H_2O_2 15%. The spectrum of chitosan was in accordance with those reported of Ssekatawa et al. (2021) and Sudianto *et al* (2020). In this study, the deacetylation degree of chitosan, which has immersed in H_2O_2 at a concentration of 20%, is 78.6% (Fig. 6). This indicated the presence of 78.6% NH_2 groups and 21.4% remaining acetyl group in the chitosan. The obtained result was lower than that of commercial chitosan. This difference can be attributed to the high purity of commercial chitin, resulting in a higher chitosan content. It also suggests that the results of the extraction process still contain substances that can dissolve in a hot, strong base solution.

The chitosan extracted from maggot pupae shell waste meets the quality standards outlined in SNI 7949-2013, as indicated in Table 2. The water content in nanochitosan can play a crucial role in determining their shelf life. Managing factors like humidity and light exposure becomes particularly important for products stored in powder form, as these conditions can affect the quality, stability, and safety of the product over time. Thorough and repeated washing during the demineralization process results in the separation of the dissolved calcium chloride component from the BSF pupae shell.

Nitrogen content determines the nature of chitosan that interacts with other groups. The presence of various compounds in chitosan, including the formation of an amine group (NH_2), causes chitosan to have a fairly high chemical reactivity, allowing it to bind water and dissolves in acetic acid (Zahiruddin et al., 2008).

CONCLUSION

The present work highlights the effect of hydrogen peroxide concentration on the deacetylation degree of chitosan extracted from maggot waste. The degree of deacetylation of chitosan by using FTIR was to be 75.3%, 77.6%, 78%, and 78.6% for a concentration of H_2O_2 of 0%, 13%, 15%, and 20%, respectively. The quality of chitosan extracted from pupae

shell of maggot waste meets the quality standards specified in SNI 7949-2013. An increase in the H_2O_2 concentration may lead to a higher deacetylation degree in chitosan extracted from maggot waste.

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