

## Antimicrobial activity and application of nanocomposite coating from chitosan and ZnO nanoparticle to inhibit microbial growth on fresh-cut papaya

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### Abstract

Fresh-cut papaya is one of the perishable minimally processed product due to microbial decay rate. In this work, a novel nanocomposite coating was developed as a possible method for maintaining the quality attributes of the samples. Chitosan and ZnO nanoparticles (ZnONP) were selected to inhibit microbial growth on fresh-cut papaya. The aims of this work were to evaluate the antimicrobial activity and effect of nanocomposite coating made of chitosan combined with ZnONP on microbial growth of fresh-cut papaya stored for 12 days at 10°C. The preliminary work was conducted to select the best formulation exhibiting the greatest bacterial inhibition effect which was chitosan combined with 0.027% ZnONP (by weight of nanocomposite solution). The results revealed that the average diameter of ZnO was 611.30 nm with polydispersion index of 0.011 confirmed by particle size analyzer (PSA). All components were completely contained in nanocomposite analyzed by Fourier-Transform Infrared (FTIR) and Scanning Electron Microscopy (SEM) for its microstructure and presence of ZnONP in the film. Selected nanocomposite application on fresh-cut papaya could suppress microbial growth significantly in comparison with uncoated papaya during storage time. Our study suggests that nanocomposite treatment provides a desirable method to maintain and improve fresh-cut papaya postharvest life.

## 1. Introduction

Minimally processed fruit products have gained great attentions and consumer's demands throughout the world (Bitencourt *et al.*, 2014). Papaya (*Carica papaya*. L) is one of the fruits which is prospectively developed to be fresh-cut product. For the last two decades, production of papaya globally is almost 10.5 million tonnes (Kandasamy *et al.*, 2012). However, fresh cut is highly perishable due to its lack of preparation stimulating microbial growth (Rojas-Grau *et al.*, 2009; Yuliani *et al.*, 2018). Finally, an alternative is strongly needed to prolong the shelf life of fresh-cut papaya.

Coating is widely known to serve retardation of fresh fruit decay and deterioration. The use of chitosan-based coating, an amino polysaccharide produced from deacetylation of chitin, is continuously improved due to its beneficial properties such as edible, biocompatible, and non-toxic (Romanazzi *et al.*, 2013). Previous works resulted that the application of chitosan-based coating could prolong the shelf-life of guava (7 days) and strawberry (8 days) (Petersen *et al.*, 1999; Hernández-Muñoz *et al.*, 2008). Interaction among amino groups of

chitosan and cell surface resulted the damage of microbial's intracellular components (Rabea *et al.*, 2003). However, single chitosan-based coating has limited inhibition against microorganism and unsatisfactory barrier characteristics (Ravi, 2000).

A nanocomposite is an innovative coating which consists of edible polymers matrix filled with nanoparticles. One of the potential inorganic materials incorporated into the matrix film is ZnONP. The application of ZnONP filler of nanocomposite to maintain fruit quality was previously developed including apples (Li *et al.*, 2011), kiwi (Meng *et al.*, 2014), and mango (Meindrawan *et al.*, 2018). The advantages of ZnONP use in the food field have been reported including a safe chemical substance and source of zinc supplement and fortification (Shi and Gunasekaran, 2008; Suyatma *et al.*, 2014). Hence, the aim of this study was to evaluate the antimicrobial activity of the chitosan combined ZnONP nanocomposite coating on fresh-cut papayas stored for 12 days at at 10° C. This work may be an alternative method to maintain the quality of fresh-cut papaya during export.

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## 2. Materials and methods

### 2.1 Materials

Papaya "California" was obtained from the local market, South Tangerang, Indonesia. Commercial ZnONP was purchased from Xuancheng Jingrul New Material co. Ltd (China), acetic acid from Merck, plate count and nutrient agar from Sigma Aldrich. Chitosan was obtained from M and H Farm, Bogor, Indonesia. *Escherichia coli* and *Staphylococcus aureus* isolates (IPB laboratory collection) as representatives for gram-negative and gram-positive bacteria respectively were used in antimicrobial test of nanocomposite.

### 2.2 Preparation of nanocomposite and application for fresh-cut papaya

Preparation of nanocomposite solution was conducted referring to the method of Kanmani and Rhim (2014) with modification. In brief, each of 0.005, 0.006, 0.010, 0.020, and 0.027% (by weight of nanocomposite solution) ZnONP was dispersed into 0.1 L of 1% acetic acid solution. At the same time, 3 g of chitosan was added into 0.3 L of 1% acetic acid at temperature 60°C and with constant stirring until completely dissolved. Afterward, each of prepared ZnONP was mixed into chitosan solution at temperature 80°C. The final solution was subsequently cooled at 29±2°C before application on fresh-cut papaya.

Fresh-cut papaya was prepared by peeling manually using a stainless-steel knife and then sliced from both sides, according to the method of Yuliani *et al.* (2018). Subsequently, flesh of papaya in pieces (3-4 cm<sup>3</sup>) was dipped in a selected nanocomposite solution for 10-20 s and drained. Papaya cube without coating was considered as a control. All samples were stored in the refrigerator for 12 days at 10°C with an interval of 4 days (0, 4, 8, and 12).

### 2.3 Characterization of nanocomposite

The particle size distribution of ZnONP was measured by using PSA, Zetasizer (model Nano ZS series Malvern Instruments, UK). SEM, JEOL 6010 LA Benchtop, at voltage of 20 kV and magnification of 40x and 180x, was employed to observe the microstructure of the nanocomposite film and the presence of ZnONP. FTIR, Thermo Fisher Scientific, iN10, scanning at range of 4000-500 cm<sup>-1</sup> was employed analysis was done to analyze the functional groups of each nanocomposite material. The antimicrobial activity of ZnONP solution was carried out with the disc diffusion agar method. The impregnated discs into nanocomposite solution were placed on the nutrient agar inoculated media with 0.5 McFarland *E. coli* and *S.aureus*. After incubation at 37°C for 48 hrs, the plates were observed for clear zone

around the discs.

### 2.4 Evaluation of nanocomposite coating to inhibit microbial growth on fresh-cut papaya

Antscope 3IN1 Digital Microscope was used to observe the nanocomposite on the surface and cross-section of fresh-cut papaya. The aerobic microbial count was determined by pour plate method using plate count agar as the medium. Sample of 10 g was dissolved until 100 mL of 0.1% peptone water and homogenized using a vortex mixer. An aliquot (1 mL) of the sample subsequently was put into a sterile petri dish followed by agar and incubated at 37°C for 3 days.

### 2.5 Statistical analysis

The data obtained in this work were analyzed using T-test for total microbial count and analysis of variance (ANOVA) for activity of ZnONP, followed by Duncan's Multiple Range Test (DMRT) when needed, used to represent results at a significance level of p<0.05 using SPSS (Statistical Product and Service Solutions) version 16.0.

## 3. Results and discussion

### 3.1 Characterization of nanocomposite

Determination of ZnONP particle size was done by PSA, Figure 1, which was 611.30 nm for ZnONP diameter average. Furthermore, it reveals that the ZnONP polydispersion index, which represents the spread distribution of particles, is 0.01. Lemarchand *et al.* (2003) explained the narrower value of polydispersion index, the smaller size distributions. The measured ZnONP were considered as aggregated ZnONP because the size tends to larger compared to commercial ZnONP (20±5 nm). It is in accordance with Shi and Gunasekaran (2008), ZnONP easily agglomerates in aqueous solution. Furthermore, Suyatma *et al.* (2014) reported a similar result which there was a slight agglomeration of ZnONP, diameter size of 100 nm, as a result of dispersing process in water.

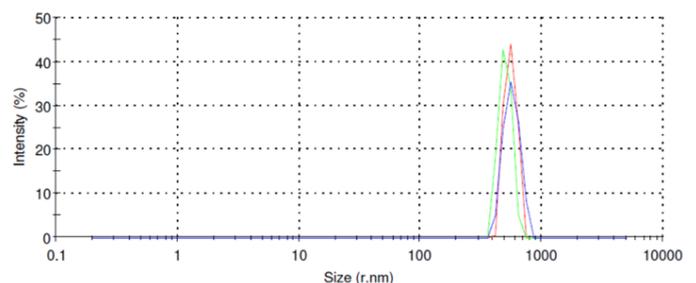


Figure 1. Size distribution spectra of ZnONP using PSA

The antibacterial activity of ZnONP was evaluated referring to the disc diffusion agar method (Table 1). It demonstrates that ZnONP is synergized substances

combined with chitosan to suppress both gram-positive and gram-negative bacterial growth. The similar results were ascertained in the previous studies in which ZnONP was able to disrupt both gram-positive and gram-negative membrane producing disorganization (Huang *et al.*, 2008; Emami-Karvani and Chehrizi, 2011). This preliminary test is considered to select the best sample which exhibits the greatest bacterial inhibition effect which is chitosan combined with ZnONP 0.027% sample.

The FTIR spectrogram of chitosan, acetic acid, ZnONP, and nanocomposite coatings are depicted in Table 1. Zone of inhibition of ZnONP for *S. aureus* and *E. coli*

Sample	<i>S. aureus</i> (mm)	<i>E. coli</i> (mm)
Chitosan	1.3±0.6 <sup>a</sup>	4.0±0.1 <sup>a</sup>
Chitosan + ZnONP 0.005%	1.3±1.2 <sup>a</sup>	5.7±1.2 <sup>a</sup>
Chitosan + ZnONP 0.006%	2.3±0.6 <sup>a</sup>	4.3±0.6 <sup>a</sup>
Chitosan + ZnONP 0.010%	2.7±0.6 <sup>ab</sup>	3.3±0.6 <sup>b</sup>
Chitosan + ZnONP 0.020%	4.0±1.0 <sup>bc</sup>	7.7±0.6 <sup>c</sup>
Chitosan + ZnONP 0.027%	4.7±0.6 <sup>c</sup>	7.3±0.6 <sup>c</sup>

Different superscript letters in the same column indicate statistically significantly different.

Figure 2. It could be observed that the characteristic peak of N-H bending in chitosan appears at 1595 cm<sup>-1</sup> and shifted to 1560 cm<sup>-1</sup> in nanocomposite. Also, the strong and broad peak of 3369 cm<sup>-1</sup> emerged in nanocomposite is inferred to O-H stretching vibration which is shifted from 3498 cm<sup>-1</sup> for chitosan and 3356 cm<sup>-1</sup> for acetic acid. ZnONP is showed by peaks below 1000 cm<sup>-1</sup> region which is shifted from 740 cm<sup>-1</sup> to 862 cm<sup>-1</sup> for composite. It is supported by Kumar and Rani (2013) that metal oxides are usually represented by the fingerprint area below 1000 cm<sup>-1</sup> as a result of inter-atomic vibrations. The typical peaks of acetic acid are exhibited by the peak at 1557 cm<sup>-1</sup> and 1415 cm<sup>-1</sup> indicating C=O and CH<sub>3</sub> bend respectively which are shifted at 1560 cm<sup>-1</sup> and 1411 cm<sup>-1</sup> for nanocomposite.

In this work, SEM was employed to observe the

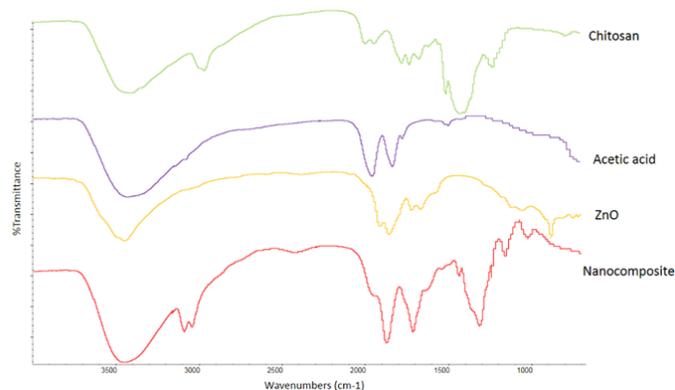


Figure 2. FTIR spectra

microstructure and presence of ZnONP in

nanocomposite edible film. Figure 3 confirms that the microstructure surface of microcomposite films is slightly smooth and relatively compact. ZnONP also emerged clearly and apparently suggested as aggregated ZnONP. Another study was reported by Wardana *et al.* (2018) that ZnONP was successfully trapped by starch matrix. Naturally, ZnONP is easily agglomerated in the solution (Shi and Gunasekaran, 2008).

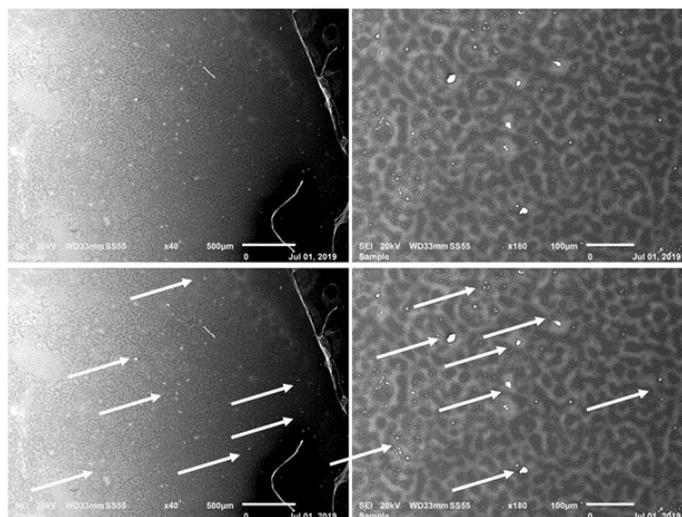


Figure 3. Scanning electron micrograph of the nanocomposite film

### 3.2. Evaluation of microcomposite coating to inhibit microbial growth on fresh-cut papaya

The surface microstructure of fresh-cut papaya was successfully depicted by microscope. Figure 4 represents that nanocomposite thoroughly coat the papaya flesh. In addition, it shows a slightly smooth and homogeneous surface covered by nanocomposite. It is in accordance with previous work that the application of nanocomposite coating was commonly miscible and homogeneous on the fruit surface (Yusof *et al.*, 2017).

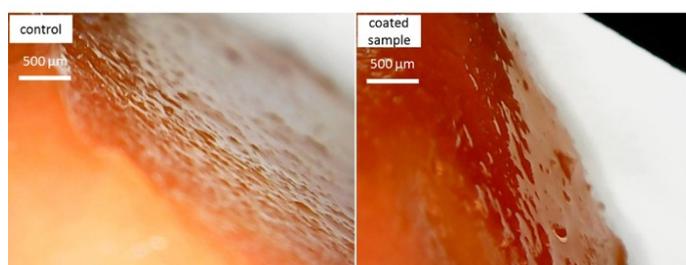


Figure 4. Surface microstructure appearance of fresh-cut papaya

Application of nanocomposite edible coating led to inhibit of the microbial growth significantly at all storage time in comparison with the control sample as exhibited in Table 2. The maximum limit of microbial count on fruit regulated by Indonesia National Agency of Drug and Food Control (BPOM) (2009) is 5.00 log CFU/g. On

day 4, nanocomposite was successfully able to suppress microbial growth below the safe threshold for consumption in comparison with uncoated sample. This is due to both chitosan and filler of ZnONP which has synergistic effect as antimicrobial agent. Interactions between amino groups contained in chitosan and the electronegative charges on the microbial cell surface cause the leakage of intracellular components (Rabea *et al.*, 2003). These results are in accordance with previous works that the application of chitosan-based coating was able to maintain the quality during storage of guava and strawberry (Petersen *et al.*, 1999; Hernández-Muñoz *et al.*, 2008). Furthermore, ZnONP could pierce into the cells of bacteria through a hole or protrusion resulting membrane disruptions and lysis cell (Yousef and Danial 2012). Other work suggested that ZnONP was able to interact with phosphorus groups in bacteria DNA so that inactivate replicating process and inhibit enzyme functions (Arabi *et al.*, 2012). Previous works showed the similar preservation effect by ZnONP coated on apples (Li *et al.*, 2011), kiwi (Meng *et al.*, 2014), and mango (Meindrawan *et al.*, 2018).

Table 2. Total aerobic microbial count of samples for 12 days

Storage time	Control (log CFU/mL)	Nanocomposite (log CFU/mL)
0	3.76±0.04	2.97±0.03
4	5.14±0.02	4.14±0.11*
8	7.11±0.02	5.05±0.01*
12	7.36±0.01	4.11±0.01*

\*Values in the same row are statistically significantly different.

#### 4. Conclusion

In this work, nanocomposite coating made of chitosan combined with ZnONP (611.30 nm) with polydispersion index of 0.011 was developed to inhibit microbial growth on fresh-cut papaya. The study revealed that all components were successfully blended producing antimicrobial nanocomposite indicated by FTIR spectra and confirmed by SEM for the microstructure and presence of ZnONP in the film. Also, the nanocomposite showed antibacterial activity on both gram-positive and gram-negative which was prospective to apply on fresh-cut fruit. After applied on fresh-cut papaya, coating film exhibited a slightly smooth and homogeneous surface representing that thorough coating of the papaya flesh. Furthermore, it could suppress microbial growth particularly at the day 4 of storage which contained microbial count below the safe threshold for consumption (5.00 log CFU/g) to 4.14±0.01 log CFU/g. At the same time, the papaya control (5.14±0.02 log CFU/g) was not feasible to be consumed on its safety aspects. By considering those of results, the development of nanocomposite coating may

provide an alternative to maintain quality of fresh-cut papaya with the aim to delay the microbial growth.

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