Coating Satsuma mandarin using grapefruit seed extract–incorporated carnauba wax for its preservation

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Abstract Grapefruit seed extract (GSE)–incorporated carnauba wax (CW) coating was developed to preserve Satsuma mandarins (*Citrus unshiu* Marc.). GSE (1.00%, w/w)–incorporated CW (GSE–CW) coating emulsions and GSE (0.50%)–oregano oil (OO, 0.50%)–incorporated CW (GSE–OO–CW) coating emulsions reduced *Penicillium italicum* disease incidence (%) on mandarin surfaces by 23.6 ± 3.6 and $25.0 \pm 5.0\%$, respectively, relative to that on uncoated mandarin samples (100%). GSE (1.00%)–CW coating emulsions exhibited a higher colloidal stability than GSE (0.50%)–OO (0.50%)–CW coating emulsions. During storage at 25 °C, GSE (1.00%)–CW coating was superior to CW coating in reducing *P. italicum* disease incidence. CW coating significantly reduced weight loss, respiration rate, and firmness loss during storage at 4 and 25 °C ($P < 0.05$). The ascorbic acid concentration and peel color were not affected by GSE–CW coating ($P > 0.05$). These results suggest that GSE–CW coating can extend the post-harvest shelf life of mandarins by inhibiting the growth of *P. italicum*.

Keywords Mandarin · Edible coating · *Penicillium italicum* · Carnauba wax · Grapefruit seed extract

Introduction

Citrus fruits are widely cultivated in tropical and subtropical regions and consumed as fresh fruit, salads, juice, desserts, and preserves, as well as used in cosmetic products [1]. In East Asia, including Korea, China (southern regions), and Japan, Satsuma mandarin (*Citrus unshiu* Marc.) is the most commercially produced citrus cultivar [1]. The annual production and per-capita annual consumption of mandarins in Korea were 672,000 tons in 2015 and 14.3 kg in 2014, respectively [2].

The genus *Penicillium* represents the most frequent fungal contaminant of mandarins [3]. Chemical fungicides, such as sodium-*O*-phenylphenol, thiabendazole, and imazalil; irradiation; and storage in controlled or ozonated atmospheres have been traditionally used for controlling post-harvest decay in citrus fruits [4]. However, the increasing public concern regarding the potential health hazards of irradiation and synthetic fungicide residues, as well as high capital investment in storage facilities with controlled conditions, have promoted the development of new post-harvest processing and preservation technologies.

Previously reported research has demonstrated the preservative effects of biopolymer-based edible coatings on citrus fruits and the formulations of coatings, including polysaccharide-based coatings and shellac-based water wax coatings applied on Valencia oranges [5] and tangerines [6]; hydroxypropyl methylcellulose-lipid edible coatings applied on ‘Clemenule’ mandarins [7] and ‘Oronule’ mandarins [8]; and chitosan-based coatings on ‘Murcott’ tangor [9] and Kinnow [10]. These coatings can cause modification of the internal atmosphere around fresh produce surfaces and can serve as carriers of antimicrobial agents and antioxidants [5]. Carnauba wax (CW), originated from the leaves of the palm *Copernicia prunifera*, is

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a natural edible coating material used for fruit coating to retard moisture loss and impart glossiness [11].

Essential oils, aliphatic aldehydes, and natural plant extracts have been studied as antimicrobial coating materials for post-harvest control in citrus fruits. A chitosan coating incorporating tea tree oil inhibited *Penicillium italicum* growth on oranges [12]. Castillo et al. [13] reported that the application of a commercial wax incorporating essential oil mixture (thymol and carvacrol) was effective in inhibiting the growth of yeast and molds and total aerobes on lemon surface.

Grapefruit seed extract (GSE) derived from *Citrus paradisi* Macf. Rutaceae is known to possess antioxidant, antibacterial, antiviral, and antifungal activities [14]. Although several reports have described biopolymer coating development for fruit preservation and reported the antifungal effects of biopolymer coatings incorporating essential oils or GSE, CW coatings incorporating GSE has not been developed and therefore the effects of the coatings on the preservation of mandarins have not been investigated. Thus, the objectives of this study were (1) to generate coating formulations with CW, GSE, and oregano oil (OO), resulting in effective antifungal activity against *P. italicum* and high colloidal stability, and (2) to study the effects of the CW coatings on *P. italicum* growth and the preservation of physicochemical properties of mandarins during storage at 4 and 25 °C.

Materials and methods

Materials

Greenhouse-grown Satsuma mandarins (*C. unshiu* Marc.) were harvested in Jeju Island, South Korea, in 2016. The harvested mandarins were immediately transported to the laboratory. The average size of the selected mandarins was 60 ± 5 mm in diameter and their average weight was approximately 65 ± 1 g. The selected mandarins were free from mechanical damage or fungal decay. The mandarins were kept at 4 °C until further experiments were performed. We used a commercial CW-based solution (Safe-pack Products, Kfar Saba, Israel) that meets the food-additive regulations of the United States Food and Drug Administration and consists of 18.1% (w/w) CW, 75.8% (w/w) water, 3.8% (w/w) morpholine salts of fatty acids, 1.5% (w/w) white shellac, and 0.8% (w/w) silicon resin. An emulsifier, Tween 80 (polyoxyethylene sorbitan mono-oleate), was purchased from Ilshinwells Co., Ltd. (Seoul, Korea). Pure OO and GSE were purchased from Quinabra (Quimica Natural Brasileira, São José dos Campos, Brazil) and Now Foods (Bloomington, IL, USA), respectively. All coating materials were of food grade.

Preparation of coating emulsions

GSE, OO, or a GSE–OO mixture (1.00%, w/w) was added to the coating solution. The ratios of GSE and OO in the GSE–OO mixture were 0.25:0.75, 0.50:0.50, and 0.75:0.25. Tween 80, used as an emulsifier, was added to the coating emulsions at 25% (w/w GSE or OO). These coating emulsions were prepared using a high-shear probe mixer (Model T25, IKA-Works Inc., Wilmington, NC, USA) for 1 min at 10,000 rpm and degassed under vacuum.

Colloidal stability

The colloidal stabilities of the GSE (1.00%)–CW (GSE–CW) and GSE (0.50%)–OO (0.50%)–CW (GSE–OO–CW) coating emulsions were studied according to the method of Lee et al. [15]. Backscattering profiles of coating emulsions in the entire length of the sample cell (40 mm) were analyzed for 7 days at 23 ± 2 °C, using an emulsion stability analyzer (Turbiscan AGS, Formulacion, Toulouse, France). Turbiscan Stability Index (TSI) was also calculated to evaluate colloid stability of coating emulsions, using the following formula [15]:

$$TSI = \sum_h [scan_i(h) - scan_{i-1}(h)]/h,$$

where h is the sample height in the measurement cell, $scan_i$ is the final backscattering (%), and $scan_{i-1}$ is the initial backscattering (%).

Fungal culture and preparation of inoculum

Penicillium italicum (KACC 40826), a strain isolated from Mandarin, was obtained from the Rural Development Administration-Genebank Information Center (Jeonju, Korea). The stock culture was maintained on potato dextrose agar (PDA, Difco, Sparks, MD, USA) slants at 4 °C. Fresh cultures were grown on PDA plates at 25 °C before use. Spore suspensions were prepared by collecting spores from 5-day-old cultures and filtered through a single layer of cheesecloth to remove fungal mycelium. The filtrate was centrifuged at $4000 \times g$ for 15 min (GyroSpin, Gyrozen, Seoul, Korea), washed twice, and suspended in 0.1% peptone water. Spores were enumerated with a hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany) and adjusted to $9.0 \log$ spores/mL. Spore suspensions were diluted to the desired concentration ($\sim 6.0 \log$ spores/mL) for inoculation.

Fungal inoculation and coating

Uniformly sized mandarins without physical injuries and signs of fungal infection were selected. On the day of the experiment, selected mandarins were lightly washed with

70% ethanol for 1 min, rinsed with running tap water for 1 min and sterile distilled water for 3 min, and air-dried for 1 h on a clean bench (HB-402; Hanbaek Co., Ltd., Bucheon, Korea). Mandarins were inoculated with *P. italicum* as described by Won et al. [16]. Needle punctures were performed on the sample surfaces, and 20 μ L of spore suspension (~ 6.0 log spores/mL) was spotted into the puncture (approximately a 1-mm depth). For coating, mandarin samples were immersed in the prepared coating emulsion for 30 s and allowed to dry for 1 h at 23 ± 2 °C and $26 \pm 3\%$ relative humidity (RH). After drying, the integrity of the coating on each mandarin was visually inspected. If uncoated areas were found, additional coating emulsion was applied across the entire surface. Mandarin coating was made before (C + I) or after (I + C) inoculating with the spores. The C + I treatment simulated a situation in which coating was made on mandarins before contamination (post-process contamination), and the I + C treatment simulated coating mandarins after contamination [17]. The thickness of the coating was approximately 0.1 mm, determined based on weight measurements before and after coating. Each sample was packaged in a sterile stomacher bag (710 mL, Nasco Whirl-Pak®, Fort Atkinson, WI, USA) and stored at 25 °C and 75–80% RH for 7 days. Fungal disease incidence rates were expressed as a percentage of mandarins showing fungal decay symptoms (visible mycelial growth) over the initial number of the mandarins.

Storage study

Among the various coatings (Table 1), GSE (1.00%)–CW coating was selected for the storage study. The antifungal effects of coating against *P. italicum* as well as the physicochemical properties, including weight loss, CO₂ concentration, firmness, and ascorbic acid concentration, were analyzed during storage at 4 and 25 °C for 7 or 28 days. Each mandarin sample was placed in a sterile bag (710 mL; Nasco Whirl-Pak®). The RH in mandarin sample-containing bags during storage at 4 and 25 °C was 80–85%, as determined using a data logger (Testo 174H, Testo AG, Lenzkirchen, Germany).

Weight loss

The weight of individual mandarins of each sample type was recorded on each sampling day, and the same samples were weighed each time on the storage day at 4 and 25 °C. The weight loss was calculated as percentage loss relative to the initial mass. The precision balance used for the weight measurements had an accuracy of 0.01 g.

Respiration rate

The respiration rate of the fruits was measured according to the method of Won et al. [16]. Four mandarin fruits were loaded into a gas container (1.5 L volume). After equilibrating in the container for 1 h at 23 ± 2 °C, CO₂ and O₂ levels (%) in the headspace were analyzed at each sampling time, using a portable gas analyzer (Check Point 2, PBI

Table 1 Effects of carnauba wax (CW) coating incorporating grapefruit seed extract (GSE) or oregano oil (OO) on the inhibition of *P. italicum* on mandarin peels

Coating	Treatment	Disease incidence (%)
No coating	C + I ¹	100 $\pm$ 1.1a ³
	I + C ²	100 $\pm$ 0.0a
CW coating (CW only)	C + I	75.0 $\pm$ 5.0b
	I + C	80.0 $\pm$ 0.0b
GSE (1.00%)–CW coating	C + I	23.6 $\pm$ 3.6g
	I + C	30.0 $\pm$ 1.0fg
OO (1.00%)–CW coating	C + I	37.5 $\pm$ 7.5def
	I + C	31.7 $\pm$ 0.3efg
GSE (0.50%)–OO (0.50%)–CW coating	C + I	25.0 $\pm$ 5.0g
	I + C	36.9 $\pm$ 2.0def
GSE (0.75%)–OO (0.25%)–CW coating	C + I	55.0 $\pm$ 5.0c
	I + C	41.7 $\pm$ 8.4de
GSE (0.25%)–OO (0.75%)–CW coating	C + I	52.5 $\pm$ 7.5c
	I + C	45.0 $\pm$ 15cd

¹Inoculation after coating on fruits

²Inoculation before coating on fruits

³Different letters within a column are significantly different ($P < 0.05$)

Dansensor, Ringsted, Denmark). An injection needle connected to a built-in pump was used to withdraw gas (10 cm³) from the container headspace, which was subsequently analyzed.

Firmness

Firmness was evaluated as the maximum force required to penetrate the whole mandarin fruit [16]. A texture analyzer (TX-Xt express 2007, Stable Micro Systems Ltd., Surrey, UK) with a cylindrical probe of 5-mm diameter was used, under the following conditions: a pre-test speed of 3.0 mm/s, a test speed of 1.0 mm/s, a post-test speed of 3.0 mm/s, and a penetration distance of 3 mm.

Ascorbic acid concentration

The ascorbic acid concentration (mg/g) in mandarin flesh was measured using a high-performance liquid chromatography system (LC-10ATvp, liquid chromatograph, Shimadzu Co., Kyoto, Japan) [16]. The mobile phase was 2% (v/v) acetic acid in acetonitrile (95:5 v/v). A Symmetry[®] C18 column (5 µm, 4.6 mm × 250 mm I.D., Waters Co., MA, USA) was placed in a column oven (Shinkwang Scientific Co., Taipei Hsien, Taiwan) set at 23 ± 2 °C. Mandarin juice was obtained from peeled mandarin flesh and blended using a food processor (Philips HR-1372, Koninklijke Philips Electronics N.V., Amsterdam, Netherlands). Mandarin juice samples were prepared by filtering the blended juice through four layers of cheesecloth. The samples (20 µL) were prepared by filtering the supernatant of mandarin juice obtained after centrifugation at 12,500×g for 10 min (23 °C). A syringe filter (Dismic[®]-25CP, cellulose acetate, pore size: 0.45 µm, Advance MFS, Inc., CA, USA) was used for filtration. The effluent was monitored using an ultraviolet–visible detector at 254 nm.

Color

The mandarin peel color was measured using a colorimeter (Chroma Meter CR-400; Minolta Camera Co., Osaka, Japan) and evaluated in terms of L^* (lightness), a^* , and b^* values or/and hue (h°), based on CIELAB coordinates. The mandarin peel color was recorded in terms of the L^* and h° values. The colorimeter was calibrated using a white standard tile (Illuminate D 65) and a 10° standard observer. The h° was calculated from L^* , a^* , and b^* values, as described below.

$$h^\circ = (\arctan[b^*/a^*]/2\pi) \times 360$$

Experimental design and statistical analyses

All experiments were performed in duplicate and were analyzed three times per sample, with each replicate. Ten samples per treatment were used for the *P. italicum*-inhibition study. Three measurement samples were used to determine weight loss, CO₂ concentration, firmness, and ascorbic acid content in each replicate. Color coordinates were recorded five times for each sample. Analysis of variance was performed to evaluate differences among means; in cases of statistical significance, means were evaluated using Duncan's multiple range test for significant differences ($\alpha = 0.05$). PASW statistics software (version 18.0.0; IBM SPSS Inc., New York, NY, USA) was used for all statistical analyses.

Results and discussion

Inhibitory activity of coatings against *P. italicum*

The inhibitory effects of the coating emulsions against *P. italicum* inoculated on coated mandarins are presented in Table 1. All coatings exhibited higher inhibitory activity against *P. italicum* compared to that in untreated mandarins ($P < 0.05$). Even the CW coating without external antimicrobials exhibited antifungal activity in both C + I and I + C treatments. The antifungal effect of CW might be caused by lytic enzymes in CW, such as chitinase and β -1,3-glucanases, capable of inhibiting the early growth of *Fusarium oxysporum*, *Colletotrichum lindemuthianum* and *Colletotrichum musae* [18]. Abd-Alla et al. [19] reported that the decay incidences of tomato fruits dipped in CW for 2 h were 35.6% for *Botrytis cinerea*, 32.9% for *Rhizopus stolonifera*, and 30.0% for *Alternaria alternate*, compared to uncoated fruits (100%).

GSE- and OO-incorporated CW coatings significantly inhibited *P. italicum* growth compared to the CW coating ($P < 0.05$). GSE or OO contained in the coating appeared to effectively inhibit the growth of *P. italicum* by diffusing on the coating surface. Previous reports have described the antifungal activities of GSE and OO. For example, Xu et al. [20] reported that intact grape berries treated by immersion in 0.5% GSE solution for 1 min showed a 9.3% incidence of *Botrytis cinerea* infection after 7 day of storage (25 °C), while this incidence with untreated grape berries was 75%. The antimicrobial activities of GSE could be attributed to its high concentration of flavonoids and its derivatives, including naringin, hesperidin, limonoid, quercetin, and kaempferol [21]. Cho et al. [22] confirmed the bactericidal activity of GSE using transmission electron microscopy, which damaged microbial cell membranes. Additionally, Xu et al. [20] demonstrated that the

germination rate of *Botrytis cinerea* spores exposed to 0.5% GSE for 1 min was reduced by 14%. Previous reports have shown that the antimicrobial effects of OO on various fruits and vegetables were higher when OO was used in a mix with other antimicrobials than when OO was used alone [23, 24]. The GSE and OO were mixed at different ratios and examined for a potential improved antifungal effect by mixing. The ratio of GSE to OO, 1:1, which was used in the formulation of the GSE (0.50%)–OO (0.50%)–CW coating, resulted in a higher inhibition than that was observed with the OO (1.00%)–CW coating when C + I treatment was applied (Table 1), agreeing with the previous reports. The results suggest that the antifungal effects depended on the ratio of the amounts of antimicrobials. There might be an optimum ratio of GSE and OO amounts in exhibiting the highest antifungal effect.

The inhibitory effects against innate *P. italicum* spores before packaging and against *P. italicum* infected after packaging were tested by the C + I and I + C treatments, respectively. Both C + I and I + C treatments effectively inhibited *P. italicum*. With the C + I treatment, *P. italicum* growth may have been inhibited after the antimicrobial compounds (GSE or OO) diffused from the coating emulsion to the smooth coating surface, with the coating creating an environment that made it difficult for hyphae of *P. italicum* to settle. With I + C treatment, the growth of *P. italicum* could be inhibited by the action of the incorporated antimicrobial compounds, GSE and OO, and the formation of oxygen barrier between the fruits and their immediate surroundings [25]. Additionally, the growth inhibition might have resulted from a failure of *P. italicum* to secure sufficient space for hyphal extension and proliferation. However, no significant differences between C + I and I + C-treated samples were found in terms of the disease-incidence rate with each coating ($P > 0.05$), except for the GSE (0.50%)–OO (0.50%) and GSE (0.75%)–OO (0.25%)–CW coatings. Therefore, the prepared antimicrobial coatings were effective not only in preventing post-harvest contamination, but also in inhibiting the growth of pre-existing *P. italicum* spores on the mandarin surface.

Colloidal stability

Colloidal stability was evaluated in emulsions formed with GSE (1.00%)–CW and GSE (0.50%)–OO (0.50%)–CW coatings, which appeared to represent the most effective coatings against *P. italicum* growth. Figure 1 presents a representative graph of the height-dependent backscattering of the GSE–CW coating emulsion (A) or the GSE–OO–CW coating emulsion (B). The GSE–CW coating emulsion was more stable than GSE–OO–CW, as judged by the lack of change in backscattering, and did not form creaming, which was studied at the top layer (38–40 mm height from

the bottom of the sample cuvette). These results can be associated with the solubility of GSE in CW solution. GSE contains many aliphatic compounds [26]. CW contained 82% wax esters, mostly present in the forms of aliphatic co-hydroxy, aliphatic cinnamic, and aliphatic diesters [27]. GSE could be evenly distributed in the CW solution due to abundance of aliphatic compounds in both GSE and CW. In contrast, backscattering of the GSE–OO–CW coating resulted in creaming, as demonstrated by a peak with the top layer [Fig. 1(B)]. A decrease in colloidal stability was also demonstrated with increased TSI [28]. The rate of TSI increase in the GSE–OO–CW coating emulsion was higher than the rate of GSE emulsion, especially after day 3 in storage [Fig. 1(C)]. An unstable emulsion solution is not suitable to form a coating barrier on food surfaces due to considerable variation in the thickness of the coating formed from the emulsion, as well as blistering and cracking generated by the instability of coating-forming solution [29]. Food applications of coating emulsions with stable disperse systems provide a high barrier against gas, volatile compounds, and moisture on the food surface; thus, GSE–CW coating could improve the quality attributes of coated fruits, which can enhance mandarin market value and consumer's preferences.

Effects of coating on *P. italicum* growth during storage

To investigate the effects of coating on *P. italicum* growth during storage, C + I treatment with GSE (1.00%)–CW coating was chosen due to its relatively high antifungal activity with a high colloidal stability. The inhibitory activities of CW coatings with and without GSE on *P. italicum* inoculated on mandarin peel are shown in Fig. 2. During storage at 4 °C, no incidence of disease symptoms in coated mandarin samples appeared until day 21 (Fig. 2). The GSE–CW-coated mandarins showed significantly higher antifungal activity than CW-coated and uncoated mandarins after day 5 at 25 °C ($P < 0.05$). The difference in the microbial counts of CW-coated and GSE–CW-coated samples was more pronounced at 25 °C than at 4 °C. This may have been due to a faster diffusion of the GSE antimicrobials at a higher temperature. Previously, Lee et al. [30], reported change of antimicrobial activity by temperature within coating emulsion due to difference in diffusion coefficient, in which the diffusion coefficient of antimicrobial substance (hypothiocyanite ion) contained in the defatted soybean meal film increased as temperature increased from 5 to 22 °C.

Fig. 1 Backscattering (BS) profiles of the carnauba wax (CW) coating incorporating grapefruit seed extract (GSE, 1.00%) (A) and GSE 0.50% and oregano oil (OO) 0.50% (B), and the variation in BS (Turbiscan Stability Index, TSI) of CW coating emulsions incorporating GSE (1.00%) and both GSE (0.50%) and OO (0.50%) in the 0–40 mm zone at 25 °C for 7 days (C)

Fig. 2 Effects of carnauba wax (CW) coating incorporating grapefruit seed extract (GSE, 1.00%) on the inhibition of *P. italicum* on mandarin peels during storage at 4 and 25 °C. Uncoated (4 °C); open circle, carnauba wax-coated (4 °C); open triangle, GSE-carnauba wax-coated (4 °C); open square, uncoated (25 °C); filled circle, carnauba wax-coated (25 °C); filled triangle, GSE-carnauba wax-coated (25 °C); filled square

Effects of coating on weight loss during storage

The effects of coating on mandarin weight loss are shown in Fig. 3(A). The weight loss of all mandarin samples at 4 °C was maintained without significant changes between groups ($P > 0.05$). No morphological changes such as wilting and pitting generated by chilling injury were visually observed with any mandarins. At 25 °C, a dramatic increase in the weight loss of uncoated mandarins appeared after day 3. These results could be explained by the increased metabolic activity of mandarins, tissue senescence, and moisture loss during storage at 25 °C. However, the weight loss rates of coated mandarins were significantly lower than those of uncoated mandarins after day 3 of 25 °C storage ($P < 0.05$). The reduced weight loss could be due to a reduction in moisture loss [17]. No significant difference between CW and GSE–CW coating was observed on the same day of sampling during storage at 25 °C ($P > 0.05$). Thus, the results suggest that CW coatings with or without GSE acted as a physical barrier to minimize weight loss of mandarins and the incorporation of GSE into coatings had no effect on weight loss.

Effects of coating on respiration during storage

Changes in the CO₂ concentration inside sample containers, representing the respiration rate of mandarins are demonstrated in Fig. 3(B). The CO₂ concentrations of uncoated mandarins at 4 °C showed higher levels than those of coated mandarins on day 28 [Fig. 3(B)] ($P < 0.05$), indicating that the coatings decreased the

respiration rate of mandarins stored at 4 °C. The coated mandarins maintained a similar CO₂ concentration throughout the entire storage period. At 25 °C, the coated mandarins had lower CO₂ concentrations than uncoated mandarins ($P < 0.05$). Such a reduction in the respiratory rate may be attributable to blocking oxygen influx by the wax barrier formed by coating [31]. Limited gas exchange due to the coating treatment could extend the shelf life by delaying the maturation of mandarins during storage. Nonetheless, GSE–CW-coated mandarins showed no significant difference in CO₂ concentrations compared to CW-coated mandarins at both temperatures, indicating that the GSE incorporation into the wax coating did not influence the respiration of the wax-coated fruit.

Effects of coating on firmness during storage

The firmness of all kinds of mandarin samples decreased during storage [Fig. 3(C)]. These changes in fruit firmness are reported to be correlated with reduced cellular turgor pressure resulting from structural degradation in fruit cell walls and water loss during fruit maturation [32]. At 4 °C, no significant difference in firmness was found among the coated and uncoated groups after storage for 2 weeks ($P > 0.05$), but higher firmness was exhibited with coated mandarins fruits after day 21. Retardation of firmness loss demonstrated by the coatings might be induced by potential carbon dioxide accumulation and oxygen depletion inside coating by increase respiration of the fruit, which can lower the activity of enzymes that responsible for structural degradation in fruit cell walls, including polygalacturonase, pectin methylesterase, pectate lyase, β -galactosidase, and cellulase [32].

The effect of coating in reducing the firmness loss was demonstrated earlier at 25 °C than 4 °C [Fig. 3(C)]. This could be due to rapid decrease in the firmness of uncoated fruits at 25 °C. Activity of the structure-degrading enzymes and moisture loss in the samples might increase at higher rates at 25 °C than 4 °C. Dramatic increase in weight loss at 25 °C compared to 4 °C was demonstrated in this study [Fig. 3(A)]. Nonetheless, the firmness values of the mandarin samples were not differed by the incorporation of GSE in the coating formulation during storage at both 4 and 25 °C, suggesting that GSE incorporation does not affect the fruit firmness.

Effects of coating on peel color during storage

Table 2 shows the effect of coating on the L^* and h° values of mandarin peels during storage at 4 and 25 °C. The L^* and h° were measured in the range of 59.6–65.2 and 3446.2–3610.7, respectively. Regardless of the storage temperature and period, no significant differences in L^* and

Fig. 3 Effects of carnauba wax (CW) coating incorporating grapefruit seed extract (GSE, 1.00%) on the weight loss (A), CO₂ concentration (B), and firmness (C) of mandarin during storage at 4 and 25 °C. Uncoated (4 °C); open circle, carnauba wax-coated (4 °C); open triangle, GSE-carnauba wax-coated (4 °C); open square, uncoated (25 °C); filled circle, carnauba wax-coated (25 °C); filled triangle, GSE-carnauba wax-coated (25 °C); filled square

Table 2 Effects of carnauba wax (CW) coating incorporating grapefruit seed extract (GSE, 1.00%) on color of mandarin peels during storage 4 and 25 °C

Storage temperature (°C)	Storage time (days)	<i>L</i> *			Hue angle		
		Uncoated	CW-coated	CW-GSE-coated	Uncoated	CW-coated	CW-GSE-coated
4	0	63.4 ± 1.1ab	64.3 ± 1.3ab	59.6 ± 11.9b	3535.7 ± 43.4a	3505.3 ± 70.5a	3590.0 ± 75.9a
	3	62.3 ± 1.2ab	62.5 ± 2.1ab	64.9 ± 1.8a	3461.5 ± 62.9a	3520.2 ± 40.0a	3579.3 ± 37.4a
	7	62.3 ± 1.6ab	62.4 ± 0.7ab	63.2 ± 0.9ab	3610.7 ± 47.8a	3548.6 ± 18.6a	3495.8 ± 23.4a
	14	63.4 ± 1.3ab	64.4 ± 1.3ab	63.3 ± 0.8ab	3527.2 ± 33.9a	3545.6 ± 34.1a	3552.2 ± 55.5a
	21	64.4 ± 0.6ab	65.2 ± 1.5a	65.1 ± 0.7a	3540.9 ± 21.0a	3441.2 ± 134.6a	3570.3 ± 143.5a
25	0	63.4 ± 1.1ab	64.3 ± 1.3ab	59.6 ± 11.9b	3535.7 ± 43.4a	3505.3 ± 70.5a	3590.0 ± 75.9a
	1	63.7 ± 1.7b	63.2 ± 0.8b	64.6 ± 0.3b	3494.9 ± 87.8a	3448.1 ± 39.7a	3572.3 ± 28.9a
	3	64.7 ± 1.7ab	62.3 ± 1.2ab	63.3 ± 1.4ab	3504.0 ± 84.7a	3432.8 ± 31.1a	3591.7 ± 56.0a
	5	65.0 ± 7.1ab	62.8 ± 1.4ab	63.6 ± 1.1ab	3446.2 ± 72.5a	3422.0 ± 56.9a	3452.1 ± 54.9a
	7	64.2 ± 1.8ab	63.3 ± 2.4ab	64.0 ± 0.9ab	3542.8 ± 168.6a	3519.3 ± 34.3a	3581.9 ± 37.5a

a, b Different letters indicate a significant difference ($P < 0.05$) within the same parameters

h° values were found between the uncoated and coated samples ($P > 0.05$). Most citrus peels contain high levels of pigments, such as chlorophylls and carotenoids [33]. Such pigments in most citrus fruits undergo various changes during ripening and senescence. Among them, chlorophyll pigments in citrus peels gradually disappear during maturation, while carotenoid pigments gradually accumulate. Accumulation of sugars, such as sucrose, also occurs during ripening, which can promote color break of citrus peel and thus the change of peel color [34]. However, no significant change in color of mandarins was observed in the present study. This finding may be due to the fact that fully ripened mandarins were used as samples, so that maturation of mandarin peel had already progressed.

Figure 4 shows the effects of mandarin coatings on the ascorbic acid content. During storage at 4 °C, ascorbic acid contents were not significantly different among samples until day 21 ($P > 0.05$), but coated mandarins had higher ascorbic acid contents than uncoated mandarins on day 28. At a storage temperature of 25 °C, the ascorbic acid contents of all mandarin samples dramatically decreased by day 7 ($P < 0.05$). Compared to that in the uncoated mandarins, the effect of coating on ascorbic acid contents at 25 °C became apparent beginning at day 3, indicating that CW coating effectively preserved the ascorbic acid, regardless of the GSE content. This result suggests that CW coating blocked the inflow of oxygen into the flesh during storage, thereby inhibiting ascorbic acid oxidation.

GSE addition to CW coatings could raise the colloidal stability and the GSE–CW coating was more effective in decreasing fungal disease caused by *P. italicum* on the mandarin surface compared to CW coatings without

Fig. 4 Effects of carnauba wax (CW) coating incorporating grapefruit seed extract (GSE, 1.00%) on the ascorbic acid concentration of mandarin flesh during storage 4 and 25 °C. Uncoated (4 °C); open circle, carnauba wax-coated (4 °C); open triangle, GSE-carnauba wax-coated (4 °C); open square, uncoated (25 °C); filled circle, carnauba wax-coated (25 °C); filled triangle, GSE-carnauba wax-coated (25 °C); filled square

antifungal agents. Most coating formulations containing GSE and/or OO demonstrated no significant difference in *P. italicum* inhibition between the I + C and C + I treatments. GSE–CW coating effectively inhibited *P. italicum* growth during storage at 4 and 25 °C where the inhibitory effect was more obviously demonstrated at 25 °C than 4 °C. The results obtained in this study demonstrate that GSE–CW coating is an effective postharvest technology

for protecting mandarins from *P. italicum* without altering the physicochemical properties of mandarins.

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