**Food Research 4 (2) : 307 - 311 (April 2020)**
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

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

Lavinia, M., Hibaturrahman, S.N., Harinata, H. and *Wardana, A.A.

*Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia 11480*

***Article history:***
Received: 14 July 2019
Received in revised form: 18 August 2019
Accepted: 21 August 2019
Available Online: 6 September 2019

***Keywords:***
Papaya,
Fresh-cut,
Nanocomposite,
ZnO nanoparticles,
Edible coating

***DOI:***
https://doi.org/10.26656/fr.2017.4(2).255

**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.

*Corresponding author.
Email: *ataaditya@gmail.com, ata.wardana@binus.edu*

eISSN: 2550-2166 

## 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 $cm^3$) 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 $cm^{-1}$ 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.

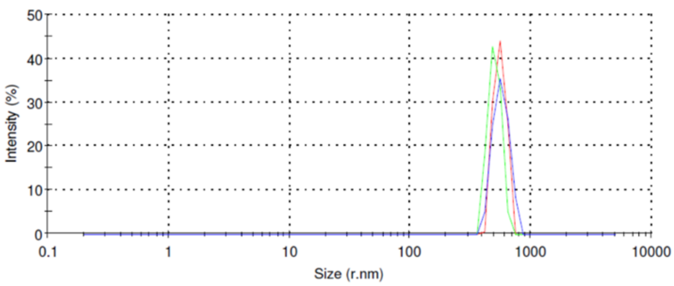


Figure 1. Size distribution spectra of ZnONP using PSA

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

eISSN: 2550-2166 

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 Chehrazi, 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 depictured in Figure 2. It could be observed that the characteristic peak of N-H bending in chitosan appears at 1595 cm$^{-1}$ and shifted to 1560 cm$^{-1}$ in nanocomposite. Also, the strong and broad peak of 3369 cm$^{-1}$ emerged in nanocomposite is inferred to O-H stretching vibration which is shifted from 3498 cm$^{-1}$ for chitosan and 3356 cm$^{-1}$ for acetic acid. ZnONP is showed by peaks below 1000 cm$^{-1}$ region which is shifted from 740 cm$^{-1}$ to 862 cm$^{-1}$ for composite. It is supported by Kumar and Rani (2013) that metal oxides are usually represented by the fingerprint area below 1000 cm$^{-1}$ as a result of inter-atomic vibrations. The typical peaks of acetic acid are exhibited by the peak at 1557 cm$^{-1}$ and 1415 cm$^{-1}$ indicating C=O and $CH_3$ bend respectively which are shifted at 1560 cm$^{-1}$ and 1411 cm$^{-1}$ for nanocomposite.

Table 1. Zone of inhibition of ZnONP for *S. aureus* and *E. coli*

| Sample | *S. aureus* (mm) | *E. coli* (mm) |
|---|---|---|
| Chitosan | 1.3±0.6[a] | 4.0±0.1[a] |
| Chitosan + ZnONP 0.005% | 1.3±1.2[a] | 5.7±1.2[a] |
| Chitosan + ZnONP 0.006% | 2.3±0.6[a] | 4.3±0.6[a] |
| Chitosan + ZnONP 0.010% | 2.7±0.6[ab] | 3.3±0.6[b] |
| Chitosan + ZnONP 0.020% | 4.0±1.0[bc] | 7.7±0.6[c] |
| Chitosan + ZnONP 0.027% | 4.7±0.6[c] | 7.3±0.6[c] |

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

In this work, SEM was employed to observe the 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).

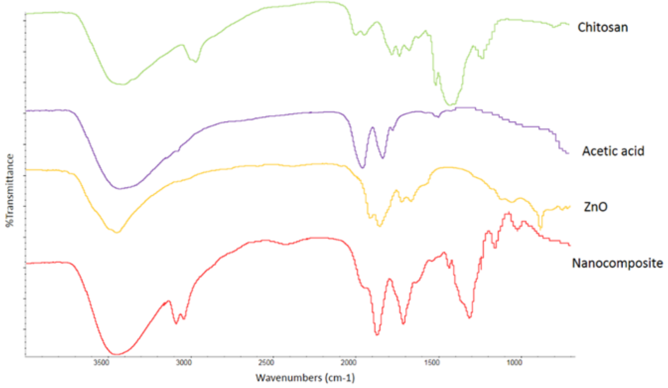


Figure 2. FTIR spectra

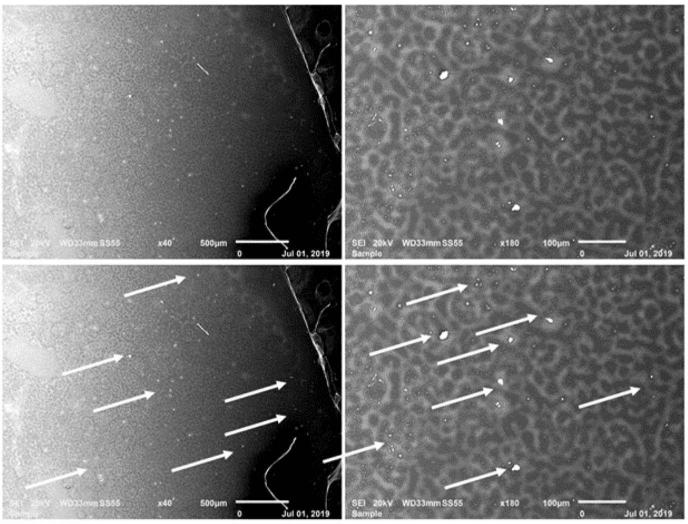

Figure 3. Scanning electron micrograph of the nanocomposite film

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

The surface microstructure of fresh-cut papaya was successfully depictured 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).

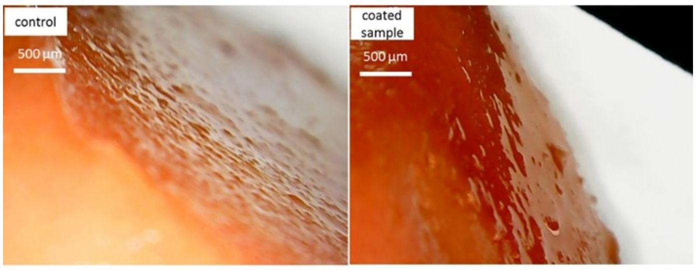


Figure 4. Surface microstructure appearance of fresh-cut papaya

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±004 | 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.

## Acknowledgements

This work was financially supported by the Ministry of Research, Technology Higher Education of the Republic of Indonesia through the grant of Student Creativity Program - Research 2018 No 204/L3/KM/2019/SPK.

## References

Arabi, F., Imandar, M., Negahdary, M., Imandar, M., Noughabi, M.T., Akbari-dastjerdi, H. and Fazilati. M. (2012). Investigation anti-bacterial effect of zinc oxide nanoparticles upon life of *Listeria monocytogenes. Annals of Biological Research*, 7, 3679-3685.

Bitencourt, R.G., Possas, A.M.M, Camilloto, G.P., Cruz, R.Z., Otoni, C.G. and Soares, N.F.F. (2014). Antimicrobial and aromatic edible coating on fresh-cut pineapple preservation. *Ciência Rural*, 44(6), 1119-1125. https://doi.org/10.1590/S0103-84782014000600027

Emami-Karvani, Z. and Chehrazi, P. (2011). Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria. *African Journal of Microbiology Research*, 5(12), 1368-1373. https://doi.org/10.5897/AJMR10.159

Hernández-Muñoz, P., Almenar, E., Valle, V.D., Velez, D. and Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry *(Fragaria × ananassa)* quality during refrigerated storage. *Food Chemistry,* 110(2), 428-35. https://doi.org/10.1016/j.foodchem.2008.02.020

Huang, Z., Zheng, X., Yan, D., Yin, G., Liao, X., Kang, Y., Yao, Y., Huang, D. and Hao, B. (2008). Toxicological effect of ZnO nanoparticles based on bacteria. *Langmuir*, 24(8), 4140-4144. https://doi.org/10.1021/la7035949

Kanmani, P. and Rhim, J.-W. (2014). Properties and characterization of bionanocomposite films prepared with various biopolymers and ZnO nanoparticles. *Carbohydrate Polymers*, 106, 190-199. https://doi.org/10.1016/j.carbpol.2014.02.007

Li, X., Li, W., Jiang, Y., Ding, Y., Yun, J., Tang, Y. and Zhang, P. (2011). Effect of nano-ZnO coated active packaging on quality of fresh-cut “Fuji” apple. *International Journal of Food Science and Technology*, 46(9), 1947-1955.

Meindrawan, B., Suyatma, N.E., Wardana, A.A. and Pamela, V.Y. (2018). Nanocomposite coating based on carrageenan and ZnO nanoparticles to maintain the storage quality of mango. *Food Packaging and Shelf Life,* 18, 140-146. https://doi.org/10.1016/j.fpsl.2018.10.006

Meng, X, Zhang, M. and Adhikari, B. (2014). The effects of ultrasound treatment and nano-Zinc oxide coating on the physiological activities of fresh-cut kiwifruit. *Food and Bioprocess Technology*, 7(1), 126-132. https://doi.org/10.1007/s11947-013-1081-0

Petersen, K., Nielsen, P.V., Bertelsen, G., Lawther, M., Olsen, M.B., Nilsson, N.H. and Mortensen, G. (1999). Potential of biobased materials for food packaging. *Trends in Food Science Technology*, 10 (2), 52-68. https://doi.org/10.1016/S0924-2244(99)00019-9

Rabea, E.I, Badawy, M.E.T., Stevens, C.V., Smagghe, G. and Steurbaut, W. (2003). Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules,* 4(6), 1457-1465. https://doi.org/10.1021/bm034130m

Ravi, K.M.N.V. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers,* 46 (1), 1-27. https://doi.org/10.1016/S1381-5148(00)00038-9

Rojas-Grau, M.A., Soliva-Fortuny, R. and Martin-Belloso, O. (2009). Edible coatings to incorporate active ingredients to fresh cut. *Trends in Food Science and Technology*, 20(10), 1-10. https://doi.org/10.1016/j.tifs.2009.05.002

Romanazzi, G., Feliziani, E., Santini, M. and Landi, L. (2013). Effectiveness of post-harvest treatment with chitosan and other resistance inducers in the control of storage decay of strawberry. *Postharvest Biology and Technology,* 75, 24-27. https://doi.org/10.1016/j.postharvbio.2012.07.007

Shi, L. and Gunasekaran, S. (2008). Preparation of pectin - ZnO nanocomposite. *Nanoscale Research Letters*, 3, 491-495. https://doi.org/10.1007/s11671-008-9185-6

Suyatma, N.E., Ishitakawa, Y. and Kitazawa, H. (2014). Nanoreinforcement of pectin flm to enhance its functional packaging properties by incorporating ZnO nanoparticles. *Advanced Materials Research*, 845, 451-456. https://doi.org/10.4028/www.scientific.net/AMR.845.451

Wardana, A.A., Suyatma, N.E., Muchtadi, T.R. and Yuliani, S. (2018). Influence of ZnO nanoparticles and stearic acid on physical, mechanical and structural properties of cassava starch-based bionanocomposite edible flms. *International Food Research Journal,* 25(5), 1837-1844. https://doi.org/10.4028/www.scientific.net/AMR.845.451

Yousef, J.M. and Danial, E.N. (2012). *In vitro* antibacterial activity and minimum inhibitory concentration of zinc oxide and nano-particle zinc oxide against pathogenic strains. *Journal of Health Sciences*, 2(4), 38-42. https://doi.org/10.5923/j.health.20120204.04

Yuliani, S., Wardana, A.A., Meindrawan, B., Suyatma, N.E. and Muchtadi. T.R. (2018). Nanocomposite edible coating from cassava starch, stearic acid and ZnO nanoparticles to maintain quality of fresh-cut mango cv. Arumanis. *Annals of the University Dunarea de Jos of Galati,* 42(2), 49-58.

Yusof, N.M., Jai, J., Hamzah, F., Yahya, A. and Pinijsuwan, S. (2017). Effect of concentration of *Curcuma longa* L. on chitosan-starch based edible coating. The 6th International Conference on Manufacturing Engineering and Process. IOP Conference Series: Journal of Physics: Conference Series, 885(2017), 012008. https://doi.org/10.1088/1742-6596/885/1/012008

 