



Original Research Article

Effect of carboxymethyl cellulose coating containing ZnO-nanoparticles for prolonging shelf life of persimmon and tomato fruit

Mooktida Saekow^{1,2}, Matchima Naradisorn^{1,3}, Wirongrong Tongdeesoontorn^{1,4} and Yasunori Hamauzu^{2*}

¹School of Agro-Industry, Mae Fah Luang University 333 Moo1 Tambon Tasud, Chiang Rai, 57100 Thailand

²Faculty of Agriculture, Shinshu University 8304 Minamiminowa-mura, Kamiina-gun, Nagano, 399-4598 Japan

³Research Group of Postharvest Technology, Mae Fah Luang University, Chiang Rai, 57100 Thailand.

⁴Research Unit of Innovative Food Packaging and Biomaterials, Mae Fah Luang University, Chiang Rai, 57100 Thailand.

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ABSTRACT

The aims of this study were to investigate the antifungal property of ZnONPs synthesized from pineapple peel extracts, and to apply carboxymethyl cellulose (CMC) coating combined with ZnONPs for maintaining of qualities and delaying black spot disease in persimmon and tomato fruits. ZnONPs was synthesized by mixing 15% (w/v) pineapple peel extract with 1 M of zinc acetate solution. Nanoparticles were characterized by using the UV spectrophotometric method and the result showed the UV absorption peak of ZnONPs at 370 nm. The antifungal activity of ZnONPs in various concentration (0, 5, 10, 20 and 40 % w/v) was studied by disk diffusion method. The minimal inhibitory concentration of ZnONPs against *Alternaria alternata* was 20% (w/v). The effect of CMC coating combined with ZnONPs on persimmon and tomato quality factors such as water loss, firmness, respiration rate, total phenolic compounds, ascorbic acid and disease severity during storage were investigated. The results showed that the application of coating with CMC and CMC + ZnONPs in persimmon and tomato reduced weight loss and respiration rate, increased fruit firmness and antioxidant compounds when compared with control during storage time. The CMC + ZnONPs coating effectively delayed the disease severity in the inoculated persimmon and tomato.

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* Corresponding author: Tel.: +81 265 77 1413; fax: +81 265 77 1700

E-mail address: hamauzu@gipmc.shinshu-u.ac.jp



INTRODUCTION

Persimmon (*Diospyros kaki* L.) fruit are rich in the bioactive compounds such as phenolics, carotenoids, dietary fibres, vitamin C, and minerals (Elabd & Gomaa, 2017). The persimmon consumption has benefits to health because it has effects on the reduction of degenerative human diseases, the protection against free radicals and the prevention of oxidative damage, and reduced risk of cardiovascular disease, diabetes, and cancer (Giordani, Doumett, Nin, & Del Bubba, 2011). Tomato (*Solanum lycopersicum* L.) fruit is also a good source of phenolic and flavonoid compounds, lycopene and ascorbic acid (Davila-Avina et al., 2014). In addition, this fruit has beneficial effects on human health. Due to the action of antioxidant compounds, tomato fruit can reduce oxidative damage in the human body (Davila-Avina et al., 2014). However, the loss of quality including phytochemical changes is the common problem of postharvest life of fruit and vegetable. This is a result of many factors such as transpiration, postharvest diseases, continued ripening, and senescence (Ali, Maqbool, Ramachandran, & Alderson, 2010; Elabd & Gomaa, 2017; Rios & Bohórquez, 2017). In Thailand, the postharvest loss is quite large problem of fruit and vegetable handling. Thailand is a tropical country that high temperature, therefore there is increased respiration and deterioration of fruit quality (Ali et al., 2010; Kumar & Kalita, 2017). Moreover, one cause of the postharvest loss in a tropical country is fruit disease, and *Alternaria alternata* is the causal fungus of black spot disease that occurs in persimmon and tomato fruit (Chen et al., 2014; Kurt, Soyulu, & Soyulu, 2010; Kwon, Ahn, & Park, 2004).

Nowaday, the coating is technology that can maintain the quality and elongate the storage life of fruits and vegetables. A coating is a thin layer of material which can be applied to many produces. The function of the coating for fresh produces is providing moisture barrier on the surface of produces, to prevent gas exchange between fresh produce and surrounding, and reduce the loss of bioactive compounds from fresh produces (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011; McHugh, 2000). Therefore, it can extend the shelf life of fresh fruits and vegetables during transportation and storage. One of the coatings widely used in industry is carboxymethyl cellulose (CMC) (Gulzar, Ishrat, & Varun, 2015).

Zinc oxide (ZnO) is inorganic materials that biosafe material, non-toxic to human cells and antimicrobial agent (Almoudi, Hussein, Abu Hassan, & Mohamad Zain, 2018; Ramy & Osama, 2013). It is listed as generally recognized as safe (GRAS) material by the U.S. Food and Drug Administration 8991. In addition, zinc oxide nanoparticle (ZnONPs) has taken a great attention due to their extensive exploitation in antibacterial activity. It can be applied as an antimicrobial agent in food packaging (Espitia et al., 2012; Sini Kuriakose, Singh, Satpati, & Mohapatra, 2013). Mechanisms of the antimicrobial action of ZnONPs have been thought to the generation of reactive oxygen species (ROS). ROS is a major factor for mechanisms including cell wall damage due to ZnO localized interaction, enhanced membrane permeability, internalization of nanoparticles due to loss of proton motive force and uptake of toxic dissolved zinc ions. These will lead to mitochondrial weakness, intracellular outflow and release in gene expression of oxidative stress which caused eventual cell growth inhibition and cell death (Sirelkhatim et al., 2015).

The nanoparticle synthesis is divided into three methods (chemical, physical and biological). The chemical method involves the use of toxic chemicals which has been proved to be hazardous to the environment, physical method involves the use of high cost of the

equipment, using high temperature and pressure for nanoparticle synthesis, whereas the biological method offers to an environment-friendly and cost-effective approach, that using bacteria, fungi, and also plant for nanoparticle synthesis (Agarwal, Venkat Kumar, & Rajeshkumar, 2017; Krol, Pomastowski, Rafinska, Railean-Plugaru, & Buszewski, 2017). Therefore, in this experiment, the biological method was selected with plant extract (pineapple peel) as a material. This is because the pineapple (cv. 'Nang Lae') is high production at Chiang Rai, Thailand, and there is a lot of pineapple peel for ZnONPs synthesis. Furthermore, the pineapple peel extract contains tannin, terpenoid, and flavonoid (Kaewsophak, Yosphan, & Pinijsuwan, 2016), these compounds in pineapple peel are biological agents expected to act as reducing agents that can reduce zinc ions to zinc oxide nanoparticles (Bhuyan, Mishra, Khanuja, Prasad, & Varma, 2015; Jamdagni, Khatri, & Rana, 2018; P. Singh et al., 2011; Sharma et al., 2016).

The aims of this study were to investigate the antifungal property of ZnONPs synthesized from pineapple peel extracts, and to apply carboxymethyl cellulose (CMC) coating combined with ZnONPs for maintaining of qualities and delaying black spot disease in persimmon and tomato fruits

MATERIALS AND METHODS

Raw materials

Persimmon (cv. 'Atago') was purchased from JA syuso and tomato (cv. 'Plum') was purchased from a local company. The fruits were uniformity as similar color, size, and shape. Non-mechanical damage and non-insect or pathogenic infection were confirmed. The maturity of persimmon is a ripe stage and tomato was a pink stage. These fruits were divided into 3 treatments (Control or uncoated, CMC coated and CMC+ZnONPs coated). Pineapple peels (cv. 'Nang lae') was collected from the pineapple farm around Mae Fah Luang University, Chiang Rai, Thailand. Pineapple peel was dried at 50°C for 24 hours, extracted with water, and then lyophilized for using as reducing agent in ZnONPs biosynthesis.

Chemicals and reagents

Carboxymethyl cellulose, Sodium hydroxide (NaOH), Zinc acetate dehydrate ($\text{Zn}(\text{CH}_3\text{COO})_2$), Metaphosphoric acid (HPO_3), Ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$), Thiourea (H_6NCSNH_6), Potato Dextrose Agar (PDA), Polyoxyethylene Sorbitan Monoleate (Tween80), 2,6-Dichlorophenoldiphenol sodium salt dihydrate, 2,4-Dinitrophenylhydrazine ($\text{C}_6\text{H}_6\text{N}_4\text{O}_4$), Sodium carbonate (Na_2CO_3), Sulfuric acid (H_2SO_4), Folin-Ciocalteu Reagent, Methanol (CH_3OH) 99.8% from nacalai tesque, INC. Kyoto, Japan. Glycerin ($\text{CH}_2(\text{OH})\text{CH}(\text{OH})\text{CH}_2(\text{OH})$) from nakarai chemical Ltd. Gallic Acid ($\text{C}_6\text{H}_2(\text{OH})_3\text{COOH}$) from wako pure chemical industries Ltd.

Pineapple peels extraction

Dried pineapple peels were ground, and extraction was conducted with distilled water (1:10, w/v) by incubation for 24 hours at room temperature. The extract was filtered through a filter paper (Whatman No. 4) and then evaporated with a rotatory evaporator at 55°C for 30 minutes and freeze-dried for 24 hours.

Biosynthesis of zinc oxide nanoparticles (ZnONPs)

The main materials for ZnONPs were zinc acetate and sodium

hydroxide. Fifty millilitres of zinc acetate (1 M) was mixed with 1 mL of 15% (w/v) pineapple peel extract solution under stirring conditions and then mixed with 50 mL of 2 M sodium hydroxide and stirred for 2 hours until white precipitate occurs. The pale white solid was centrifuged at 4500 rpm for 15 min, washed twice with distilled water and dried at 60°C overnight (Singh et al., 2011).

Antifungal activity

The qualitative measurement of antifungal activities against *Alternaria alternata* of the ZnONPs was determined using the disc diffusion method (Bauer, Kirby, Sherris, & Turck, 1966). A spore suspension (1.5 x spore/ml) of *A. alternata* isolated from persimmon (100 µl) was spread on PDA plates. Then sterilized filter paper discs (diameter = 0.6 cm) containing ZnONPs (0, 1, 5, 10, 20 and 40% w/v) was placed on PDA plate. The antifungal activity was visualized by a clear zone that formed around the disc samples on the plate and diameter was recorded as an indication of inhibition against *A. alternata*.

Preparation of carboxymethyl cellulose (CMC) coating with or without ZnONPs and application of the coatings on persimmon and tomato fruits

CMC-based edible coatings (2% w/v) with or without ZnONPs (20%, w/v) were prepared by dissolving in distilled water with glycerol (30% v/v) and tween 80 (0.1% v/v). The mixture was heated until the coating materials were dissolved. The composition of the coatings is shown in **Table 1**.

Table 1: Composition of coating solution

	Condition			
	CMC	Tween 80	Glycerine	ZnONPs
CMC	2% (w/v)	0.1% (v/v)	30% (v/v)	-
CMC+ZnONPs				20%(w/v)

Fresh fruits were a similar color, size and shape, no fungal infection, and no mechanical damage. Fruits in each group (Control, CMC, and CMC+ZnONPs) were disinfected by immersion in 0.1% sodium hypochlorite solution for 2 minutes, and air dried. Fruits for coating were dipped in each coating condition in **Table 1** for 2 minutes, air-dried at room temperature. The coated and control (uncoated) persimmon fruits were stored at 25°C, 65-70% relative humidity (RH) and tomato fruits were stored at 10°C, 80-85% RH for 15 days. Quality factors of fruits, i.e. weight loss, firmness, respiration rate, ascorbic acid, total phenolic content (TPC), were measured. Three fruits (persimmon) and five fruits (tomato) from each treatment or control were used for measurement on each sampling day.

Weight loss measurement

The effect of coated persimmon and tomato with/without ZnONPs on weight loss (%) was determined by measuring the weight of the fresh fruits on a digital balance and then comparing the difference with initial weight before storage (Wo) with final weight after storage in the indicated period (Wf). The percentage of weight loss was calculated by the following equation

$$\text{Weight loss (\%)} = \frac{(W_o - W_f) \times 100}{W_o}$$

Firmness measurement

Firmness of fruit was measured as penetration force (N) from a 6 mm diameter round probe which penetrated 10 mm depth at the middle location of each fruit was measured using a digital force gauge (Model: ZP-100N, IMADA, Japan).

Respiration rate measurement

Respiration (CO₂ production) rate was measured by using a TCD-Gas chromatograph system (GC-8A) with Porapak Q column (50-80 mesh, Water), carrier gas, Argon G3 (>99.999%); pressure, 40 kPa; Column temperature = 50°C; detector temperature = 100°C; current = 80 mA. One mL of headspace gas was injected after the gas sample taken from each bottle containing 3 fruits (for persimmon) or 6 fruits (for tomato) after 2-hour accumulated period at 25 ± 1°C.

$$S = \text{Total volume of container} - \text{Volume of FAV in container}$$

$$\Delta \text{CO}_2 = \frac{(\% \text{CO}_2 \text{ after } t \text{ hours} - \text{ambient CO}_2 \%) \times S}{100}$$

$$\text{CO}_2 \text{ production rate (mg CO}_2 \text{ /kg h)} = \frac{\Delta \text{CO}_2 \times K}{t \times W}$$

$$S = \text{Volume of head space (mL)}$$

$$K = \text{Coefficient}$$

$$t = \text{Time (h)}$$

$$W = \text{Weight of fruits (kg)}$$

Ascorbic acid content

One gram of fruits was added with 5 mL of 5% (w/v) metaphosphoric acid and homogenized for 1 minute. The homogenate was centrifuged 12000×g at 4°C for 15 min and the supernatant was kept for ascorbic acid analysis.

The diluted sample extract (400 µL) was mixed with 200 µL of 2,6-dichlorophenolindophenol solution, 400 µL of 2% (w/v) thiourea and 200 µL of 2% (w/v) 2,4-dinitrophenylhydrazine, then incubated 3 hr at 37 °C. After that the solution was mixed with 1000 µL of 85% sulfuric acid, then incubated 30 minutes at room temperature. The absorbance of the reaction mixture was measured at 540 nm (International Organization of Standardization, 2005).

Total phenolic content (TPC)

One gram of fruits was added with 5 mL of 70% (v/v) methanol and homogenized for 1 minute. After that the solution was centrifuged 15000×g at 4 °C for 15 min and the supernatant was used for total phenolic content analysis.

The total phenolic content was determined by Folin-Ciocalteu assay using UV-spectrophotometer (UV-1800 spectrophotometer). Gallic acid was used as a standard. The sample extract 250 µL was mixed with 1250 µL of Folin-Ciocalteu reagent and 1000 µL of 7.5% Na₂CO₃, then incubated for 60 minutes at room temperature. The absorbance of the reaction mixture was measured at 765 nm.

Disease severity

The fruit was wounded on the skin by puncturing that was already sterilized. Then each wound site was inoculated with 20 µL of spore suspension (1.5 x spore/ml) of *A. alternata*. Treated and control fruits were placed on a plastic tray, maintaining and incubated at room temperature. The disease development was evaluated by visual observation for 15 days and measurement of diameter of damaged area.

Statistical analysis

Data are the mean ± SD of three replications. Different letters indicate significant differences at P ≤ 0.05 according to Duncan's multiple range test using SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

Biological synthesis and characterization of ZnONPs

The information of ZnONPs was confirmed by UV-vis spectroscopy. Figure 1 showed the absorption spectra of pineapple peel extract, biologically synthesized ZnONPs and zinc acetate in the wavelength range 300-500 nm. The absorption peak of ZnONPs solution was observed at 370 nm. The existence of absorption peak between 348-380 nm indicates the successful synthesis of ZnONPs by biological method because this characteristic of ZnONPs can be seen in previously reported studies (Abdelhady, 2012; Chauhan, Reddy, & Abraham, 2014; Nafchi, Nassiri, Shebani, Ariffin, & Karim, 2013).

According to previous study Suttiporn et al., 2016, FESEM confirmed that the ZnONPs synthesized with 'Nanglae' pineapple peel extract was the rod shaped, and the size of ZnONPs were in range of 26-55 nm.

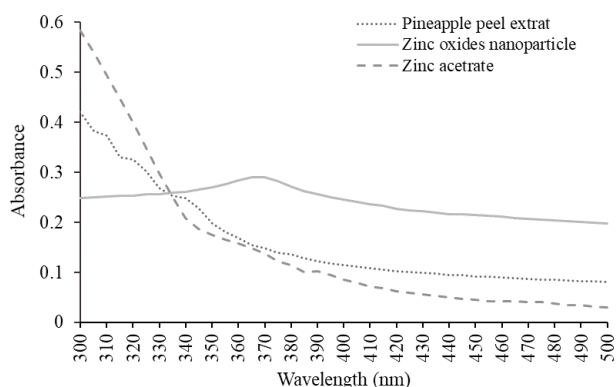


Figure 1 : UV-vis spectra of ZnO nanoparticles solution

Antifungal activity of synthesized ZnONPs

The antifungal activity of ZnONPs was tested against *A. alternata* by Bauer-Kirby disk diffusion assay in various concentration including 0, 5, 10, 20 and 40 % w/v. The inhibition zone determined was shown in Table 2. The results suggested that the least concentration of ZnONPs have the antifungal effect was 20% w/v. In the previously reported studies, an antifungal activity of ZnONPs was evaluated by inhibitory effect in the germination of *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* (Wani & Shah, 2012).

Table 2: Antifungal activity of ZnO nanoparticle determined by dimension of clear zone in the disc diffusion method

Condition	Dimension of clear zone (mm)
Control	0
1 % (w/v)	0
5 % (w/v)	0
10 % (w/v)	0
20 % (w/v)	0.78 ± 0.05 a
40 % (w/v)	0.82 ± 0.09 ab

Weight loss of fruits treated by CMC-based coatings

The weight loss of persimmon and tomato fruits increased all of treatment during storage time. However, both of coated persimmon and tomato showed a lesser weight loss than that of control (uncoated fruits). Although the highest weight loss of the control persimmon and tomato fruit was around 14.8% and 1.7%, respectively, the highest weight loss of coated persimmon and tomato in CMC and CMC+ZnONPs treatments was 10.4 and 9.9%, respectively in persimmon, and 1.4 and 1.2 %, respectively in tomato. The effect of the coatings on reduced weight loss of fruits might be due to coating created a modified atmosphere around fruit surface and the surrounding (Elabd & Gomaa, 2017).

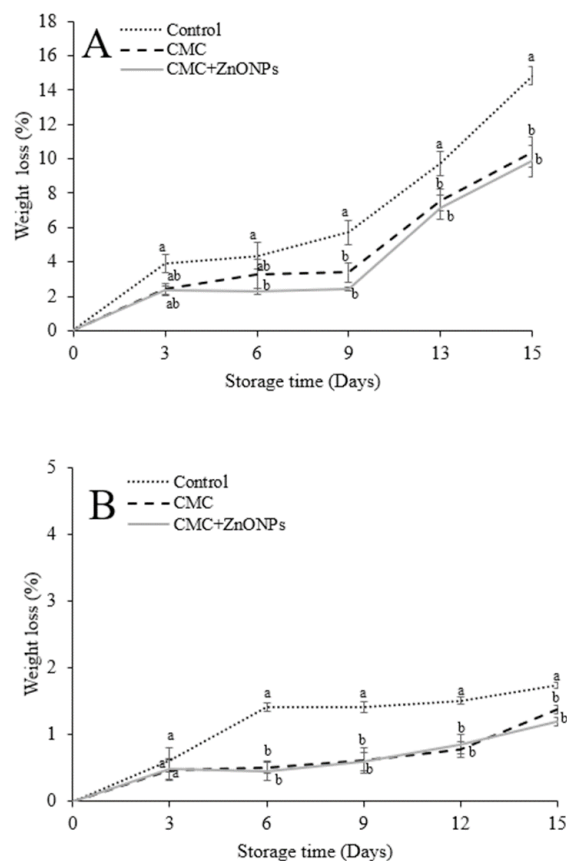


Figure 2: Changes in weight loss during storage of persimmon (A) and tomato (B) fruits at 25°C and 10°C, respectively.

Fruit firmness

The flesh firmness is an important factor for the postharvest quality of fruit and vegetables. As shown in Figure 3, the coated fruits were significantly delayed the firmness loss. The maximum firmness loss was observed in control persimmon and tomato fruits at the end of storage time (The flesh firmness was 15.6 and 19.4 N, respectively). Meanwhile, the firmness of persimmon coated with CMC and CMC+ZnONPs was maintained higher than that of control (26.8 and 27.8 N, respectively), and in case of tomato, the firmness of coated fruit was 28.7 and 27.6 N, respectively. The maintenance of flesh firmness of the coated fruits might be due to reducing respiration and other ripening processes during storage (Ali, Muhammad, Sijam, & Siddiqui, 2011).

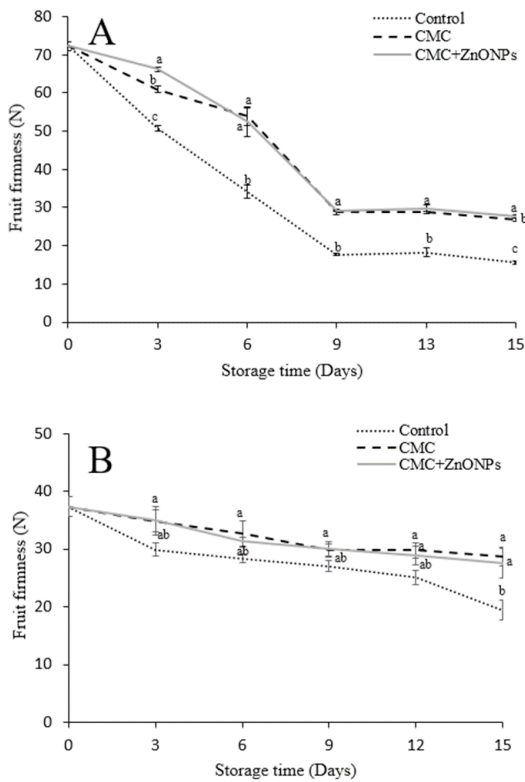


Figure 3: Changes in fruit firmness during storage of persimmon (A) and tomato (B) fruits at 25°C and 10°C, respectively.

Respiration rate

The respiration rate in fresh fruit and vegetables is a good indicator for estimation of storage life. Figure 4 A represents the change in respiration rate of persimmon fruit with or without coatings. The lowest respiration rate was observed in CMC+ZnONPs coated fruit followed by CMC coating and control. The coatings provide a semi-permeable barrier against gas movement that leads to reduce the respiration rate (Ali, Maqbool, Ramachandran, & Alderson, 2010). The respiration rate of tomato is shown in Figure 5 B. The value of control fruit was higher than that of coated fruits. There was a peak value on day 6 and it decreased thereafter until the end of storage time. The temporal increase of CO₂ production might relate with the climacteric respiratory peak (Mustafa, Ali, Manickam, & Siddiqui, 2013).

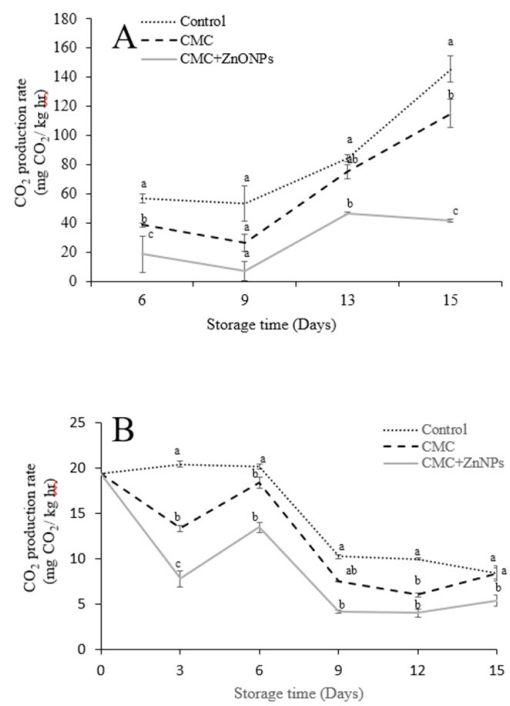


Figure 4: Changes in respiration rate during storage of persimmon (A) and tomato (B) fruits at 25°C and 10°C, respectively.

Ascorbic acid content

The effects of coating treatment on ascorbic acid content of persimmon was shown in Figure 5A. Ascorbic acid decreased gradually during storage time in control persimmon and also in CMC and CMC+ZnONPs coated fruits. However, the coated persimmon retained higher ascorbic acid content than the control especially former half of storage time. In case of tomato, ascorbic acid content of CMC and CMC+ZnONPs coated fruit was increased till 9 days of storage and thereafter it decreased (Figure 5B). Because ascorbic acid content of control fruit started to decrease earlier, the coated fruit maintained higher ascorbic acid content. This might be due to coating retarded oxidation process and also delayed the metabolism of ascorbic acid in the fruit. In the result of tomato, ascorbic acid content initially increased. The similar change has been reported that the ascorbic acid content increased more slowly in tomatoes during the storage time (Davila-Avina *et al.*, 2014). These results of persimmon and tomato have similarly to found in another study. The ascorbic acid content increased with the fruit ripening and when it fully ripe the ascorbic acid content starts to decrease (Wilhelmin, 2005).

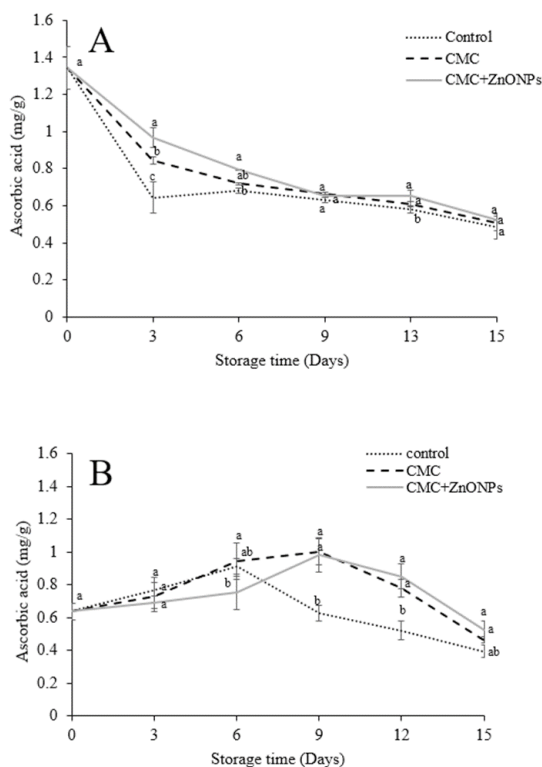


Figure 5: Changes in ascorbic acid content during storage of persimmon (A) and tomato (B) fruits at 25°C and 10°C, respectively.

Total phenolic contents (TPC)

The effect of coatings on changes in TPC of persimmon and tomato during 15 days of storage time can be seen in Figure 6A and 7B, respectively. The TPC of coated persimmon increased and reached a peak value at day 9 in CMC+ZnONPs coated fruit and day 13 in CMC coated fruit. The TPC in coated fruits was entirely higher value compared with control. After that slight decrease was observed during latter part of storage time. In figure 6B, Similar to the result of persimmon, TPC of all coated tomato was higher than control. The increase of TPC by coating treatment might be due to the coating can produce abiotic stress on fruit. The stress caused by the coating can modify metabolisms affecting to the increase of secondary metabolites (Gonzalez *et al.*, 2009).

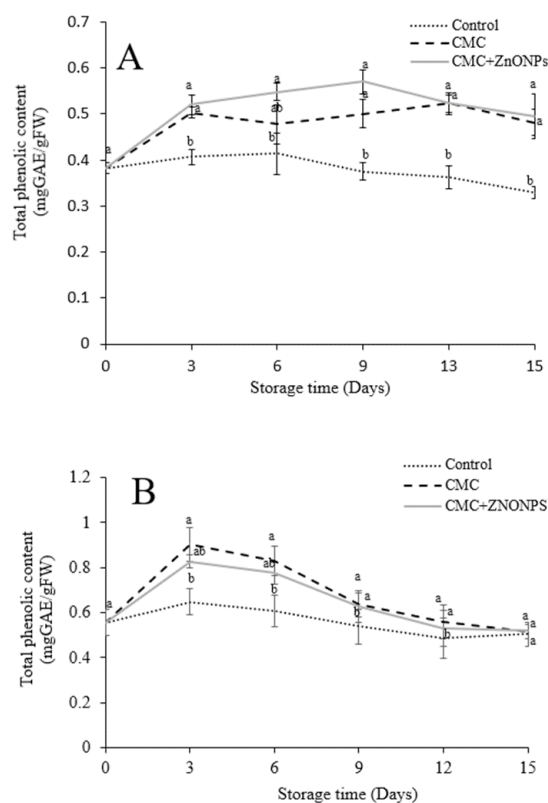


Figure 6: Changes in total phenolic content (TPC) during storage of persimmon (A) and tomato (B) at 25°C and 10°C, respectively.

Disease severity

Changes in disease severity of persimmon and tomato fruit that inoculated *Alternaria alternata* before storage are shown in Figure 7. The results showed that control had a progressive disease development in early stage (at day 3) of the storage period. Contrary to the control, CMC and CMC+ZnONPs coated persimmon and tomato remained lower level of the disease development. It has been reported that ZnONPs are also effective to inhibit disease development caused by strains of plant pathogenic fungi *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum* and *Fusarium oxysporum* (Vinay, Neeraj, & Narashans, 2015). Because of ability to reduce the disease development, those treatments may be effective to maintain postharvest quality and to extend shelf-life of these fruits.

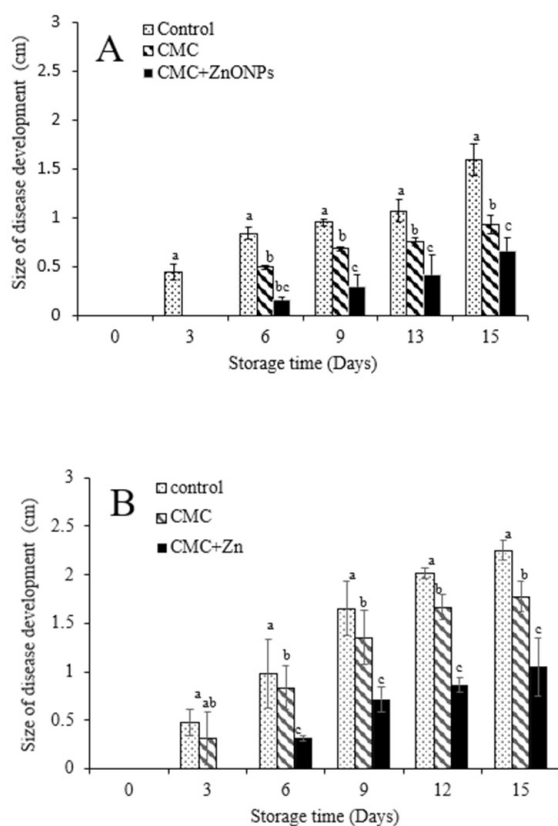


Figure 7: Antifungal activities of coatings against *Alternaria alternata* in persimmon (A) and tomato (B) fruits during storage

Effect of CMC contain ZnONP

Coating with CMC containing ZnONPs affected the qualities such as weight loss, firmness, and respiration rate of persimmon and tomato. The CMC+ZnONPs coating can reduce the weight loss and firmness of fruits because ZnONPs addition on the film or coating decrease water vapor transmission (WVT). Therefore, CMC+ZnONPs film or coating can protect the water loss and extend the shelf life of fruit and vegetable (Li, Xing, Li, Jiang, & Ding, 2010). Furthermore, Koushesh et al., 2017 reported that ZnONPs addition can enhanced semi-permeable barrier of coating to reduce oxygen uptake which

can reduce the respiration rate of fruit and vegetable. The uses of coatings contain ZnONPs considerably reduces apparent changes in fruits such as Chinese jujube, kiwifruit and strawberry (Li et al., 2009; Meng, Zhang, & Adhikari, 2013; Ommol Banin Sogvar, Mahmoud Koushesh Saba, Aryou Emamifar, & Rahman Hallaj, 2016).

CONCLUSIONS

From the results, zinc oxide nanoparticle can be obtained by biosynthesis method using pineapple peel extracts. Zinc oxide nanoparticles showed effective delay of disease development (*A. alternata*) in *in vitro* and *in vivo*. Application of coatings based on CMC and CMC + ZnONPs on persimmon and tomato fruits showed beneficial effect in retarding the ripening behavior, elevated total polyphenols and extending shelf-life (Li et al., 2011; Petriccione et al., 2015; Sagili Jhansi Lakshmi et al., 2018).

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