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# Fabrication and Characterization of Chitosan Film Incorporated with ZnO and Patchouli Oil for Food Packaging

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**ABSTRACT**

This study aimed to fabricate and analyze a chitosan-based film incorporating ZnO and patchouli oil, and to elucidate its physicochemical and bioactive properties. The film was synthesized via the phase inversion method, utilizing 1 g of chitosan, 0.15 g of ZnO, and 0.25 mL of patchouli oil. An enhancement in the mechanical properties of the resultant film was observed, with the tensile strength escalating from an initial 2.59 kgf/mm$^2$ to 25.28 kgf/mm$^2$. Fourier transform infrared (FTIR) spectroscopy affirmed the successful integration of ZnO and patchouli oil within the chitosan matrix. X-ray diffraction (XRD) analyses indicated a decrease in the crystallinity of the chitosan film post-addition of ZnO and patchouli oil. Furthermore, the modified chitosan film exhibited augmented antibacterial activity, with the inhibition zone diameters against *Staphylococcus aureus* and *Escherichia coli* expanding from 0 mm to 7.75 mm and 9.55 mm, respectively. Practical application of this chitosan-ZnO-patchouli oil film as a cover for grapes demonstrated its efficacy in preserving the freshness of the fruit over an extended period in comparison to conventional plastic. The results suggest that this modified chitosan film presents potential as a novel, natural, high-strength, antibacterial packaging material.

## 1. INTRODUCTION

The surge in research focused on the development of active food packaging from biofilms can be attributed primarily to their biodegradability and inherent antioxidant and antimicrobial activities [1]. Economically viable food packaging films can be realized from the by-products of various food industries, harnessing the biodegradable nature of these active films to enhance food shelf-life while promoting health safety.

Among the myriad natural polymers available, such as starch and cellulose, chitosan stands out as the most widely employed in food packaging applications, owing to its biodegradability, ease of modification, and notable antimicrobial properties [2]. However, biofilms derived from polysaccharides often exhibit deficient mechanical properties. These limitations can be addressed through the incorporation of nanoparticles, thereby enhancing the mechanical strength of these composite films.

Numerous studies have reported on the utilization of fillers in the fabrication of composite films, including ZnO nanoparticles [3], e-polylysin [4], chlorella biomass [5], zeolite [6], polypyrrole [7], epigallocatechin gallate conjugates [8], gelatin [9], carboxymethyl cellulose [10], $TiO_2$ [11], melissa essential oil [12], and lemon essential oil [13]. Yet, no known research to date has reported on the combined use of ZnO and patchouli oil to modify chitosan film.

Renowned for its antioxidant and antimicrobial bioactivity, patchouli oil, primarily composed of patchouli alcohol (30-40%) and nor-patchoulienol (0.3-0.4%) [14, 15], represents an exciting potential additive. The integration of ZnO can enhance the film's antibacterial, barrier, and hydrophobic properties, with films containing ZnO demonstrated to effectively reduce bacterial contamination [16]. This stems from the ability of ZnO particles to generate oxygen radicals or hydrogen peroxide ($H_2O_2$) via oxygen defect sites, thus inducing antibacterial activity.

This study aimed to prepare chitosan composite films using the phase inversion method, with a ratio of chitosan, ZnO, and patchouli oil of 1/0.15/0.25 (g/g/mL). The films were characterized using FTIR, XRD, SEM-EDX, and tensile tests. The potential antibacterial activity of the films was evaluated using *Staphylococcus aureus* and *Escherichia coli* bacteria.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Patchouli oil was collected from Aceh, Indonesia. Tokyo Chemical Industry Co., Ltd. in Japan provided analytical quality shrimp shell-derived chitosan with a deacetylation degree of 75.0-85.0%. Sigma-Aldrich in Selangor, Malaysia, supplied all analytical grade chemicals.

### 2.2 Preparation of films

1 g chitosan was dissolved in 100 mL of 2% acetic acid solution and stirred for 2 hours at room temperature using a magnetic stirrer. Dope solution was poured into a 17 × 12 cm

glass plate and dried at 40°C in an oven for 48 hours. After the film was formed, it was removed from the glass plate. The procedure was repeated for preparation of chitosan-ZnO, chitosan-patchouli oil, and chitosan-ZnO-patchouli oil films with weight ratio of 1/0.15 (g/g), 1/0.2 (g/mL), and 1/0.15/0.25 (g/g/ml), respectively.

### 2.3 Characterization of films

The chitosan composite films were analyzed for their molecular structure using a Shimadzu Fourier Transform-Infrared (FT-IR) 8400 (Kyoto, Japan) after being oven-dried at 40°C overnight and crushed with KBr. Shimadzu XRD-700 Series X-Ray Diffractometer (Kyoto, Japan) was used to scan the samples from 10° to 70° at 8°/min using CuK radiation (30 mA; 40 kV). The surface of the sample was then analyzed for morphology using scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDS), observed with 500x magnification at 10 kV. Antibacterial activity was determined by the disc diffusion method using *staphylococcus aureus* and *Escherichia coli* bacteria. The parameter evaluated was diameter of the inhibition zone of the films against *Staphylococcus aureus* and *Escherichia coli* bacteria. Organoleptic studies were performed for grapes placed in plastic cups and covered with chitosan composite films and compared with conventional plastics.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Mechanical properties of films

A tensile test was carried out to investigate the mechanical characteristics of chitosan, chitosan-ZnO, chitosan-patchouli oil, and chitosan-ZnO-patchouli oil films. The procedure was performed according to ASTM (American Standard Testing Material) D638 TYPE IV. Table 1 displays the results.

**Table 1.** Mechanical properties of chitosan and modified chitosan films

| Film | Tensile Strength (kgf/mm$^2$) | Elongation (%) |
|---|---|---|
| Chitosan | 2.59 | 32.95 |
| Chitosan-ZnO | 9.95 | 23.40 |
| Chitosan-patchouli oil | 40.35 | 7.64 |
| Chitosan-ZnO-patchouli oil | 25.28 | 12.48 |

Based on Table 1, tensile strength of chitosan film increases after modification with ZnO and patchouli oil. Tensile strength of chitosan film without modification was 2.59 kgf/mm$^2$ and elongation was 32.95%. After addition ZnO, the tensile strength of chitosan film was increased, where the tensile strength of chitosan-ZnO film was 9.95 kgf/mm$^2$ with elongation of 23.40%. The improvement of tensile strength was also found in patchouli oil addition on chitosan film preparation, where tensile strength of chitosan-patchouli oil was 40.35 kgf/mm$^2$ with elongation 7.64%. Addition of ZnO and patchouli oil in combination produced chitosan-ZnO-patchouli oil film with tensile strength of 25.28 kgf/mm$^2$ and elongation of 12.48%. These tensile strength values were higher than chitosan film without modification. ANOVA statistical analysis revealed a significant difference in tensile strength with Sig. value of 0.021. It implies that ZnO and patchouli oil act as good fillers for chitosan composite film. Tensile strength was increased due to intermolecular interactions between chitosan, ZnO, and patchouli oil in the composite film. The increase in tensile strength film also indicates a better orientation of the molecular segments within it [16].

### 3.2 FT-IR analysis

Determination of the functional groups can be carried out using the FT-IR analysis. The results of FT-IR analysis are shown in Figure 1. Figure 1(a) depicts an FT-IR spectrum of chitosan film exhibiting characteristic chitosan absorption bands. Absorption band at wave number 3264.91 cm$^{-1}$ indicates the presence of -OH groups and –NH groups in chitosan [17, 18]. Absorption band of C=O stretching vibration with respect to –NH appears at wavenumber 1542.05 cm$^{-1}$. CO and CH vibrations are observed at wave number 1022.61 cm$^{-1}$ and 407.24 cm$^{-1}$, respectively.

Absorption bands of chitosan-ZnO film (Figure 1(b)) slightly different compared to the absorption bands of the chitosan film. Absorption band at wave number 3264.91 cm $^{-1}$ (chitosan film) shifts to wave number 3247.38 cm$^{-1}$ from wave number 1542.05 cm$^{-1}$ to wave number 1542.09 cm$^{-1}$. These shifts indicate an interaction between chitosan and ZnO. Figure 1(c) shows the FT-IR spectrum of chitosan modified with patchouli oil exhibits a shift in absorption band at wavenumber 3247.12 cm$^{-1}$, and broadening of absorption band at wave numbers 1541.95 cm$^{-1}$, 1406.94 cm$^{-1}$, and 1020.33 cm$^{-1}$. It indicates interaction between –CNOH and –OH groups in patchouli oil.

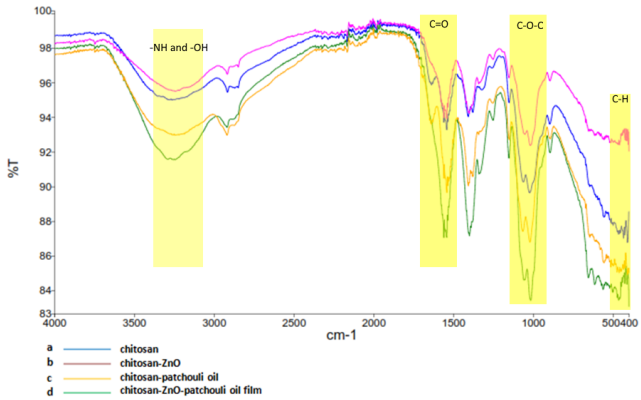


**Figure 1.** FT-IR spectra of chitosan film (a) chitosan-ZnO film (b) chitosan-patchouli oil film (c) and chitosan-ZnO-patchouli oil film (d)

Figure 1(d) is FT-IR spectrum for chitosan modified with ZnO and patchouli oil (chitosan-ZnO-patchouli oil film). It is observed a shift in absorption band from wave number 3264.91 cm$^{-1}$ to 3247.10 cm$^{-1}$ and broadening of the absorption bands at wave numbers 1560.18 cm$^{-1}$, 1400.32 cm$^{-1}$, 1015.65 cm$^{-1}$ and 464.28 cm$^{-1}$. These changes indicate interaction of the functional groups present in chitosan with ZnO and patchouli oil and an increase in the amount of -OH due to the inclusion of patchouli oil.

### 3.3 XRD analysis

Characterization using XRD aims to determine the crystalline phase present in the sample. The intensity was directly proportional to the number of X-ray photons detected by the detector.

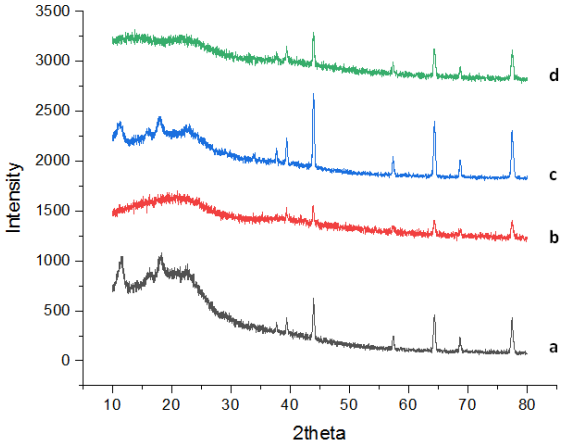


**Figure 2.** Diffractograms of chitosan film (a), chitosan-patchouli oil film (b), chitosan-ZnO film (c), and chitosan-ZnO-patchouli oil film (d)

Based on Figure 2(a), XRD pattern of chitosan shows a typical semi-crystalline structure of chitosan indicates by broad peaks, at 2 theta 11.4086°, and 18.16°. The semi-crystalline of chitosan occurs due to intermolecular interactions and interactions between the hydrogen bonds in–$NH_2$ which are in the $C_2$ position and the hydroxyl groups [10]. XRD pattern of chitosan-patchouli oil film (Figure 2(c)) shows a slightly decrease in crystallinity, where similar peaks are observed with lower intensity than XRD pattern of chitosan film. It indicates patchouli oil molecules have no significant effect on the crystallinity of chitosan film. XRD pattern of chitosan-ZnO film (Figure 2(b)) shows an amorphous structure where typical peaks of chitosan are not observed. It is probably due to ZnO restricts incorporation of chitosan polymer chains during film formation. Similar result is also found in the XRD pattern of the chitosan-ZnO-patchouli oil film (Figure 2(d)), where typical peaks of chitosan are also not observed and the intensities are lower than the other films. The presence of ZnO and patchouli oil affects the formation of the chitosan films which accordingly affects the crystallinity.

### 3.4 SEM-EDX analysis

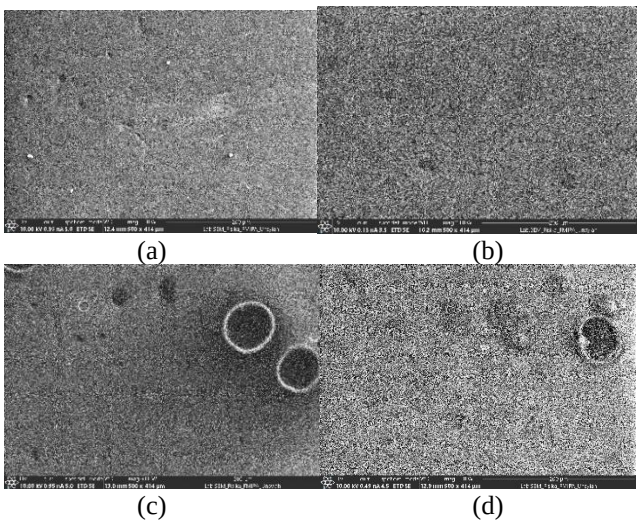


**Figure 3.** SEM images of chitosan film (a), chitosan-ZnO film (b), chitosan-patchouli oil film (c), and chitosan-ZnO-patchouli oil film (d)

Morphological analysis was conducted using Scanning Electron Microscopy (SEM) with 500X magnification on the top of the film which aims to determine the homogeneity of the chitosan film and the modified chitosan films. The morphology of the films can be seen in Figure 3.

The surface image of chitosan-ZnO film (Figure 3(b)) shows relatively less dense surface compared to chitosan film (Figure 3(a)). It was due to the ZnO particles were evenly distributed among polymer chains of the chitosan film, which proves a good interaction between filler and matrix in composite polymer. This interaction produce a film with higher tensile strength value as shown in Table 1. It is evident that good compatibility between the particles and the polymer matrix can effectively improve the overall properties of the composite film [16].

Figure 3(c) is SEM image of chitosan-patchouli oil film which exhibits a smoother film surface compared to the chitosan film (Figure 3 (a)) indicating that the patchouli oil can bond or enter into the chitosan structure. Although in some parts of the film were found to be inhomogeneous because the presence of non-dispersed parts of patchouli oil or the existence of air in the dope solution, the film produced has higher tensile strength than chitosan film. Meanwhile, SEM image of chitosan-ZnO-patchouli oil film (Figure 3(d)) shows a combination of Figure 3(b) and Figure 3(c).

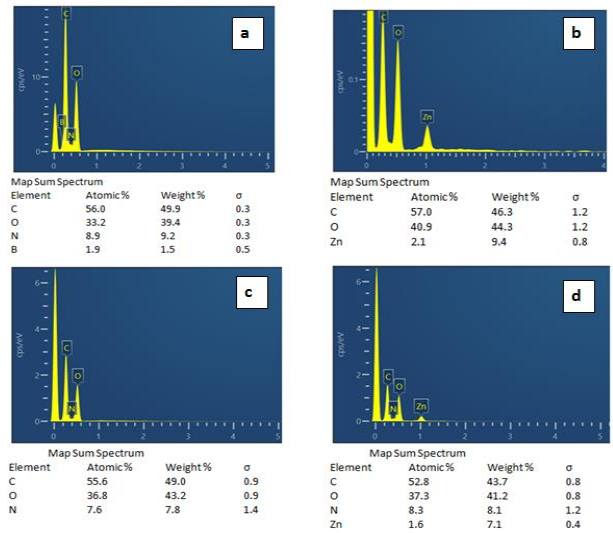


**Figure 4.** Results of energy dispersive X-ray (EDX) analysis of chitosan film (a), chitosan-ZnO film (b), chitosan-patchouli oil film (c), and chitosan-ZnO-patchouli oil film (d)

Incorporation of ZnO and patchouli oil was also observed by EDX analysis and the results are shown in Figure 4. The dominant elements of the chitosan film (Figure 4(a)) are C and O which are the main constituents of chitosan. Where the content of elements C and O in the chitosan film was 56% and 33.2%, respectively. An increase in O element and the decrease of C and N elements were observed in chitosan-patchouli oil film (Figure 4(c)). The-OH groups of patchouli oil contributed to the increase in O element. It confirmed the incorporation of patchouli oil in chitosan film. Meanwhile, Zn element was found in chitosan-ZnO film (Figure 4(b)) that confirm the incorporation of ZnO in chitosan film. The combination of these was found in chitosan-ZnO-patchouli oil film (Figure 4(d)) that confirm the formation of chitosan-ZnO-patchouli oil film.

### 3.5 Antibacterial activity of films

The method used to determine antibacterial activity is the disc diffusion method using Mueller Hinton Agar (MHA) media. The chitosan film was cut approximately the size of a blank disc paper. Blank discs were soaked in sterile distilled water as a negative control and soaked in amikacin as a positive control for 30 minutes. Furthermore, the chitosan film, negative control, and positive control were placed on top of the media that had been scratched with *Staphylococcus aureus and Escherichia coli* bacteria on their respective media. Observations were performed after incubation for 24 hours at 37°C using an incubator. Inhibition zone/clear zone was observed around the disc paper (Figure 5). This indicated that the samples were able to inhibit bacteria. Table 2 shows the results of observations after 24 hours.

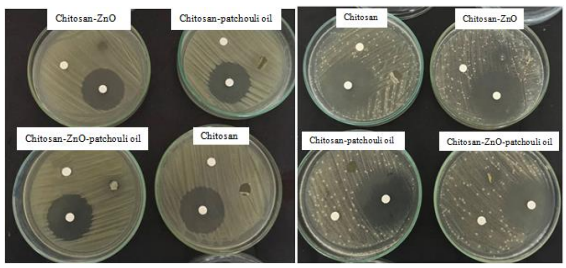


**Figure 5.** Antibacterial activity of films againt (a) *Staphylococcus aureus* and (b) *Escherichia coli*

**Table 2.** Antibacterial activity of chitosan and modified chitosan films

| Sample | Positive Control | | Negative Control | | Film | |
|---|---|---|---|---|---|---|
| | S. *aureus* | E. *coli* | S. *aureus* | E. *coli* | S. *aureus* | E. *coli* |
| | Diameter of the inhibition zone (mm) | | | | | |
| Chitosan | 33.30 | 33.10 | 0 | 0 | 0 | 0 |
| Chitosan-ZnO | 32.65 | 36.00 | 0 | 0 | 9.20 | 0 |
| Chitosan-patchouli oil | 33.00 | 36.65 | 0 | 0 | 0 | 9.15 |
| Chitosan-ZnO-patchouli oil | 32.55 | 35.85 | 0 | 0 | 7.75 | 9.55 |

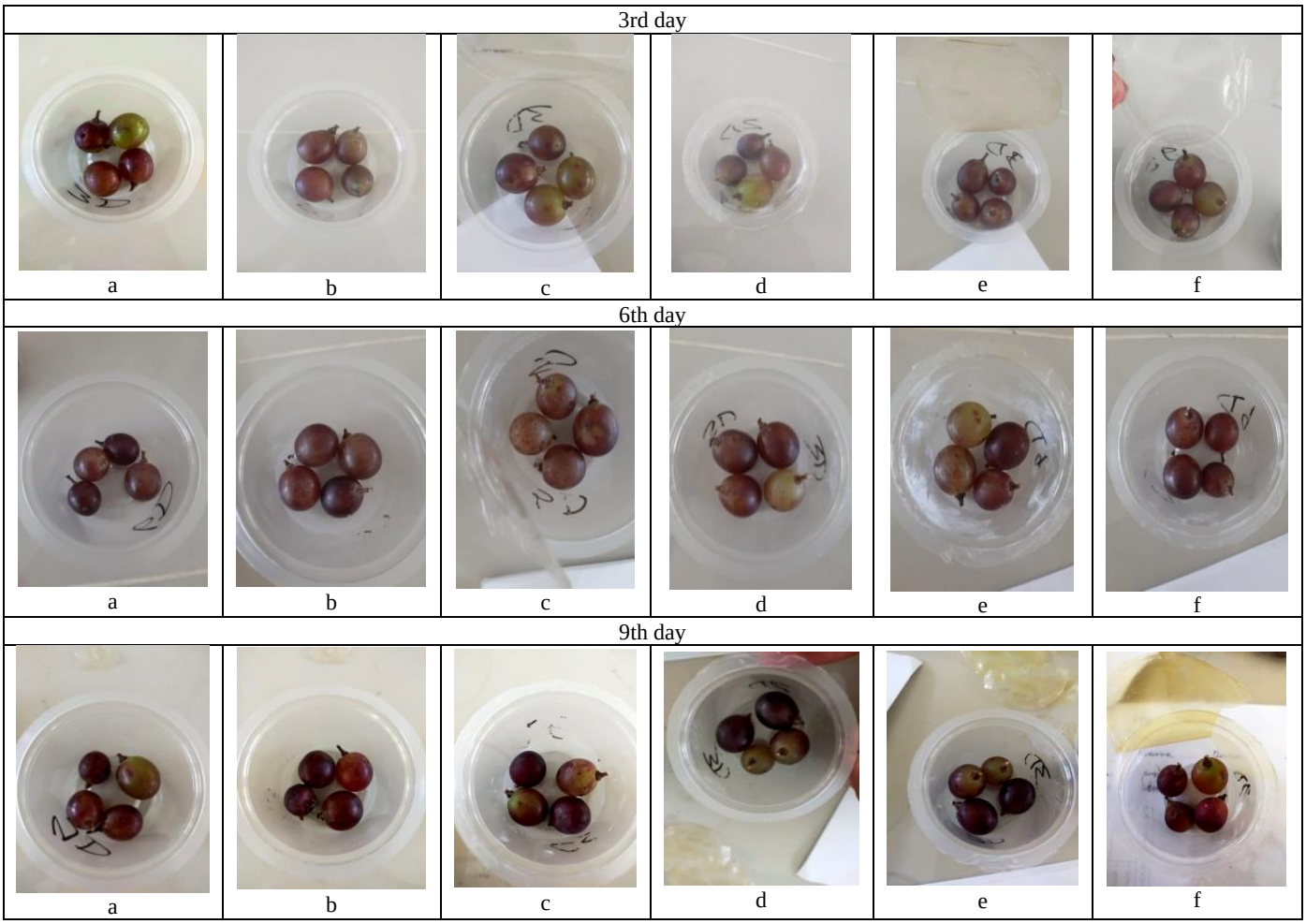


**Figure 6.** Grapes in plastic cups without cover (a) cover by conventional plastic (b) chitosan film (c) chitosan-ZnO film (d) chitosan- patchouli oil film (e) chitosan-ZnO- patchouli oil film and (f) after 3, 6 and 9 days

Figure 5(a) shows the anti-bacterial activity of chitosan film and modified chitosan film against *Staphylococcus aureus*. The chitosan film and chitosan-patchouli oil film did not show the inhibition zone against bacteria. Meanwhile, the chitosan-ZnO film had an inhibition zone for bacteria of 9.20 mm and the chitosan-ZnO-patchouli oil film had an inhibition zone for bacteria of 7.75 mm (Table 2). Figure 5(b) shows the antibacterial activity chitosan film and modified chitosan films against *Escherichia coli* bacteria. Chitosan film and chitosan-ZnO film did not show inhibition zone against the bacteria. The chitosan-patchouli oil film and chitosan-ZnO-patchouli oil film showed inhibition zone of 9.15 mm and 9.55 mm, repectively.

Based on these results, initially chitosan film did not have antibacterial activity. After modification with ZnO and patchouli oil, chitosan film showed antibacterial activity for *Staphylococcus aureus* and *Escherichia coli* bacteria. ZnO has antibacterial activity because $Zn^{2+}$ ions disrupt the bacterial cell wall and nano ZnO generates oxidative stress in the bacterial cell wall through the formation of active $H_2O_2$ species on its surface [16, 19].

### 3.6 Organoleptic analysis

Organoleptic analysis of grapes covered by various films was carried out to study the effectiveness of chitosan film and modified chitosan films on food packaging. Grapes were placed in plastic cups and covered by chitosan film, chitosan-ZnO film, chitosan-patchouli oil film, chitosan-ZnO-patchouli oil film. As a control, the grapes were also covered by conventional plastic and without cover. Figure 6 depicts the results of an organoleptic test that lasted 9 days.

On the 3rd day, the grapes covered by chitosan film, chitosan-ZnO film, chitosan-patchouli oil film, chitosan-ZnO-patchouli oil film had the same taste, color and smell with initial grapes. The texture of grapes without cover and covered by conventional plastic turned a little soft. There was no significant change in organoleptic of grapes on the 6th day, where the observation results on the 6th day the same as the 3rd day. The significant change was observed on the 9th day, where grapes without cover and covered by conventional plastic changed in the texture become wrinkled, and the taste was slightly bland. Whereas, the taste, texture, color, and smell of grapes covered by chitosan film, chitosan-ZnO film, chitosan-patchouli oil film, and chitosan-ZnO- patchouli oil film were hardly changed. These findings suggest that modified chitosan films could be used for food packaging.

## 4. CONCLUSIONS

Based on the results, modification chitosan with ZnO and patchouli oil improved mechanical properties and antibacterial activity of chitosan. The formation of chitosan-ZnO-patchouli oil film was confirmed by FT-IR spectra. XRD analysis shows the addition of ZnO and patchouli oil has decreased the crystallinity of chitosan film. Homogeneous surface of modified chitosan films observed by SEM analysis implies a good compatibity between filler and matrix polymer. Addition of ZnO and patchouli oil has given antibacterial activity to chitosan film. Chitosan-ZnO-patchouli oil film was able to maintain fruit freshness better than conventional plastic. This implies that chitosan-ZnO-patchouli oil film has the potential to be used as a friendly antibacterial packaging film to replace the use of conventional plastic which is not environmentally friendly. However, before its implementation, some studies should be performed such as evaluating barrier properties and testing on real food samples.

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