Menara Perkebunan 2024, 92(2), 103-111
p-ISSN: 0125-9318/ e-ISSN: 1858-3768
http://dx.doi.org/10.22302/iribb.jur.mp.v92i2.575
Accreditation Number: 164/E/KPT/2021

Optimization of the demineralization process of black soldier fly (*Hemertia illucens*) pupa shell maggot chitosan and the physicochemical characteristics

Mira MAULIDINA[1*)], Muhammad RIFQI[1)] & SISWANTO[2)]

[1)]Halal Food Science Faculty, Djuanda University, Jl. Tol Jagorawi No.1 Ciawi, Bogor 16720, West Java, Indonesia
[2)]Indonesian Oil Palm Research Institute- Bogor Unit, Jl. Taman Kencana No.1, Bogor 16128, West Java, Indonesia

Received 23 Mar 2024 / Revised 27 May 2024 / Accepted 22 Oct 2024

Abstract

Chitosan is a derivative compound of chitin that has undergone deacetylation. Chitosan has three stages of the manufacturing process, including demineralization, deproteinization, and deacetylation. Chitin is also found in the black soldier fly maggot pupae, but maggot pupae contain high minerals content that can affect the purity of the resulting chitosan. Therefore, demineralization treatment is necessary to remove minerals from maggot pupae shells. This study aims to optimize the demineralization process by finding the best type of acid solvent, the best incubation time, and combination treatments. The black soldier fly (BSF) maggot pupa shell was soaked using various formic acid, hydrochloric acid, and nitric acid solutions with incubation times of 60, 120, and 180 minutes. Chitosan characterization was carried out following SNI 7949:2022, including water content, ash content, nitrogen content, pH, deacetylation degree, characterization of functional groups with FT-IR, and as an antimicrobial comparison is formalin. The best demineralization treatment was obtained at 0.5 M nitric acid treatment with an incubation time of 120 minutes. The characterization of chitosan produced 7.81% water content, 0.56% ash content, 4.73% nitrogen content, pH 7.39, and 75.14% deacetylation degree. Characterization of groups on chitosan with FT-IR resulted in the absorption of O-H and N-H groups 3484 cm^{-1} and 3152 cm^{-1}; C-H 2877 cm^{-1}; and C=O 1653 cm^{-1}. The inhibitory power against *E. coli* of the BSF maggot pupa shells chitosan is better compared to chitosan standard but not better than formalin.

[Keywords: antimicrobial, BSF maggot pupa shell, characterization, chitosan, demineralization]

*) Corresponding author: miramaulidina10@gmail.com

Introduction

Formalin is a food preservative that has side effects as a poison but is often misused by food producers in Indonesia to preserve products such as fresh noodles, salted fish, fresh fish, tofu, chicken, beef, and so forth (Budianto, 2011). The use of formalin as a food preservative is prohibited in Indonesia, as stated in the Indonesian Ministry of Health Regulation No.1168/Menkes/Per/X/ 1999, which was updated from Indonesian Ministry of Health Regulation No.722/Menkes/ Per/IX/1988, Ministry of Industry Regulation No. 24/MInd/Per/5/2006, and Indonesian Government Regulation No. 28 Year 2004 (Yulisa et al., 2014).

As an alternative, chitosan can be used as a safer food preservative, with the expectation that it can provide antimicrobial properties comparable to formalin. According to Damayanti et al. (2016), chitosan exhibits antimicrobial properties by inhibiting pathogenic bacteria and spoilage microorganisms, including fungi, Gram-positive and Gram-negative bacteria. Damayanti et al. (2016) also state that chitosan has a higher antibacterial ability against *Escherichia coli* (Gram-negative bacteria).

Chitosan is a compound derived from chitin that has undergone deacetylation (Aranaz et al., 2021). Chitin can also be found in the pupae of black soldier fly (*Hermetia illucens*) (BSF) maggots. Maggot farming in Indonesia is increasingly popular, typically used as animal feed due to its high protein content, ranging from 30-45% (Azir et al., 2017). With the increase in maggot production, more pupae are generated. BSF maggots contain about 12.4% chitin, while their pupae contain approximately 25.5% chitin (Triunfo et al., 2022). According to Yanuar (2013), crab shells contain 20-30% chitin, whereas shrimp shells contain 15-20% chitin (Altschul, 1976).

Menara Perkebunan is DOAJ indexed Journal and accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3125)
How to Cite: Maulidina, M., Rifqi, M. & Siswanto (2024). Optimization of the demineralization process of black soldier fly (*Hemertia illucens*) pupa shell maggot chitosanand the physicochemical characteristics. *Menara Perkebunan, 92*(2), 103-111
http://dx.doi.org/10.22302/iribb.jur.mp.v92i2.575

However, among these sources of chitin-containing raw materials, black soldier fly (BSF) pupae have a relatively short lifespan of around 40-43 days (Tomberlin et al., 2002), thus producing more raw material faster compared to crabs and shrimp, resulting in abundant waste shells suitable for utilization. Chitosan is obtained through several processing steps, including demineralization, deproteinization, depigmentation, and deacetylation. According to Triunfo et al. (2022), maggot pupae contain 16.0% minerals, a significant amount that can affect the purity of the resulting chitosan. Therefore, demineralization treatment is necessary to remove minerals from maggot pupae shells.

This study aims to optimize the demineralization method of BSF pupae shells by determining the most optimal types of acidic solutions and incubation times. It will also characterize chitosan from BSF pupae and assess its antimicrobial properties, which will be compared with the antimicrobial activity of formalin. In accordance with SNI 7949:2022 on "Chitosan - Quality Requirements and Processing" (BSN, 2022), the characteristics to be evaluated include color, shape, moisture content, ash content, nitrogen content, pH, and degree of deacetylation. Functional group characterization will also be conducted using Fourier-transform infrared spectroscopy (FT-IR).

Materials and Methods

Materials

The materials used in this study were the shells of BSF maggots from the Indonesian Oil Palm Research Institute (Unit Bogor). Formic acid, HCl, HNO_3, hydrogen peroxide, and distilled water used in this research was from pure analytical reagent.

Demineralization treatment

The demineralization process was done by soaking 100 g of BSF maggot pupae shells in an acid solution (material: acid solution = 1:10/ v:v). This study used three types of acids (formic acid / HCOOH, hydrochloric acid / HCl, and nitric acid/ HNO_3), each at a concentration of 0.5 M (v/v), and incubation times of 60 minutes, 120 minutes, and 280 minutes with three replications at 40°C. The difference from previous research lies in the type of acid solution and the incubation time used. Subsequently, the samples were washed until neutral pH (6.5 – 7.5) and dried in an oven at 65°C. Ash content analysis was performed to determine the acid solution that provides the best demineralization efficiency (DME). After demineralization, the BSF maggot pupae shells with the highest DME value will proceed to deproteinization. The demineralization efficiency (DME) can be calculated using the following formula (1) (Hahn et al., 2022):

$$\%\text{DME} = 100 - (\frac{A}{B} \text{ x } 100) \text{.....(1)}$$

Note:
A= The ash content after demineralization
B= The ash content of raw materials

Chitosan production

Chitosan production from BSF pupa shells was carried out through extraction with chemical solutions. The method used refers to the research by Triunfo et al. (2022), Hahn et al. (2022), and Wahyuni et al. (2021). The extraction process began with demineralization according to the experimental design. The demineralized BSF pupa shells with the best DME value was proceed to deproteinization. Deproteinization involves soaked 50 g demineralized BSF pupa shells in a 3 M NaOH solution (material: NaOH = 1:10/ v:v) and incubated at 90°C for 240 minutes. Next, the sample was washed to a neutral pH (6.5 – 7.5) and dried in an oven at 65°C.

Subsequently, the depigmentation process was done by soaking 25 g of deproteinized chitin in 5% hydrogen peroxide. Incubation was conducted at 60°C for 60 minutes. Next, the sample was washed to a neutral pH (6.5 – 7.5) and dried in an oven at 65°C. The deacetylation process was followed by soaking 50 g of demineralized BSF pupa shells in a 12 M NaOH solution (material: NaOH = 1:20/ v:v). Incubation was conducted at 95°C for 240 minutes. Finally, the sample was washed to a neutral pH (6.5 – 7.5) and dried in an oven at 65°C.

Chitosan characterization

The produced chitosan was then subjected to chemical characterization according to SNI 7940:2022 with parameters including moisture content by gravimetric method, ash content by gravimetric method, nitrogen content by Kjeldahl method, pH, and degree of deacetylation by volumetric method.

a. Water content analysis

Water content analysis was calculated according to AOAC (2006). An aluminum dish was dried in an oven for 15 minutes, cooled in a desiccator for 10 minutes, and then weighed (A). Approximately 1 g of the sample was weighed in the dish (B). The dish and contents were dried in an oven at 100ºC for 6 hours. The dish was then transferred to a desiccator, cooled, and weighed.

The dish and contents were dried again until a constant weight was obtained (C). Water content calculation (2):

$$\text{Water content } \left(\%\frac{b}{b}\right) = \frac{B-(C-A)}{B} \text{ x } 100\% \text{.....(2)}$$

Note:
A = The weight of the empty dish (g)
B = The weight of the sample (g)
C = The weight of the dish and sample after heating (g)

b. Ash content analysis

A dish was prepared for ashing by drying in an oven for 15 minutes, then cooled in a desiccator and weighed (A). Approximately 1 g of the sample was weighed in the dish (B) and then burned in a fume hood until it no longer emits smoke. Ashing was carried out in an electric furnace at 400-600ºC for 4-6 hours until white ash or a constant weight was obtained. The ash and dish were cooled in a desiccator and weighed (C) (AOAC, 2006). Ash content calculation (3):

$$\text{Ash content } \left(\%\frac{b}{b}\right) = \frac{C-A}{B} \text{ x } 100\% \text{.....(3)}$$

Note:
A = The weight of the empty dish (g)
B = The weight of the sample (g)
C = The weight of the dish and sample after heating (g)

c. Nitrogen content analysis

Protein analysis used the semi-micro Kjeldahl method (AOAC, 2006). Approximately 1 g of sample was weighed and placed into a 30 mL Kjeldahl flask. Then, a mixture of Selen (0.9 g K_2SO_4, 40 mg HgO) and 10 mL H_2SO_4 was added. The sample was boiled for 1–1.5 hours until the solution became clear. The solution was then transferred to a distillation apparatus, rinsed with distilled water, and 15 mL of 30% NaOH solution was added. The NH_3 gas generated from the reaction in the distillation apparatus was captured by 20 mL of H_3BO_3 in an Erlenmeyer flask to which three drops of BCG: MM indicator (a mixture of 2 parts 0.2% methyl red in alcohol and 1 part 0.2% methylene blue in alcohol) have been added. The condensate was then titrated with 0.05 N standardized HCl until the color changed from green to pink. A blank determination was carried out using the same method as the sample determination. Nitrogen content calculation (4):

$$\text{Nitrogen content } = \frac{(V1-V2)\ x\ N\ HCl\ x\ 14\ x\ fp}{B} \text{ x } 100\% \text{ (4)}$$

Note:
V1 = volume of 0.05 N HCl required for titrating the sample (mL)
V2 = volume of 0.05 N HCl required for titrating the blank (mL)
N = normality of HCl
Fp = dilution factor
B= sample weight (mg)

d. Degree of deacetylation analysis

The degree of deacetylation test was conducted using the titration method (SNI, 2016). As much as 0.2 g of chitosan was weighed and placed into an Erlenmeyer flask, then 20 mL of 0.1 N HCl was added and shaken for 15 minutes. Then, 50 mL of distilled water was added gradually while shaking until homogeneous. Subsequently, 2-3 drops of PP indicator were added and titrated with 0.1 N NaOH until a faint pink endpoint was obtained. Calculation of deacetylation degree (5):

$$\text{Deacetylation degree}(\%) = \frac{(V1\ x\ C1)-(V2\ x\ C2)}{\text{M x } 0.0994} x\ 0.016\ x\ 100\text{.....(5)}$$

Note:
V1 = Volume of HCl solution (mL)
V2 = Volume of NaOH solution (mL)
C1 = Concentration of HCl solution (N)
C2 = Concentration of NaOH solution (N)
M = Constant weight of chitosan (g)

e. pH measurement

As much as 5 g of the finely ground sample was placed into a 100 mL glass beaker, then add 25 mL of distilled water. Then, the sample was homogenized using a magnetic stirrer or a stirrer until homogenous. The pH meter probe was immersed into the sample solution, and record the pH reading on the pH meter screen once it was stable.

Functional group identification using FT-IR

FT-IR analysis is one of the infrared spectroscopy methods that can identify functional groups, chemical structures, absorption bands, and polymer bonding information in a compound. This technique utilizes the infrared spectrum of 400-4000 cm^{-1} (Kauppinen & Partanen, 2001). In FT-IR spectroscopy, the traditional monochromator was replaced by an interferometer. This method offers several advantages, including relative speed, the fact that it does not require pure samples and high precision. Pellets of BSF pupa shell chitosan samples were prepared with Kalium bromide (KBr) with diameters less than 2 µm, then scanned in the frequency range from 4500 cm^{-1} to 500 cm^{-1}.

Antibacterial activity test against E. coli

The antimicrobial activity test was conducted using the well-diffusion method described by Halimathussadiah et al. (2021). Sterile Nutrient Agar (NA) medium (10 mL) was poured into sterile Petri dishes as the base layer and allowed to solidify halfway. Next, 1 mL of the bacterial suspension was spread onto the medium and homogenized. Then, 5 mL of medium was added as the second layer and homogenized again. After solidification, wells were created using a sterile punch with a 6 mm diameter. The test samples were pipetted into the wells, and the plates were incubated at 37°C for 24 hours. The inhibition zones formed were measured using a

screw micrometer. The inhibition test was performed with two replicates.

Data analysis

The data obtained in this study were analyzed using SPSS 21 software. The statistical test used was one-way ANOVA. If $p < 0.05$ (significant difference), then further analysis was conducted using the Duncan Multiple Range Test (DMRT) with a confidence level of 5%.

Result and Discussion

Chitosan demineralization

Demineralization is one of the stages in the production of chitosan, with the intention of reducing the mineral content in the material to produce pure chitosan as much as possible. This study conducted experiments using several acidic solutions, including formic acid, hydrochloric acid, and nitric acid. The use of these acidic solutions functions to hydrolyze the raw materials so that the minerals contained in the materials can dissolve into the acidic solution (Cahyono et al., 2018). Additionally, experiments were conducted to determine the incubation time of the materials with the solutions, with treatments of incubation for 60, 120, and 180 minutes. The % DME (Demineralization Efficiency) results from each treatment can be seen in Table 1.

The ANOVA test showed that the acidic solution treatment significantly affected the %DME value (Table 1). However, the different incubation time treatments did not significantly affect the %DME value. Nevertheless, the interaction between acidic solution treatment and incubation time significantly affected the %DME value. The Duncan post-hoc test results showed that the 0.5 M formic acid treatment for 120 minutes was significantly different from all other treatments. The 0.5 M formic acid treatments for 60 and 180 minutes were not significantly different, as were the 0.5 M hydrochloric acid treatments for 60 and 180 minutes. Additionally, the 0.5 M nitric acid and 0.5 M hydrochloric acid treatments with an incubation time of 120 minutes were not significantly different. According to Hahn et al. (2022), all three acids—formic acid, hydrochloric acid, and nitric acid—have high mineral solubility.

The hydrochloric and nitric acid treatments showed that they were not significantly different. This finding is also supported by the research of Septiani et al. (2018), which stated that nitric acid and hydrochloric acid have the same efficiency in dissolving minerals. In contrast, the treatment with formic acid was significantly different from nitric acid and hydrochloric acid because formic acid is an organic acid, whereas hydrochloric acid and nitric acid are inorganic acids. According to Sasongko et al. (2020), organic acids are generally weak acids with lower hydrolysis capabilities than strong inorganic acids, resulting in a lower ability to dissolve minerals than inorganic acids.

The treatment with 0.5 M nitric acid and an incubation time of 120 minutes was chosen as the best treatment because it had the highest %DME value. This indicates that this treatment was the most effective in reducing the mineral content in the BSF maggot pupal shells. Additionally, there were visual differences in the appearance of the maggot pupal shells for each treatment, as shown in Figure 1. The visual results indicated that the higher the %DME value, the more the color of the maggot pupal shells faded from dark brown to yellowish-brown.

Yield of BSF pupal chitosan

The chitosan yield from BSF maggot pupal shells produced in this study was 5.37 g from 50 g of raw material, resulting in a yield percentage of 10.74%. The chitosan yield from BSF maggot pupal shells obtained in this study was lower than the result from Pratiwi et al. (2022), which was 11.93% that used higher hydrochloric acid (3M) for the demineralization process. The type of acid and the acid concentration used can cause differences in the yield obtained.

Characterization of BSF pupal chitosan

In this study, chemical characterization tests were conducted on chitosan produced from BSF maggot pupal shells. The characterization results were then compared with the Indonesian National Standard (SNI) 7949-2022 on "Chitosan - Quality Requirements and Processing". A comparison of the chemical characterization analysis results of chitosan from BSF maggot pupal shells with SNI 7949-2022 can be seen in Table 2. Additionally, the chitosan produced exhibited a whitish-brown color, as shown in Figure 2.

The moisture content of the obtained chitosan was 7.81%. This means that the chitosan's moisture content meets the SNI 7949-2022 requirement (BSN, 2022), which specifies a maximum moisture content of 12%. The moisture content obtained can be influenced by the duration of chitosan drying and the surface area of the drying equipment (Zahiruddin et al., 2008). The decrease in moisture content also occurs during the demineralization process because the mineral content in the BSF maggot pupal shells decomposes due to the acidic solution used, causing $CaCO_3$ to form soluble calcium salts in water, releasing molecules of H_2O and CO_2 gas (Younes & Rinaudo, 2015).

Table 1. Demineralization efficiency percentage during demineralization

Type of acid solution (0.5 M)	Incubation time (minutes)			Average
	60	120	180	
Formic acid (HCOOH)	71.31$^{b*)}$	65.58^{a}	73.03^{b}	69.97^{p}
Hydrochloric acid (HCl)	80.88^{cd}	81.97^{d}	79.54^{cd}	80.80^{q}
Nitric acid (HNO_3)	77.63^{c}	83.55^{d}	80.20^{cd}	80.46^{q}
Average	76.61^{x}	77.03^{x}	77.59^{x}	

*) Numbers followed by different letters in the same row indicated significantly different according to DMRT with a level of 5%.

Figure 1. Visual appearance of BSF maggot demineralization treatment; A1B1=Formic acid 0.5M 60 minutes, A1B2=Formic acid 0.5M 120 minutes, A1B3=Formic acid 0.5M 180 minutes, A2B1=HCl 0.5M 60 minutes, A2B2=HCl 0.5M 120 minutes, A2B3=HCl 0.5M 180 minutes, A3B1=HNO_3 0.5M 60 minutes, A3B2= HNO_3 0,5M 120 minutes, A3B3= HNO_3 0.5M 180 minutes

Table 3. Chemical characterization of maggot pupa shell chitosan compared with SNI 7949-2002

Parameter	BSF pupae chitosan	Standard
Water content	7.81% ± 0.13	Max. 12
Ash content	0.56% ± 0.01	Max. 5
Nitrogen content	4.73% ± 0.23	Max. 5
pH	7.39% ± 0.02	7 - 8
Degree of deacetylation	75.14% ± 0.07	Min. 75

Figure 2. Chitosan from black soldier fly maggot pupae shell

The ash content of the chitosan produced in this study is 0.56%, which indicates that the ash content of the obtained chitosan meets the SNI 7949-2022 requirement (maximum ash content of 5%). The level of ash content is influenced by the demineralization process (Cahyono et al., 2014). This is because the acidic solution used in this process can dissolve the mineral content in the material, thereby reducing its ash content. The different types of acids used result in the chitosan's final ash content levels. Based on the findings of this study, mineral acids such as hydrochloric acid and nitric acid are more effective in reducing ash content than organic acids such as formic acid.

The nitrogen content determines the properties of chitosan that interact with amine groups (NH_2). The nitrogen content in chitosan obtained in this study is 4.73%, while according to SNI 7949-2022 (BSN, 2022), the maximum nitrogen content should be 5%. This is influenced by the deproteinization stage using NaOH base, which results in sodium proteinates soluble in water. The Na^+ ions can bind to the ends of protein chains with negative charges, thus dissolving in NaOH solvent (Rochima, 2007).

The degree of deacetylation is one of the critical parameters in determining the quality of chitosan. This analysis aims to determine the percentage of acetyl groups lost from the BSF maggot pupal shells. The analysis of the degree of deacetylation using volumetric methods showed a result of 75.14%. When compared to SNI 7949-2022, the chitosan from BSF maggot pupal shells meets the requirements of SNI 7949-2022. According to Erika et al. (2006), a higher degree of deacetylation of chitosan indicates that more acetyl groups have been removed, resulting in more free active amide groups (-NH2), thus increasing the purity of chitosan. The conditions of pretreatment, demineralization, deproteinization, and deacetylation processes can influence the high degree of deacetylation.

Functional group identification using FT-IR

FT-IR is a tool used to detect frequencies in combined signals, thereby determining the molecular structure of chemical compounds by identifying functional groups that make up the compound. The advantages of using FT-IR testing include being relatively fast, not requiring pure samples, and having a high level of precision (Pavia et al., 2009). FT-IR testing was conducted on the final samples of chitosan from BSF maggot pupal shells, obtained from the best demineralization treatment using 0.5 M nitric acid with an incubation time of 120 minutes at 40°C.

Functional group identification using FT-IR was conducted within the 800-4000 cm^{-1} wavelength range (Figure 3). The hydroxyl group O-H is absorbed within the wavelength range of 3200-3400 cm^{-1} (bonded H) and 3650-3600 cm^{-1} (free hydroxyl group). The amino group N-H absorbs within the wavelength range of 3500-3100 cm^{-1} (stretching vibration) and 1640-1550 cm^{-1} (bending vibration). The amino group C-N absorbs within the 1350-1000 cm^{-1} wavelength range. The carbonyl group C=O is found within the 1300-1000 cm^{-1} wavelength range. The methyl group C-H absorbs within the wavelength range of 3000-2850 cm^{-1} (Pavia et al., 2009). The FT-IR spectrophotometer readings of chitosan from BSF maggot pupal shells at wavelengths 3484 cm^{-1} and 3152 cm^{-1} indicate the presence of OH- and NH groups. Compared to the chitosan from BSF reported by Pratiwi et al. (2022), OH- and NH- groups were observed at a wavelength of 3433.08 cm^{-1}. At 2877 cm^{-1}, a C-H group was detected, whereas for chitosan from BSF in Pratiwi et al. (2022), it was at 2926.14 $cm^{-1.}$ Additionally, the absorption band of the C=O group in chitosan from BSF maggot pupal shells was observed at 1653 cm^{-1}. This result closely aligns with the literature indicating the absorption band of C=O at 1626.95 cm^{-1} (Pratiwi et al., 2022). Overall, the absorption bands of functional groups in chitosan from BSF maggot pupal shells show similarities with those from other BSF studies. Yet, slight differences in the observed wavelengths are likely influenced by the range of absorption values for each functional group.

Antibacterial activity test against E. coli

In this study, a test was conducted to evaluate the inhibition activity of chitosan against *E. coli*. The inhibition activity of chitosan against the bacteria was compared to that of formalin, with an additional comparison made using standard chitosan from Sigma. The microbial inhibition test asses the activity of chitosan against the test bacteria, as the appearance of an inhibition zone or a clear area around the test medium indicates the antimicrobial activity of chitosan (Figure 4). The results of the inhibition test against *E. coli* for the three samples can be seen in Table 4.

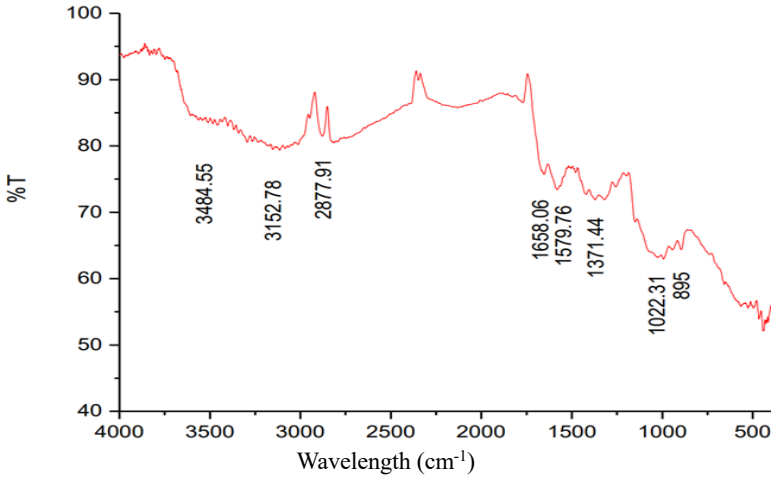

Figure 3. FT-IR spectrum of chitosan from black soldier fly maggot pupae shell

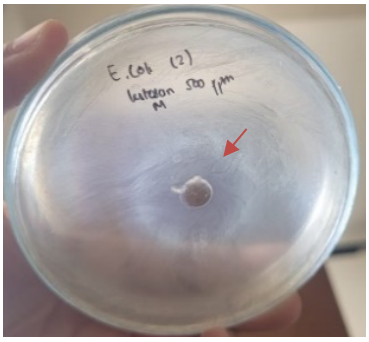

Figure 4. *Escherichia coli* inhibition test result with 500 ppm chitosan

Table 4. Inhibition activity of chitosan and formalin against *E. coli*

Test solution	Clear zone (cm)		
	500 ppm	1000 ppm	1500 ppm
Chitosan BSF maggot puppae shells	2.5$^{d*)}$	2.4^{cd}	2.9^{e}
Chitosan standard (Sigma)	2.1^{bc}	1.7^{ab}	1.5^{a}
Formalin	2.4^{cd}	3.1^{e}	3.4^{e}

*)Numbers followed by different letters in the same row indicated significantly different according to DMRT with a level of 5%.

Based on the results of the inhibition test against *E. coli*, it was found that treatments with chitosan from BSF maggot pupal shells, standard chitosan, and chitosan significantly affected *E. coli* growth inhibition. Overall, each treatment showed significant differences in inhibiting *E. coli* growth. Specifically, the treatment with chitosan from BSF maggot pupal shells at a concentration of 1500 ppm significantly differed from those at 500 ppm and 1000 ppm. However, the inhibition results of chitosan from BSF maggot pupal shells at 1000 ppm did not significantly differ from those of formalin at 500 ppm. According to Table 4, treatment with chitosan from maggot pupal shells showed better effects than standard chitosan but was not as effective as formalin treatment. The optimal concentration of chitosan from BSF maggot pupal shells for inhibiting *E. coli* bacteria growth was 1500 ppm. The antimicrobial properties of chitosan are supported by Damayanti et al. (2016) who found that chitosan at a concentration of 2% exhibited the best antibacterial ability and the longest product shelf life.

The antimicrobial properties of chitosan or its derivatives stem from the positively charged molecules of chitosan binding aggressively to the surface of microbial cells, leading to gradual shrinkage of the cell membrane and ultimately causing cell death. According to Jardine & Sayed (2018), antimicrobial activity involves polycationic chitosan molecules interacting predominantly with the anionic components of the microbial cell wall (lipopolysaccharides and proteins). This interaction results in intracellular component leakage due to changes in membrane permeability, prevents nutrients from entering the cell, binds to DNA upon entering the cell, inhibits RNA and protein synthesis, and binds through hydrophobic interactions.

Conclusion

The 0.5 M nitric acid solution with an incubation time of 120 minutes at 40°C was the optimal treatment for demineralization, yielding a demineralization efficiency (%DME) of 83.55%. In this study, the chitosan yield was 10.74%. The chemical characterization results of chitosan from BSF maggot pupal shells, compared to the Indonesian National Standard (SNI) 7949-2022 on "Chitosan and Its Processing," met all parameters. Characterization of functional groups in chitosan using FT-IR revealed absorption peaks of O-H and N-H groups at wavelengths 3484 cm^{-1} and 3152 cm^{-1}, absorption of C-H groups at 2877 $cm^{-1,}$ and absorption of C=O groups at 1653 cm^{-1}. In this study, the antimicrobial activity against *E. coli* exhibited by chitosan from BSF maggot pupal shells was superior to standard chitosan but not as effective as formalin. However, chitosan is preferable to formalin due to its safer consumption, as it is non-toxic to the human body.

Acknowledgment

Thank you to the Indonesia Oil Palm Research Institute-Bogor Unit and Djuanda University Bogor for funding this research.

References

[AOAC] Association of Official Analytical and Chemistry (1995). *Officials Methods of Analysis of AOAC Internasional*. Virginia (US): Association of Official Analytical and Chemistry Inc.

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Caballero, A. H., & Acosta, N. (2021). Chitosan: An overview of its properties and applications. *Polymers Journal,* 13(19), 1-27.

Azir, A., Harris, H., Bayu, R., & Haris, K. (2017). Produksi dan kandungan nutrisi maggot (*Chrysomya megacephala*) menggunakan komposisi media kultur berbeda. *Jurnal Ilmu-ilmu Perikanan dan Budidaya Perairan,* 12(1), 34-40.

[BSN] Badan Standarisasi Nasional (2022). *SNI 7949:2022 tentang Kitosan – syarat mutu dan pengolahan.* Badan Standarisasi Nasional, Jakarta

Budianto, A. (2011). Formalin dalam kajian undang undang kesehatan, undang-undang pangan dan undang - undang perlindungan konsumen. *Fakultas Hukum Universitas Pelita Harapan*, 1(1), 151-172

Cahyono, E. (2018). Karakteristik kitosan dari limbah cangkang udang windu (*Panaeus monodon*). *Jurnal Akuatika Indonesia*, 3(2), 96-102.

Cahyono, E., Suptijah, P., & Wientarsih, I. (2014). Development of a pressurized hydrolysis method for producing glucosamine. *Asian Journal of Agriculture and Food Sciences* 2(5), 390-396.

Damayanti, W., Rochima, E., & Hasan, Z. (2016). Aplikasi kitosan sebagai antibakteri pada filet patin selama penyimpanan suhu rendah. *JPHPI*, 19(3), 321–328.

Erika, I., Rojas, D., Waldo, M., Arguelles, M., Inocencio, H.C., Javier, H., Jaime, L.M. & Francisco, M.G. (2006). Determination of chitin and protein contents during the isolation of chitin from shrimp waste. *Macromolecular Bioscience,* 6(1),340–347.

Fauzi, R.U., & Sari, E.R. (2018). Analisis usaha budidaya maggot sebagai alternatif pakan lele. *Jurnal Teknologi dan Manajemen Agroindustri,* 7(1),39-46.

Hahn, Thomas, Elena T., Nils von S., Patrizia F., Rosanna S., Jannik T., Eva F., Malgorzata F., Eric S., Linus S., & Susanne Z. (2022). Purification of chitin from pupal exuviae of the black soldier fly. *Waste and Biomass Valorization* 13, 1993-2008.

Halimathussadiah, Rahmawati, D., & Indriyanti, N. (2021). Uji aktivitas minyak atsiri daun pala (*Myristica fragrans* houtt.) sebagai antibakteri. *Proceeding of Mulawarman Pharmaceuticals Conference,* 13(1), 85–91.

Jardine, A. & Sayed, S. (2018). Valorisation of chitinous biomass for antimicrobial applications. *Pure Appl. Chem Journal* 90(1), 293–304.

Kauppinen, Jyrki & Jari P. (2001). *Fourier Transform in Spectroscopy*. New York : Wiley-VCH.

Pavia, D.L., Lampman, G.M., Kriz, G.S., & Vyvyan, J.R (2009). *Introduction to Spectroscopy. Fourth Edition*. Belmont, USA.

Pratiwi, Sivia N., Nastiti, U. & Prashinta, N.D. (2022). Karakterisasi kitosan dan pembuatan nanopartikel kitosan dari cangkang pupa black soldier fly (*Hermetia illucens*). *Medical Sains: Jurnal Ilmiah Kefarmasian*, 7(4), 963-972.

Rochima, E. (2007). Karakterisasi kitin dan kitosan asal limbah rajungan Cirebon Jawa Barat. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 10(1), 9-22.

Sasongko, Ashadi & Legahati, N. (2020). Pengaruh konsentrasi asam sitrat dan daya ultrasonik pada produksi oligosakarida dari biji *Salacca zalacca* dengan metode Ultrasonic Assisted Acid Hydrolysis (UAAH). *SNITT Poltekba,* 4(1), 395-400.

Septiani, Mimin, Santoso, K. &Majid, R. A. (2018). Efektivitas asam nitrat (HNO_3) sebagai pelarut alternatif pada proses acid wash terhadap plate electrolyzer di PT Kaltim Nitrate Indonesia. *Journal of Chemical Process Engineering*, 3(2), 17-21.

Tomberlin, J.K., Sheppard, D.C. & Joyce, J.A. (2002). Selected life-history traits of black soldier flies (Diptera : Stratiomyidae) reared on three artificial diets. *Ann. Entomol. Soc. Am.* 95(3), 379–386. https://doi.org/10.1603/0013-8746(2002)095[0379:slhtob] 2.0.co;2

Triunfo, M., Tafi, E., Guarnieri, A., Salvia, R., Scieuzo, C., Hahn, T., Zibek, S., Gagliardini, A., Panariello, L., Coltelli, M. B., De Bonis, A., & Falabella, P. (2022). Characterization of chitin and chitosan derived from *Hermetia illucens*, a further step in a circular economy process. *Scientific Reports* 12(1), 1-17.

Wahyuni, S., Fauziah, R., Aziz, M., Eris, D., Prakoso H., Priyono & Siswanto (2021). Sintesis komposit kitosan berbasis selongsong black soldier fly (BSF) dengan ekstrak daun kipahit dan uji penghambatannya terhadap *Xanthomonas oryzae*. J*urnal Rekayasa Bahan Alam dan Energi Berkelanjutan*, 5(2), 16-23.

Yanuar, V. (2013). Karakteristik tepung cangkang rajungan berdasarkan metode penepungan yang berbeda. *Juristek*, 1(2), 1–10.

Yulisa, N., Asni, E., & Azrin, M. (2014). Uji formalin pada ikan asin gurami di pasar tradisional pekanbaru. *Jurnal Jom FK* 1(2), 1-12.

Younes, I & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources, structure, properties, and applications. *Mar Drugs Journal* 13(3), 33-74.

Zahiruddin, W., Ariesta, A., & Salamah, E. (2008). Karakteristik mutu dan kelarutan kitosan dari ampas silase kepala udang windu (*Penaeus monodon*). B*uletin Teknologi Hasil Perikanan* 11(2), 25-29.