Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 10, No. 4, October 2025

Research Paper

The Utilization of Chitosan from Maggot (*Hermetia illucens*) Exuvia as Edible Coating for Tomatoes (*Lycopersicon esculentum*) and Edible Film with the Addition of Honey as an Antibacterial Agent

Daniel[1*], Saibun Sitorus[1], Subur P. Pasaribu[1], Eva Marliana[1], Rita Hairani[1], Ritbey Ruga[1], Ritson Purba[1], Chairul Saleh[1], Djihan Ryn Pratiwi[1], Agustina Rahayu Magdaleni[2], Indra Masmur[3], and Hestina[4]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, 75123, Indonesia*
[2]*Department of Medicinal Education, Faculty of Medicine, Mulawarman University, Samarinda 75123, Indonesia*
[3]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[4]*Department of Chemistry, Universitas Sari Mutiara Indonesia, Medan, 20123, Indonesia*
***Corresponding author**: daniel_trg08@yahoo.com

Abstract
The use of chitosan from maggot (*Hermetia illucens*) exuvia as an edible coating for tomatoes (*Lycopersicon esculentum*) and an edible film with honey added as an antibacterial agent was investigated. This study aimed to determine the yield of chitosan from black soldier fly (BSF, *Hermetia illucens*) maggot exuviae as an edible coating. The chitosan was produced from chitin by deacetylation, and its effect on the weight loss and vitamin C levels of tomatoes for 7 days after treatment was investigated. The chitosan coating was characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy. The antibacterial activity was evaluated using a disk diffusion method. The result showed that by deacetylation, conversion of chitin produced chitosan with a yield of 72% and a deacetylation degree of 75.05%. The chitosan coating significantly affected the weight loss of tomatoes, with the best concentration being 2%. However, the treatment did not significantly affect the decrease in vitamin C levels. The edible chitosan film from BSF maggot exuviae with added honey had a thickness, water content, and water vapor transmission rate of 0.156 mm, 16.9913%, and 30.45 g/m^2/24 hours, respectively. SEM characterization showed a relatively dense surface structure, which was slightly smooth and porous. Regarding antibacterial activity, the edible chitosan film inhibited *Staphylococcus aureus* with an inhibition zone of 10.37 mm; however, *Escherichia coli* was not inhibited. These results suggest that chitosan from BSF maggot exuviae has potential as an effective edible coating for reducing weight loss in tomatoes despite showing limited antibacterial properties.

Keywords
Chitin, Chitosan, *Hermetia illucens*, Edible Coating, Edible Film

Received: 17 February 2025, Accepted: 2 September 2025
https://doi.org/10.26554/sti.2025.10.4.1232-1241

1. INTRODUCTION

Hermetia illucens, belonging to the Stratiomyidae family, is commonly known as the black soldier fly (BSF). It is currently attracting attention from the scientific and industrial community in Indonesia due to BSF cultivation being relatively safe for humans. It can also reduce the population of houseflies and waste pollution. Moreover, BSF larvae produce biomass rich in protein and carbohydrate, such as chitin, an abundant biopolymer extracted from maggot exuviae in the exoskeleton form (Siddiqui et al., 2024).

Chitin [$(C_8H_{13}NO_5)$n] is a carbohydrate polymer of n-acetyl-D-glucosamine and D-glucosamine units. It is found in many living organisms, including insects, mollusks, and mushrooms. The BSF larvae contain chitin with an α-configuration. The α-chitin molecule exhibits an antiparallel configuration of carbohydrate chains and is the most thermodynamically stable form of chitin (Kaczor et al., 2023).

The deacetylation of chitin using an alkali solution at high temperatures produces chitosan [$(C_6H_{11}NO_4)$n], which is more water-soluble than chitin. Additionally, chitosan is a non-toxic compound and easily degraded. It can be applied in many fields, such as agriculture, environment, biomedical, foods, chemicals, feeds, cosmetics, biodiesel, and the paper industry, and in producing films (Bhavsar et al., 2021).

Chitosan is useful in the food industry as an edible coating that prevents fruits and vegetables from dehydrating and is semipermeable to some gasses due to its potential to replace plastic as a food product coating. Chitosan has also been used

as a finishing agent due to its broad-spectrum antibacterial activity against various pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* (Hahn et al., 2019; Huang et al., 2018). Another study reported that an approximately 2% (w/v) chitosan coating on green grapes could maintain physical characteristics such as color, aroma, and texture for 7 days, while a 1% (w/v) chitosan coating maintained vitamin C levels for up to 4 days of storage (Aranaz et al., 2021).

Based on this information, it is important to explore the potential of BSF maggot exuviae as a sustainable solution to environmental challenges such as waste pollution and pest control. By studying the extraction and application of chitosan from BSF maggot exuviae, this research can contribute to reducing reliance on harmful chemicals and plastic packaging, providing a natural alternative to synthetic preservatives. Additionally, the antibacterial properties of chitosan could enhance food safety and extend shelf life, addressing global concerns about food waste and environmental sustainability. Therefore, this research was conducted to extract the chitin content from BSF maggot exuviae, which was then converted into chitosan. The chitosan obtained was then applied as an edible coating on tomatoes as a means to use maggot exuviae in the food sector. The product was also applied as an edible chitosan film from BSF maggots based on thickness, water content, water vapor transmission rate (WVTR), Fourier Transform-Infra Red (FT-IR) spectroscopy, scanning electron microscopy (SEM) characterization, and antibacterial properties with and without honey.

2. EXPERIMENTAL SECTION

2.1 Materials

Materials used in this research were BSF maggot exuvia, tomatoes, HCl 3 M, NaOH 3.5%, CH_3COOH 1%, $KMnO_4$, $H_2C_2O_4$, *Staphylococcus aureus*, *Escherichia coli*, nutrient agar, Luria-Bertani broth, ampicillin, EtOH, glycerol, and distilled water. Additionally, the instruments used in this research included an analytical balance, glassware, an oven, freezer, incubator, desiccator, grinder, pH Universal, silica gel, a magnetic stirrer, pipette, spatula, hot plate, reflux kit, filter paper, ose needle, laminar flow, autoclave, petri dish, micropipette, scanning electron microscope (JEOL JSM 6510 LA), and Fourier transform infrared spectrometer (FT-IR, IRPrestige-21 SHIMADZU).

2.2 Methods

2.2.1 Sample Preparation

BSF maggot exuviae samples were washed until clean, dried using an oven, and then ground with a grinder. As much as 150 g of maggot exuviae powder was soaked in a 3 M HCl solution using a ratio of 1:10 (w/v) at room temperature with occasional stirring for up to 36 hours. The products were filtered and rinsed with distilled water until neutral. The residue obtained was mineral-free chitin, which was then dried using an oven at 60°C and weighed. The demineralized residue was placed in a 3.5% (w/v) NaOH solution using a ratio of 1:10 (w/v), then heated at 65°C and stirred with a magnetic stirrer for 2 hours. The products were filtered and rinsed with distilled water until neutral. The residue obtained was chitin free of minerals and proteins, which was then dried using an oven at 60°C and weighed. The deproteinized residue was soaked in a 1% (w/v) $KMnO_4$ solution at room temperature for 2 hours, then soaked in a 1% (w/v) $H_2C_2O_4$ solution at room temperature for 2 hours (Aparna and Geetha, 2024; Utama et al., 2022).

2.2.2 Application of Chitosan as Edible Coating

Chitosan powder, 1, 1.5, and 2 g, was added to 100 mL of 1% (v/v) CH_3COOH solution and homogenized at 30°C for 15 minutes using a magnetic stirrer. However, variations of this solution were obtained at 1%, 1.5%, and 2% (w/v). Tomatoes were dipped in each chitosan solution for 10 minutes, removed, and dried at room temperature, followed by storage under room conditions. Changes were then observed on the 3rd, 5th, and 7th days of storage (Utama et al., 2022). Observations of tomato characteristics included color and texture, where samples coated with 1%, 1.5%, and 2% (w/v) chitosan edible coating and those without coating were observed for changes on the 3rd, 5th, and 7th days of storage, using an analytical balance. Weight loss was determined by comparing the initial weight (after coating) and the final weight (3rd, 5th, and 7th days of observation). The percentage of weight loss was determined using Equation (1) (Aparna and Geetha, 2024; El-Araby et al., 2023; Thambiliyagodage et al., 2023)

$$\%\,\text{Weight Loss} = \frac{\text{Initial Weight (g)} - \text{Final Weight (g)}}{\text{Initial Weight (g)}} \times 100\% \quad (1)$$

2.2.2.1 Determination of Vitamin C Levels in Tomatoes Before Coating

As much as 50 g of pureed tomatoes was placed in a 100 mL measuring flask coated with aluminum foil, then distilled water was added to the boundary mark and the mixture was homogenized. Furthermore, the sample solution was filtered, and 2 mL of the filtrate was taken and placed in a 50 mL measuring flask coated with aluminum foil. Distilled water was then added to the boundary mark, and the mixture was homogenized. However, a dilute extract was obtained. This was measured for its absorbance at the maximum wavelength, and the vitamin C concentration was determined by substituting the absorbance into the linear regression equation (Noviyanto et al., 2025). The data was then analyzed using Equation (2).

$$\%\,\text{Vitamin C} = \frac{C \times V \times FP}{W \times 1000} \times 100\% \quad (2)$$

Here, C = concentration of sample solution after dilution (mg/L), V = sample volume (L), FP = dilution factor, and W = sample weight (g).

2.2.2.2 Determination of Vitamin C Levels in Tomatoes After Coating

On the 3rd, 5th, and 7th days of storage, each tomato coated with 1%, 1.5%, and 2% (w/v) chitosan and without chitosan coating had its vitamin C level measured using the same procedure as described in point 2.2.2.1.

2.2.3 Application of Chitosan as Edible Film

2.2.3.1 Preparation of Edible Chitosan Film

BSF chitosan weighing 2.5 g was dissolved with 100 mL of 1% CH_3COOH in a beaker until a homogeneous mixture was formed and stirred using a magnetic stirrer for up to 45 minutes with heating at 80°C. Furthermore, 1 mL of glycerol was added, then the mixture was stirred again until it was homogeneous, poured into a petri dish, and dried at room temperature for 3-4 days (Kusumawati et al., 2025; Noviyanto et al., 2025).

2.2.3.2 Preparation of Edible Chitosan Film with Added Honey

BSF chitosan, 2.5 g, was dissolved in 100 mL of 1% CH_3 COOH in a beaker until a homogeneous mixture was formed; this was stirred with a magnetic stirrer for 45 minutes with heating at 80°C, removed, and cooled. Moreover, 1 mL of glycerol and 1 g of honey were added, and the mixture was stirred again for 30 minutes. After being poured into a petri dish and dried at room temperature for 3-4 days, it was tested for water content and thickness and subjected to a water vapor transmission test and SEM characterization (Kusumawati et al., 2025; Noviyanto et al., 2025).

2.3 Antibacterial Activity Test

2.2.4.1 Sterilization of Equipment

The petri dish was cleaned with water, then wrapped in paper, placed in an autoclave, and sterilized for 1 hour at 121°C. Other tools, such as ose, stirring rods, and tweezers, were heated on a Bunsen burner to sterilize them.

2.2.4.2 Preparation of Media

The media used were solid nutrient agar (NA) and Luria-Bertani liquid media. The solid NA media was produced by dissolving 5 g of NA in 200 mL of distilled water, mixing with a stirrer, and then sterilizing in an autoclave for 15–20 minutes at 121°C. After sterilization, the NA media was poured into a sterile petri dish in a laminar airflow and left until it solidified. For the Luria-Bertani liquid media, 1 g of NaCl, 0.5 g of yeast, and 1 g of tryptone were dissolved in 100 mL of distilled water, stirred, and sterilized by autoclaving for 1 hour at 121°C (Al-Musawi et al., 2020).

2.2.4.3 Preparation of Bacteria Tested

Each test bacterium, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, was cultured by bacterial inoculation. Pathogenic bacteria, 10 µL, turned into glycerol stock, were placed in 5 mL of sterile Luria-Bertani liquid media and then left at room temperature for 24 hours. The cultured bacteria were then inoculated into sterile NA solid media using the swab technique. Cotton swabs were inserted into the test bacterial suspension and then aseptically scratched directly/zigzag in NA solid media until smooth (Popescu et al., 2022).

2.2.4.4 Determination of Antibacterial Activity

Edible film samples, chitosan, and chitosan with added honey, were cut with a diameter of 7 mm and then sterilized initially under ultraviolet radiation for 30 minutes. Sterile samples were placed aseptically on the agar surface using a spatula and tweezers. All petri dishes were left for 24 hours at room temperature, and the positive control test used ampicillin antibiotics, while the negative control test used sterile distilled water. The appearance of an inhibition zone around the sample disk showed the presence of antibacterial agents. Subsequently, the diameter of the clear zone, as an inhibition zone appearing in each isolate, was measured directly using a ruler (Muñoz-Tebar et al., 2023).

3. RESULTS AND DISCUSSION

3.1 Chitin Extraction and Chitosan Synthesis

The basic material used in this study was BSF maggot exuviae washed, dried, and ground into powder. The chitin extraction used a chemical method that included demineralization, deproteination, and depigmentation. Furthermore, chitosan was synthesized from chitin through the deacetylation (Alemu et al., 2023).

The demineralization using 3 M HCl solution aimed to release inorganic minerals in the BSF maggot exuviae, which were generally calcium carbonate and calcium phosphate. Bubbles of CO_2 gas indicated a demineralization reaction ((Alemu et al., 2023), which is shown in Figure 1.

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

$$Ca_3(PO_4)_2\,(s) + 6HCl\,(aq) \longrightarrow 3CaCl_2\,(aq) + 2H_3PO_4\,(aq)$$

Figure 1. Reaction of Demineralization

The next step was deproteination using a 3.5% NaOH solution. This step aimed to remove the proteins attached to the chitin. Here, Na^+ ions from the NaOH reacted with the negative ends of the protein chain, forming sodium proteinate. The deproteination occurred when the solution turned reddish and became slightly thicker. This change was due to the protein detaching from the chitin to form sodium proteinate (Mora-López et al., 2023), the deproteination reaction is presented in Figure 2.

The depigmentation was performed to remove the brown color (pigment) from the sample. This was achieved by soaking it in a 1% $KMnO_4$ solution and then in a 1% $H_2C_2O_4$ solution. The $KMnO_4$ solution was intended to act as an oxidizing agent to remove the pigments stuck to the chitin, while the $H_2C_2O_4$ solution was intended to remove the remaining $KMnO_4$. Af-

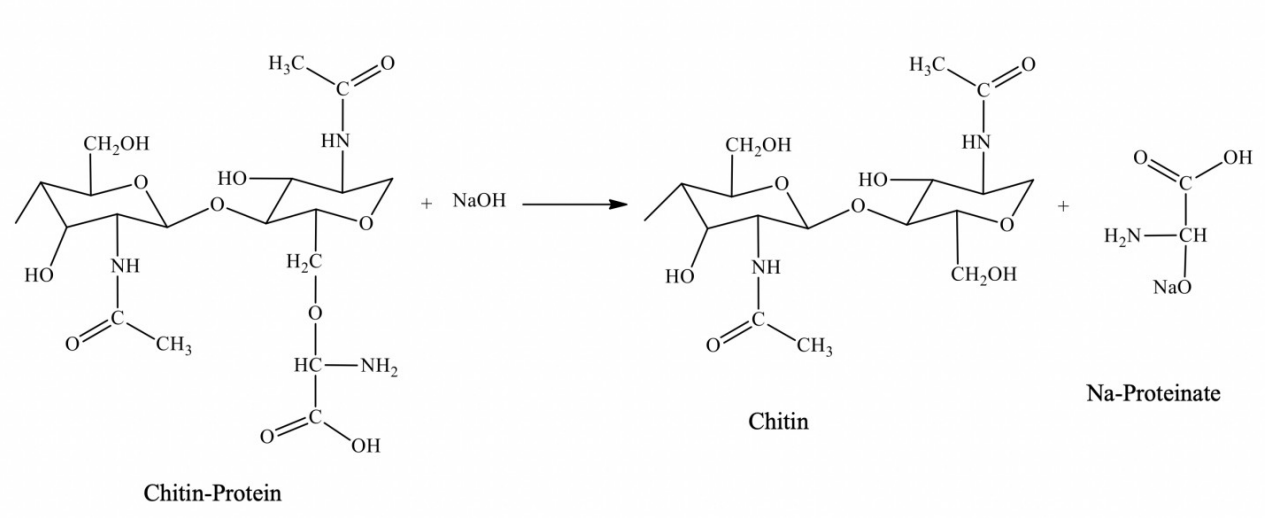

Figure 2. Reaction of Deproteination

Table 1. FT-IR Absorption Peaks of Chitin and Chitosan-BSF

Functional Group	Wavenumber (cm^{-1})			
	Chitin		Chitosan-BSF	
	Standard*	Study	Standard*	Study
OH stretching	3440.35	3448.72	3442.60	3448.72
OH (vs) NH_2 overlap	–	–	3442.60	3448.72
NH ($–NHCOCH_3$) stretching amide I	3268.63	3271.27	3442.60	3448.72
$CH(CH_3)$ stretching	2961.32	2924.09	2922.80	2924.09
C=O ($–NHCOCH_3$) stretching amide I	1661.50	1627.92	1660.55	1635.64
NH ($–NHCOCH_3$) bending amide II	1558.90	1558.48	1587.94	1597.06
CN ($–NHCOCH_3$) stretching	1259.54	1257.59	1259.54	1257.59
NH ($R–NH_2$) bending	–	–	1587.94	1597.06
CH ($–CH_2$) bending sym	1320.34	1381.03	1377.11	1381.03
C–O (–C–O–C–O–) stretching asym	1155.12	1072.42	1154.64	1149.57
C–O (–C–O–C–O–) stretching sym	1026.33	1026.13	1026.23	1033.85
β-1,4-glycosidic	752.33	894.97	897.41	894.97

ter depigmentation, the sample changed color from brown to yellowish white, as shown in Figure 3.

The chitin was then converted into chitosan using a 50% NaOH solution. This process was intended to remove the acetyl group ($-COCH_3$) attached to the nitrogen atom of chitin (Elsabagh et al., 2023). The 50% NaOH solution was used because it was the most appropriate concentration to change the acetyl group attached to the nitrogen into an amine group ($-NH_2$). Whether the transformation occurred could be determined by comparing the chitin spectrum with the spectrum after deacetylation at some wavenumbers (Elsabagh et al., 2023). The chitosan produced from the deacetylation of chitin from BSF maggot exuviae was a yellowish-white powder, and the yield was 12% (Reshad et al., 2021; Santi et al., 2020).

Characterization using FT-IR spectroscopy aimed to identify functional groups in the sample. Table 1 and Figure 4 show the FT-IR analysis results for chitin and chitosan based on BSF maggot exuviae.

3.2 Effect of Chitosan as Edible Coating

3.2.1 Physical Characteristics

This study used tomatoes with an average ripeness of 70 days after planting (DAP). The physical characteristics, including color and texture, influenced consumers because these could be observed visually. Table 2 shows changes in the tomato fruit color from before treatment to 7 days after treatment (DAT). Meanwhile, Table 3 shows changes in the tomato fruit texture from before treatment to 7 DAT.

The treatment with edible chitosan coating at concentrations of 1.5% and 2% (w/v) maintained the tomato fruit color better than the treatment with 1% chitosan coating (w/v) and without coating. The edible coating could maintain the amount of CO2 abundant in the fruit as well as inhibit chlorophyll degradation and the formation of beta-carotene. However, the

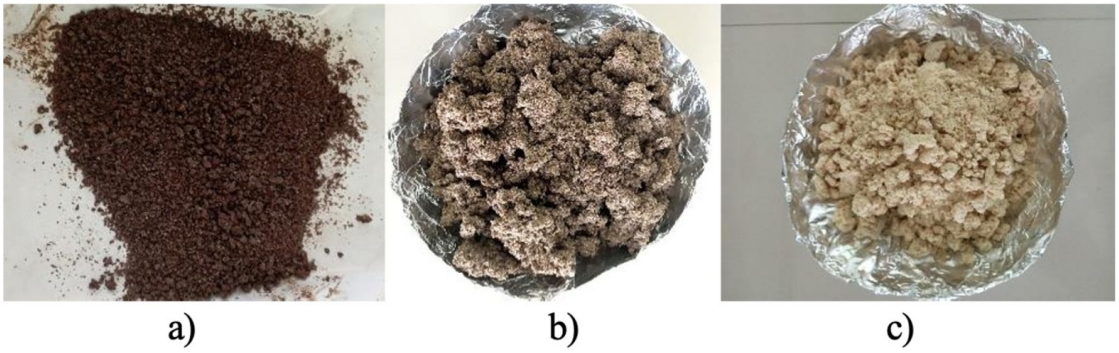

Figure 3. Chitin Extraction Results. a) Demineralization, b) Deproteination, c) Depigmentation

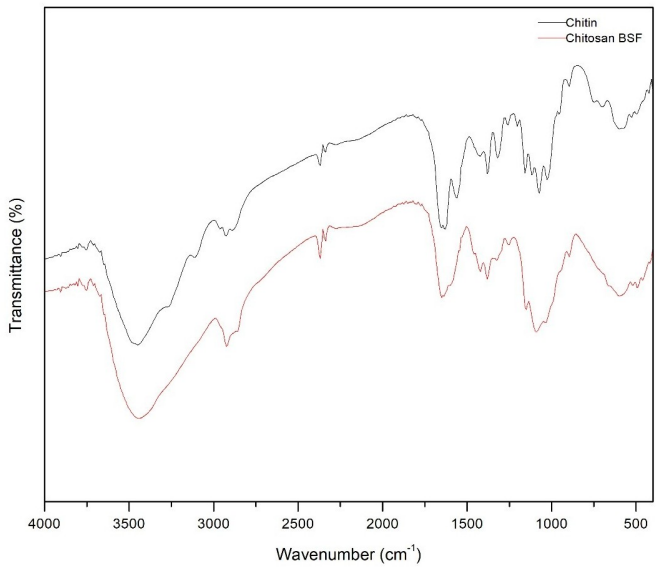

Figure 4. FT-IRSpectrum of Chitin and Chitosan-BSF

edible coating was useful for maintaining the fruit color during ripening. When the fruit respiration rate decreased, the physiological changes in the fruit slowed and the fruit could be protected from attack by rotting microorganisms. Chitosan was known to maintain the fruit quality against rotting by inhibiting bacterial growth through amine groups (NH_2) bound to negatively charged bacterial cell walls (Sree et al., 2020).

The fruit softened when damage occurred to the cell structure, intracellular composition, and cell walls, as well as through degradation of protopectin into water-soluble pectin, which reduced the cohesion between cell walls. The edible coating could prevent oxygen from entering the tissue, reducing the activity of enzymes active in respiration. The coating could also maintain the fruit texture by reducing the WVTR and protopectin degradation. This coating on fruit could suppress the fruit respiration rate because it reduced the amount of oxygen entering the tissue, decreasing the fruit softening rate during storage (Sree et al., 2020; Yadav et al., 2022).

Table 2. Changes in Tomato Fruit Color During Storage

Functional Group	Color changes			
	0 DAT	3 DAT	5 DAT	7 DAT
Without coating	Light Red	Red	Overripe	Overripe
Chitosan 1%	Light Red	Red	Red	Overripe
Chitosan 1.5%	Light Red	Red	Red	Red
Chitosan 2%	Light Red	Red	Red	Red

DAT = Days After Treatment

Table 3. Changes in Tomato Fruit Texture During Storage

Treatment	Texture changes			
	0 DAT	3 DAT	5 DAT	7 DAT
Without coating	Hard	Hard	Soft	Soft
Chitosan 1%	Hard	Hard	Moderate	Moderate
Chitosan 1.5%	Hard	Hard	Hard	Hard
Chitosan 2%	Hard	Hard	Hard	Hard

DAT = Days After Treatment

3.2.2 Weight Loss

Post-harvest, the fruit decreased in weight during storage; this weight decrease was caused by respiration that transformed sugar into H_2O and CO_2. Figure 5 shows the results of the tomato fruit weight loss test with 1%, 1.5%, and 2% (w/v) chitosan coating treatments and treatments without coating at 3 to 7 DAT. Based on the ANOVA results, a significant difference generally existed in the effect of the concentration of the chitosan edible coating on tomato fruit weight loss over 7 DAT.

3.2.3 Vitamin C Levels

Ascorbic acid, the chemical name for vitamin C, is easily soluble in water and easily damaged. The decrease in the compound

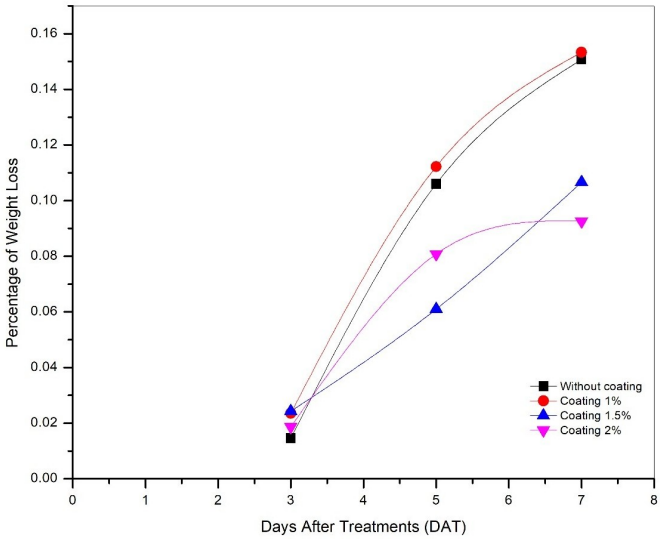

Figure 5. The Effect of Chitosan Concentration on Tomato Fruit Weight Loss During 7 DAT

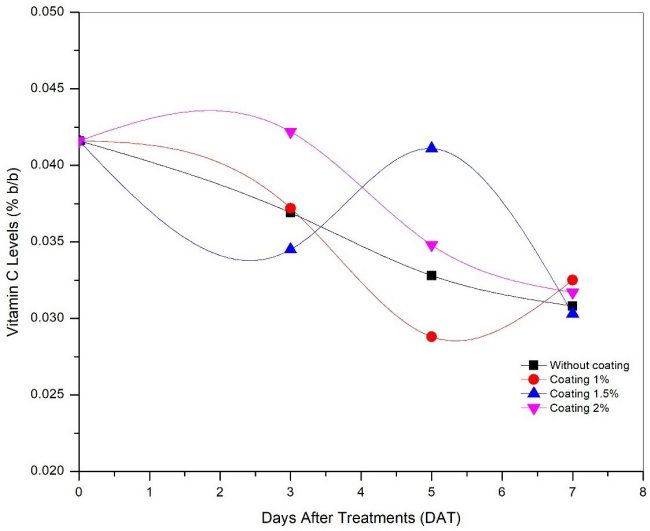

Figure 6. The Effect of Chitosan Concentration on Tomato Fruit Vitamin C Levels During 7 DAT

Table 4. Thickness Test Results of Edible Film

Edible film	Thickness (mm)	Average (mm)
Chitosan	0.15	0.154
	0.15	
	0.16	
	0.15	
	0.16	
	0.15	
Chitosan-honey	0.16	0.156
	0.16	
	0.15	
	0.15	
	0.17	

levels in food was usually caused by long-term storage at high temperatures. During storage, the oxidation of vitamin C was influenced by light, temperature, and air. Figure 6 shows the vitamin C levels (%b/b) determined in tomatoes from the beginning of storage to 7 DAT.

The decrease in vitamin C levels in fruit was caused by the oxidation of ascorbic acid into dehydroascorbic acid, which then underwent further transformation into diketogluconic acid. The fruit cells contained an ascorbic acid oxidase enzyme, which contributed to increasing the oxidation reaction rate, accelerating the decrease in vitamin C levels (Sree et al., 2020). However, according to a previous study (Abral et al., 2021), sour fruit could stabilize vitamin C. Nevertheless, even when the storage period was extended, it could not significantly affect the decrease in vitamin C levels in the fruit. This study used tomatoes aged 70 DAP, which were light red. At this age, the tomatoes were thought to be ripe, and the vitamin C levels became stable (Suriati et al., 2023).

3.3 Application of Chitosan as Edible Film

Edible film from chitosan dissolved in a CH_3COOH cloud had improved mechanical properties. This condition could be related to lactic and citric acid remaining in the layer, changing its properties, which did not occur with CH_3COOH. The film obtained through chitosan dissolved in CH_3COOH was resistant, while a very fragile but elastic edible film formed when lactic acid was used. Therefore, depending on this function, it was important to select the appropriate acid and amount to obtain properties matching the function of the edible film (Abdul-Rahman and Abass, 2021). Glycerol was widely used in drug formulations such as oral, ophthalmic, topical, and parental preparations (Fatullayeva et al., 2022). Besides its use as a humectant and ergoline, glycerol was used as a plasticizer in the manufacture of the edible film; as a plastic former, glycerol could also improve the quality of the edible film (Kedir et al., 2022).

Honey is a traditional medicine containing substances active against bacteria. Additionally, its strong antibacterial properties originated from the osmotic effect, acidity, hydrogen peroxide, and phytochemical factors. This activity worked well in preventing the growth of *Escherichia coli*, *Shigella* sp., *Helicobacter pylori*, and *Salmonella* sp. (Lam et al., 2023).

The edible film was manufactured using the casting plate method, where a homogeneous edible film mass was poured into a petri dish, allowed to dry for 3-4 days, and then released. The edible film obtained was a transparent, thin sheet, smelling slightly of honey, and yellowish.

3.3.1 Edible Film Test

Thickness could be interpreted as a physical property affecting the tensile strength, WVTR, and elongation percentage. The increased thickness of the edible film made the resulting film

Table 5. Water Vapor Transmission Test Results of Edible Film

Edible film	Initial weight (grams)	Final weight (grams)	Surface area (m^2)	Delta weight (grams)	Test duration (hours)	WVTR (g/m^2/24 hours)
Chitosan	11.9444	12.1891	0.00035	0.2447	24	29.48
Chitosan-honey	11.9431	12.1960	0.00035	0.2529	24	30.45

structure stiffer and harder; however, this condition was beneficial because it could protect the packaged product. Based on Table 4, adding honey could increase the thickness of the edible film. Adding honey with an increase in concentration of 50%–200% significantly increased the film thickness from 0.060 to 0.109 mm. This indicated that the solute proportion became more concentrated because adding certain compounds also increased the total dissolved solids, increasing the thickness. According to the Japanese Industrial Standard (JIS), the international standard value for the edible film thickness was >0.25 mm. In this study, the thicknesses of both edible films fulfilled the JIS (Budiati et al., 2024; Nasution and Idris, 2023; Rachmawaty et al., 2023).

3.3.2 Water Vapor Transmission Test

The WVTR was related to the ability of the edible film to withstand the migration of water vapor from the packaged product. The storage duration of the packaged product was relatively long when the WVTR was low (Nasution and Idris, 2023).

Based on Table 5, the WVTR of the edible chitosan film was 29.48 g/m^2/24 hours, while the edible chitosan film with honey administered had a WVTR of 30.45 g/m^2/24 hours. Based on the data obtained, administering honey could provide a higher WVTR in the edible film from chitosan. The presence of honey modified the intermolecular interactions between polymer chains by expanding the molecular network to allow water molecules to interact and diffuse easily, resulting in a higher WVTR. According to the JIS, the standard WVTR of the edible film was required to be >7 g/m^2/24 hours (Sulaeman et al., 2024). However, the WVTR in this study did not fulfill this standard. This condition occurred due to the use of glycerol as a hydrophilic plasticizer; when this was bound to the edible film matrix, the tension between its molecules could decrease. The distance between the molecules increased; however, the edible film was more easily penetrated by water vapor. The higher glycerol concentration used could increase the permeability of the edible film to water vapor (Tampubolon et al., 2023; Xia et al., 2022).

3.3.3 SEM Characterization

SEM was used to analyze the surface morphology of the edible film obtained. The working principle of SEM is to emit radiation toward the set holder (Rachmawaty et al., 2023). The samples analyzed were edible chitosan film and chitosan with honey, with magnification of 1000× to 10000×, as shown in Figure 7.

Based on Figure 7, the surface was slightly detached from the molecular structure of the edible chitosan film, where cracks were thought to occur due to the presence of chitosan fibers with relatively large particles, namely 20 to 30 mesh, which resulted in imperfect dissolution. Furthermore, the looser cracks or structure of the fibers resulted in more water being absorbed. The figure also showed a porous and rougher surface, indicating that the edible film was less homogeneous during stirring (Yadav et al., 2022). Meanwhile, the morphology of the edible chitosan film with the added honey showed a relatively dense molecular structure as well as a relatively smooth and slightly porous surface. This was due to the ability of honey to bind free water in the edible film suspension; however, evaporation did not occur during drying. The presence of free water that evaporated caused pore formation on the surface of the edible film. Additionally, the homogeneous stirring could cause the formation of a satisfactory surface on the edible chitosan film with added honey (Chawla et al., 2021).

3.3.4 Antibacterial Activity Test

The antibacterial activity test was evaluated using the disk diffusion method, where the diameter of the clear zone around the edible film sample disk with added honey could be calculated. The bacteria provided were Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*). As a comparison, ampicillin was provided as a positive control and sterile distilled water as a negative control. The use of ampicillin as a positive control could inhibit the growth of and kill Gram-positive and negative bacteria (Shakoori et al., 2024).

Based on Table 6, the edible chitosan film sample with added honey showed a clear zone only in the *Staphylococcus aureus* microbial media with an average diameter of 10.34 mm, while it did not show a clear zone in the *Escherichia coli* microbial media. Additionally, the edible chitosan film sample without honey showed no clear zone in the *Staphylococcus aureus* and *Escherichia coli* microbial media. However, the edible chitosan film with honey could inhibit the activity of Gram-positive bacteria categorized as relatively weak. The difference in inhibition power was due to differences in the sensitivity of the two bacteria to antimicrobial substances because they had different cell compositions and structures. *Escherichia coli* was a Gram-negative bacterium that was less susceptible to several antibiotics. This condition occurred because the bacterial cell wall structure tended to be more complex and comprised three layers, with an inner layer of peptidoglycan, then a middle layer of lipopolysaccharide, and an outer layer of lipoprotein. *Staphy-*

Table 6. Antibacterial Activity Test Results of Edible Film

Sample	Inhibition zone diameter (mm)							
	Staphylococcus aureus				*Escherichia coli*			
	I	II	III	Average	I	II	III	Average
Edible chitosan film	-	-	-	-	-	-	-	-
Edible chitosan-honey film	10.8	10.8	9.5	10.37	-	-	-	-
Control (+)	13.5	16.3	16.5	15.37	18	15.5	13.8	15.77
Control (-)	-	-	-	-	-	-	-	-

Diameter of disc is 6 mm

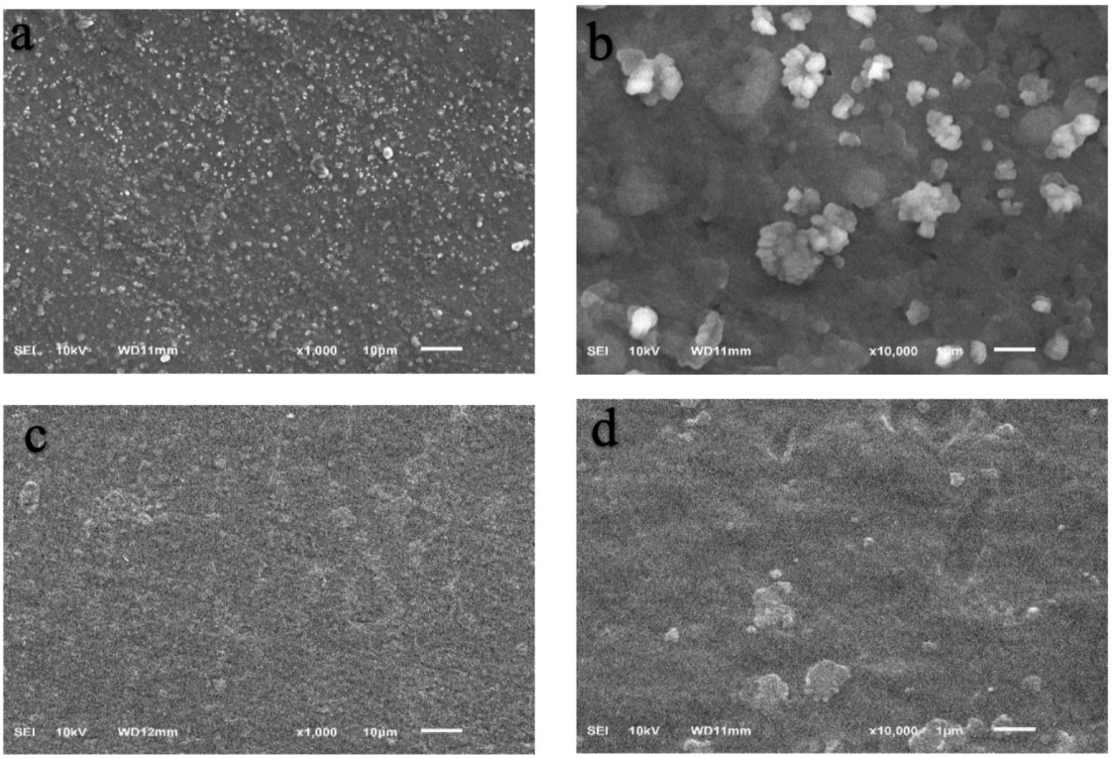

Figure 7. Morphology of Edible Film a) Chitosan (1000×), b) Chitosan (10000×) c) Chitosan-honey (1000×), and d) Chitosan-Honey (10000×)

lococcus aureus was classified as a Gram-positive bacterium with a thicker peptidoglycan in its cell wall, which could create a more rigid structure (Wang et al., 2022). Furthermore, this had a cell wall with a relatively simple structure, which facilitated the entry of antibacterial compounds such as honey into cells.

The efficacy of honey was based on its effect as an antibacterial agent. Several influencing factors were high sugar levels inhibiting bacterial growth, high acidity reducing bacterial life and growth, and the presence of organic compounds (flavonoids, polyphenols, glycosides, and inhibins) with antibacterial properties. Moreover, this active ingredient could damage the integrity of bacterial cell walls, killing or inhibiting bacteria (Miksusanti et al., 2022; Sanchez-Ortega et al., 2014; Siddiqui et al., 2024)

4. CONCLUSIONS

In conclusion, the chitosan coating derived from BSF maggot exuviae significantly impacted the reduction of weight loss in tomatoes, with an optimal concentration of around 2%. However, it did not significantly influence the decline in vitamin C levels during the 7-day assessment. The physical properties of the chitosan film, which included added honey, revealed a relatively dense, slightly smooth, and slightly porous structure with promising moisture retention characteristics. The film also exhibited a moderate inhibition zone against *Staphylococcus aureus*, although it did not inhibit *Escherichia coli*. However, the chitosan film without honey had a rougher, more porous surface with cracks and did not show antibacterial activity against either of the bacterial strains tested. These findings suggest that chitosan coatings from BSF maggot exuviae could be effective

in reducing weight loss in tomatoes; however, their antimicrobial properties are limited, and further enhancements might be required for broader food preservation applications.

5. ACKNOWLEDGMENT

We are thankful for financial support from PNBP Faculty of Mathematics and Natural Sciences, Mulawarman University (Grant no. 1559/UN17.7/PT/2024).

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