Effect of carboxymethyl cellulose coating enriched with clove oil on postharvest quality of ‘Xinyu’ mandarin oranges

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Abstract – Introduction. Attempts to exploit natural preservatives to control postharvest diseases as an alternative technology to synthetic fungicides in citrus fruit have been drawing much attention. Materials and methods. The combined effects of carboxymethyl cellulose (CMC) coating and/or clove oil on the qualitative properties of cold-stored ‘Xinyu’ mandarin oranges stored at 5 °C for 120 days were investigated. Results and discussion. The results showed that the addition of clove oil as an antifungal component to the CMC coating had a good effect on the inhibitory growth of fungal decay, and the coating treatments significantly decreased the decay rate and weight loss, as well as the maleic dialdehyde (MDA) concentration compared with control samples. The clove oil-carboxymethyl cellulose (CO-CMC) coating significantly maintained commercial quality and inhibited respiration. Meanwhile, the results showed that the activities of superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) in fruit treated with CO-CMC coating were higher than in those treated with CMC coating and control samples. Conclusion. The CO-CMC coating has good potential for application as an alternative to synthetic fungicides for improving postharvest quality and prolonging the shelf life of ‘Xinyu’ mandarin oranges during cold storage.

Keywords: China / mandarin / Citrus reticulata / CMC / postharvest quality / integrated disease management / clove oil / enzyme activity / shelf life

Résumé – Effet de l’enrobage au carboxyméthyl de cellulose enrichi avec de l’huile de clou de girofle sur la qualité des mandarines ‘Xinyu’ après récolte. Introduction. Les tentatives visant à exploiter les conservateurs naturels ont beaucoup attiré l’attention dans la perspective de contrôler les maladies sur agrumes après récolte comme technologie alternative aux fongicides de synthèse. Matériel et méthodes. Les effets combinés du carboxyméthyl de cellulose (CMC) en enrobage avec ou sans huile de clou de girofle ont été étudiés sur les propriétés qualitatives des mandarines ‘Xinyu’ stockées à 5 °C pendant 120 jours. Résultats et discussion. L’ajout d’huile de clou de girofle comme composant antifongique dans l’enrobage au CMC a eu un effet positif sur l’inhibition de croissance de la pourriture fongique, et les traitements d’enrobage ont diminué de manière significative le taux de décroissance, la perte de poids, ainsi que la teneur en dialdéhyde maléique (MDA) par rapport aux échantillons témoins. La combinaison huile de clou de girofle-CMC a sensiblement maintenu la qualité commerciale et inhibé la respiration. Dans le même temps, les activités enzymatiques de la superoxyde dismutase (SOD), de la catalase (CAT), de la polyphénol oxydase (PPO) et de la phénylalanine ammonialyase (PAL) étaient plus élevées au sein des fruits enrobés (huile de clou de girofle-CMC) que dans les échantillons enrobés au CMC seul ou dans ceux du témoin. Conclusion. L’enrobage huile de clou de girofle-CMC présente un fort potentiel d’utilisation comme alternative aux fongicides de synthèse pour améliorer la qualité post-récolte et prolonger la durée de conservation des mandarines ‘Xinyu’ pendant le stockage au froid.

Mots clés : Chine / mandarine / Citrus reticulata / CMC / qualité post-récolte / gestion intégrée des maladies / activité enzymatique / durée de conservation

1 Introduction

‘Xinyu’ mandarin oranges (Citrus reticulata Blanco cv. Tangerine), which are well known for their uniform color, consistent size, delicate flesh, rich juice, less dregs and delicious taste, are among the economically important fruits endemic to Xinyu City in China [1]. This is one of the main citrus cultivars in Jiangxi province, and is popularly known as ‘Nanfeng’ mandarin. However, these citrus fruits ripen early in November; the rapid postharvest physiological changes account for a short harvest period, and pose great challenges for
both picking and marketing [2]. Quality loss, including the losses of nutrient substance, water and disease resistance, are the most important characteristics indicating the deterioration of ‘Xinyu’ mandarin orange; these changes directly affect the quality of the fruit, as well as their storability, transportability and marketability.

At present, citrus fruits destined for long-term storage generally rely mainly on the use of chemical fungicides, especially imazalil, prochlordaz, thiabendazole, fludioxonil, pyrimethanil or different mixtures of these compounds. However, their use is increasingly restricted because of concerns regarding the potentially harmful impact on human health, environmental pollution, and the development of fungicide-resistant pathogens. Therefore, safer and more eco-friendly alternative treatments of citrus postharvest diseases have become an urgent need for controlling postharvest decay of citrus fruit.

Clove oil, distilled from the flower buds of the clove (Eugenia caryophyllata Thunb.), is widely used and well known for its antioxidant, antimicrobial, antifungal and antiviral activity [3], and has been listed as a “generally regarded as safe” substance by the United States Food and Drug Administration (FDA) when administered at dosages not exceeding 1,500 ppm in all food categories [4]. The main chemical compositions of clove oil are eugenol (76.8%), β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%) [3], which are natural and harmless for people and the environment. Clove oil and its active substances have been tested for inhibitory activity against Penicillium italicum [5], Penicillium digitatum [6], Laetiporus sulphureus [7], Aeromonas hydrophila and Enterococcus faecalis [8].

Carboxymethyl cellulose (CMC) from sugar beet pulp cellulosic is the most important water-soluble cellulose derivative that has received a great deal of attention, with several examples of applications in many fruits and vegetables. A novel edible coating formulation based on CMC and other coalescing agents has, for instance, been applied to strawberries [9], jujubes [10], pears and peaches [11], and many other fruits. Although polysaccharide film and/or essential oil were applied to several fruits and vegetables, no data are available of CMC coating enriched with clove oil and their effect on the quality of ‘Xinyu’ mandarin orange, to the best of our knowledge. The objectives of this study were to investigate the effects of CMC enriched with clove oil on quality properties and pertinent enzyme activities of ‘Xinyu’ mandarin oranges during storage at 5 °C for 120 days.

2 Materials and methods

2.1 Fruit material

The ‘Xinyu’ mandarin oranges (Citrus reticulata Blanco cv. Tangerine) used throughout this study were harvested from a local orchard located in the Yushui district of Xinyu City (Jiangxi Province, China). The fruit was picked on the basis of consistent size and uniform color, and without bruises or disease. Then, the fruit was immediately carried back to the Jiangxi Key Laboratory for Postharvest Technology and Nondestructive Testing of Fruits and Vegetables within 2 h.

2.2 Evaluation of antifungal activity

2.2.1 Plant pathogens

The Penicillium italicum, Penicillium digitatum, Geotrichum candidum var. citri-aurentii and Alternaria citri used in this investigation were provided by the Jiangxi Key Laboratory for Postharvest Technology and Nondestructive Testing of Fruits and Vegetables (Nanchang, China). All the test strains were maintained on potato dextrose agar (PDA) plates at 4 °C to maintain their pathogenicity. The concentration of the Penicillium spore suspensions (10⁵ spore mL⁻¹) was determined with the aid of a hemocytometer.

2.2.2 In vitro antifungal activity assay of clove oil

The antifungal activity assay of clove oil was determined by using a modified agar-well diffusion assay method with slight modifications. Briefly, clove oil was dissolved in sterile distilled water containing 0.1% (v/v) Tween 80, and then added to the sterile culture medium (PDA) at the specified concentrations selected based on the preliminary experiments done in the laboratory. Following thorough mixing, the media were poured into Petri dishes (90 mm). Thereafter, the agar-mycelial plugs (6 mm diameter) infected with fungi were placed in the center of each PDA plate, and then the Petri dishes were sealed with parafilm and incubated at 25 °C in the dark. Colony growth diameters were measured until the fungal hyphae reached the edge of the control plate without any additives. The experiments were carried out with three replicates per treatment and 5 plates were used in each replicate. The minimum inhibitory concentration (MIC) was established as the lowest concentration that resulted in no visible growth of the tested fungi incubated at 25 °C for 48 h [12]. The antifungal activity of clove oil was expressed as percentage inhibition of mycelial growth (IMG) using the following formula:

\[
IMG(\%) = \frac{(dc - dt) \times 100}{(dc - 6)}
\]

where \(dc\) and \(dt\) were the averages from three replicates of mycelium diameters (mm) of the control and the treatment, respectively.

2.2.3 In vivo antifungal activity assay of clove oil

The antifungal assay of clove oil against P. italicum in vivo on citrus fruit was evaluated according to the modified method of Xu et al. [13] (fruit pathology test). The selected fruit of the same size and color, without any injuries or infections, were dipped in 1% sodium hypochlorite solution for 2 min, rinsed with sterile water, and air-dried before wounding.

Fruit was wounded in the equatorial region with a sterile puncher to generate a uniform wound (4 mm diameter, 2 mm deep) and then divided randomly into six groups and placed in containers (35 cm × 26 cm white plastic boxes). A volume of 15 μL clove oil at 0.5, 1.0, 2.0, 4.0 and 8.0 mL L⁻¹, along with sterile distilled water as the control, were pipetted
into individual wounds. After 30 min, 15 μL spore suspension of P. italicum (5 × 10⁴ spore mL⁻¹) was inoculated into each wound. The lesion diameters of decayed fruits were recorded after 7 days of incubation at 25 °C, with the formula:

\[
\text{Inhibition (\%)} = \left(\frac{\text{lesions diameters of control} - \text{lesions diameters of treatment}}{\text{lesions diameters of control}}\right) \times 100
\]

Ten fruits were used in a treatment and each treatment was carried out with three replicates.

2.3 Preparation of coating and treatment

CMC solution (1.0%, w/v) was prepared by dissolving 10.0 g CMC powder in 1,000 mL distilled water, containing 0.07% citric acid, 1.0% sucrose esters and 1.0% calcium propionate, with agitation for 8 h. All the film-forming aids were food-grade. Five mL clove oil (Jian Shenada Perfume Ltd., Jiangxi, China) was added to the coating, with agitation for 1 h.

The selected fruits were washed with tap water and air-dried at room temperature (25 ± 1 °C), then coated by dipping in the CMC coating and/or clove oil for 2 min, while the control group was washed and left without coating. After drying, the coated as well as control fruits were film-packaged individually, and then pre-cooled (10 °C, 12 h). Finally, all fruits were stored at 5 °C and 90% RH.

2.4 Measurement of weight loss and decay rate

A total of 150 fruits were divided randomly into three groups to measure the weight loss and decay rate. Ten fruits per treatment were used to measure weight loss, and another 40 fruits were used to measure the decay %, respectively. The weight loss of the samples was measured during storage every 20 days and compared with the initial weight. The decay % was expressed by the percentage of fruits indicating fungal disease.

2.5 Determination of fruit quality

The total soluble solid (TSS) concentration in the pulp was assayed by a RA-250 WE digital Brix meter (KYOTO, Tokyo, Japan) and the value was expressed in °Brix. The titratable acidity (TA) in the pulp (5.0 g) was titrated to pH 8.1 with 0.1 N NaOH and expressed as a percentage of citric acid. For the analysis of vitamin C, pulp tissue (4.0 g) was well homogenized with 20 mL of 2% (w/v) oxalic acid and centrifuged at 12,000 g at 4 °C for 10 min. The vitamin C (VC) concentration in the supernatant was titrated with a standard 2,6-dichlorophenolindophenol and the results were expressed as mg ascorbic acid per 100 g fresh fruit pulp.

2.6 Measurement of MDA concentration and respiration rate

Peel tissue (2.0 g) from 10 fruits in each treatment was homogenized in 10 mL of 50 mM phosphate (PBS) buffer at pH 7.8 and then centrifuged at 12,000 g for 20 min. The maleic dialdehyde (MDA) concentration was measured according to the method of Hodges et al., with slight modifications [14]. The supernatant was collected and 2.5 mL was mixed with 2.5 mL of 0.5% thiobarbituric acid. The mixture was heated to 100 °C for 30 min, quickly cooled and centrifuged at 6,000 g for 10 min. The supernatant was collected to test absorbance at 450, 532 and 600 nm. The MDA concentration (mmol g⁻¹ FW) was calculated according to the formula:

\[
\text{MDA} = \left[6.452 \times (A_{532} - A_{600}) - 0.559 \times A_{450}\right]
\]

The measurement of the CO₂ concentration was made on 10 fruits from each treatment, which were placed in an airtight Plexiglas jar with a metal probe in the headspace for 2 h at 25 °C prior to gas sampling. The CO₂ concentration was recorded by a GHX-3051H infrared CO₂ fruit and vegetable breathing apparatus (Jingmi Scientific LLC., Shanghai, China). The respiration rate was expressed as mg CO₂ kg⁻¹ h⁻¹ fresh weight (FW) and calculated by using the following equation:

\[
\text{Respiration rate} = \frac{\Delta \text{CO}_2}{100} \cdot \frac{V_{\text{headspace}}}{m} \cdot 1000 \cdot \frac{60}{t}
\]

where \(m\) is the mass of Xinyu mandarin oranges (g), \(V_{\text{headspace}}\) is the empty volume of the jar (mL), \(\Delta \text{CO}_2\) is the difference between the initial and final concentration of CO₂, and \(t\) is the recording time (min).

2.7 Measurement of enzyme activity

All steps in the proposal for crude enzymes were carried out at 4 °C. Peel samples (2 g) from 10 fruits in each treatment were homogenized in 25 mL of ice-cold 50 mM phosphate buffer at pH 7.8, containing 0.5 mM ascorbic acid, 1 mM ethylenediaminetetraacetic acid (EDTA) and 2% polyvinylpyrrolidone (PVP, m/V) and centrifuged at 12,000 g for 30 min at 4 °C. For polyphenol oxidase (PPO), a 1-g peel sample was extracted with 100 mM ice-cold sodium acetate buffer at pH 5.5, containing 1 mM polyethylene glycol (PEG), 4% PVP (m/V) and 1% Triton X-100 (m/V). To measure phenylalanine ammonia lyase (PAL) activity, 2-g peel samples were ground with 50 mM ice-cold Tris-HCl buffer at pH 8.8, containing 15 mM β-mercaptoethanol, 5 mM EDTA, 5 mM ascorbic acid, 1 mM phenylmethanesulfonyl fluoride (PMSF) and 4% PVP (m/V).

Total SOD activity was determined by using a “SOD Detection Kit”, following the manufacturer’s instructions (NJBI, Nanjing, China). The absorbance was monitored at 550 nm (Shimadzu UV-1800, Japan). One unit of SOD is the amount of extracts that gives 50% inhibition of reduction of xanthine. CAT activity determination was performed according to the method of Havir and Rober, with slight modifications [15].
CAT activity was expressed as U μL⁻¹, where one unit was 0.01 absorbance change min⁻¹ at 240 nm. PPO activity was based on the determination of catechol oxidation at 420 nm. One unit of PPO activity was defined as the amount of enzyme extract causing absorbance increase per minute under the conditions of the assay. PAL activity was determined according to the method described by Ballester [16]. One unit was defined as the amount of 1 μg trans-cinnamic acid released h⁻¹.

### 2.8 Statistical analysis

The experimental data were analyzed as a completely randomized design with three replicates. All statistical analysis was performed using Duncan’s multiple range test (SPSS version 17.0) and the least significant difference (LSD) at \( P = 0.05 \).

### 3 Results and discussion

#### 3.1 *In vitro* antifungal activity

The effect of clove oil on mycelial growth of *P. italicum*, *P. digitatum*, *G. candidum* var. *citri-auroanti* and *A. citri* was observed on PDA plates. As shown in Table I, the growth of *P. italicum* was completely inhibited when the concentration of clove oil reached 1,000 μL L⁻¹ (v/v) and the value of MIC was 200 μL L⁻¹. With 1,000 μL L⁻¹ clove oil, the IMG and MIC of *P. digitatum* and *G. candidum* var. *citri-auroanti* were 96.3% and 91.6%, and 500 μL L⁻¹, respectively. These results indicated that there was a significantly positive correlation between the concentration of clove oil applied and the IMG of four kinds of postharvest fungal decay in citrus fruit.

Attempts to exploit natural preservatives to control postharvest diseases as an alternative technology to synthetic fungicides in citrus fruit have been drawing much attention [26]. Hence, the presence of antifungal compounds in plant essential oils of extracts has been recognized for a long time, and the inhibitory effects of plant essential oils or crude extracts and their active ingredients against pathogenic fungi have been demonstrated in numerous studies. Our previous work has already verified that clove oil has strong antifungal activity against the main fungal diseases in citrus fruit, such as *P. italicum*, *P. digitatum*, *G. citri-auroanti* and *A. citri*. These results were in accordance with Yahyazadeh et al. [6], who reported that thyme and clove essential oils completely inhibited

### Table I. *In vitro* effect of clove oil on inhibition of mycelial growth (IMG, in %) of *Penicillium italicum*, *Penicillium digitatum*, *Geotrichum citri-auroanti* var. *citri-auroanti* and *Alternaria citri*. Each value represents the mean ± standard error (n = 5).

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>IMG (%) at various concentrations (μg L⁻¹)</th>
<th>MIC (μg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td><em>P. italicum</em></td>
<td>21.6 ± 1.57 i</td>
<td>50.2 ± 0.95 g</td>
</tr>
<tr>
<td><em>P. digitatum</em></td>
<td>4.0 ± 0.55 i</td>
<td>35.5 ± 1.61 b</td>
</tr>
<tr>
<td><em>G. citri-aurantii</em> var. <em>citri-auroanti</em></td>
<td>36.7 ± 1.80 b</td>
<td>49.4 ± 1.44 c</td>
</tr>
<tr>
<td><em>A. citri</em></td>
<td>11.3 ± 1.69 b</td>
<td>28.7 ± 1.35 i</td>
</tr>
</tbody>
</table>

* Means followed by different lowercase letters are significantly different according to Duncan’s new multiple range test (\( P < 0.05 \)).

* MIC determined by testing different concentrations in plates (MICz).

#### 3.2 *In vivo* antifungal activity

The application of clove oil was effective for controlling blue mold of citrus fruit caused by *Penicillium italicum* (figure 1 and table II). As shown in figure 1, when the lesion diameters of control citrus fruit reached 58.1 mm after incubation for 7 days at 25 °C, the inhibition percentage was between 6.5% and 60.8% at concentrations of clove oil ranging from 0.5 to 8 mL L⁻¹ (figure 2). The value of the IC₅₀ obtained by statistical analysis was 4.96 mL L⁻¹ (table II).

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**Figure 1.** Effects of clove oil (CO) on (A) the lesion diameter (in mm) and, (B) the inhibition percentage of citrus fruit after 7 days of incubation with *Penicillium italicum* at 25 °C.

*P. digitatum* growth in an *in vitro* mycelial growth assay when added to the medium at a rate of 600 μL L⁻¹. Similarly, the efficacy of *Mentha spicata* and *Lippia scaberrima* essential oils in controlling citrus green mold caused by *Penicillium digitatum* was also reported by du Plooy et al. [27].

Many studies have documented the in vitro efficacy of essential oils against common postharvest diseases of citrus fruit [6, 28, 29]. To be considered as potential natural fungicides, high inhibition of disease development, a low additive dose, and a positive effect on fruit quality are necessary. In the present study, we conclude that the IC₅₀ value of clove oil for controlling citrus blue mold was 4.96 mL L⁻¹ (table II and figure 2). Similarly, Regnier et al. [30] documented that Lippia scaberrima essential oil had high fungistatic activity against Botryosphaeria parva and Colletotrichum gloeosporioides, and reduced fungal infection of mango fruit.

### 3.3 Effect of CMC enriched with clove oil on the weight loss and decay rate

Weight loss is one of the most critical quality attributes of the postharvest life and quality of fruit during storage. As can be inferred from figure 3A, the weight loss of all samples increased gradually in both coated and uncoated fruit throughout the cold storage (P < 0.05) due to the loss of water caused by evaporation, transpiration and respiration processes. It was found that both the CO-CMC- and CMC-coated fruits had less weight loss during storage than the control group (figure 3A, P < 0.05). At the end of storage, the control group clearly showed the highest weight loss (6.83%), while the weight loss of the CO-CMC- and CMC-coated fruits reached 4.64% and 5.07%, respectively. It is universally accepted that migration of water from fruit to the environment is the major cause of weight loss of fruit [17]. Our results were consistent with previous studies indicating that the use of menthol and thymol essential oils in sweet cherry led to a reduction in weight loss [18].

### Table II. Effect of clove oil (CO) against Penicillium italicum of citrus fruit under in vivo conditions. Colony diameter was recorded after 7 days of incubation at 25 °C. Data are means ± standard errors (n = 5).

<table>
<thead>
<tr>
<th>CO treatments (g L⁻¹)</th>
<th>Average diameters of lesions (mm)</th>
<th>Inhibition over control (%)</th>
<th>IC₅₀ (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>22.8 ± 1.03</td>
<td>60.8 ± 0.84</td>
<td>4.96</td>
</tr>
<tr>
<td>4.0</td>
<td>31.2 ± 1.47</td>
<td>46.3 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>41.1 ± 0.88</td>
<td>29.2 ± 1.01</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>48.5 ± 1.58</td>
<td>16.5 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>54.3 ± 1.33</td>
<td>6.5 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>0 (Control)</td>
<td>58.1 ± 1.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The different lowercase letters within columns indicate significant differences according to Duncan’s new multiple range test (P < 0.05).

Figure 2. Control efficacy of clove oil at concentrations from 0.5 to 8.0 mL L⁻¹ against blue mold caused by Penicillium italicum.

Figure 3. Changes in (A) weight loss (in %) and (B) decay rate (in %) of ‘Xinyu’ mandarin oranges stored at 5 °C for 120 days. Bars indicate standard deviation of three replicates (CO: clove oil, CO-CMC: clove oil-carboxymethyl cellulose).

The decay % is another major determining factor that affects the preservation effect of horticultural produce. There was no visible sign of decay in coated or control fruits up to 40 days of the storage period (figure 3B). Thereafter, the coatings significantly (P < 0.05) reduced decay compared with the control group. Many of the control fruits (23.4%) were rotten at the termination of the storage, while fruits treated with CO-CMC coating and CMC coating exhibited a significantly lower decay % than the control group at the level of P < 0.05 (13.7% and 16.8%, respectively). Fruit decay in ‘Xinyu’ mandarin oranges is usually caused by fungi, with blue mold (Penicillium italicum) and sour rot (Geotrichum candidum) being identified as the two most common postharvest diseases.

### 3.4 Effect of CMC enriched with clove oil on fruit quality

The changes in the concentrations of soluble solids (TSS), acidity (TA) and vitamin C (VC) are shown in figure 4. The TSS concentration increased continuously during the early stage of storage and decreased slightly in the subsequent storage period. The TSS concentration in the control group reached 11.60 ± 0.10 °Brix after 20 days, while the coated groups reached their highest sugar level (12.14 ± 0.06 and 11.9 ± 0.10 °Brix, respectively), delaying the onset of disease 20 days longer than the control group, being stored for 40 days. There are significant differences among the three peaks. During the later storage stage, the TSS concentration of fruit
treated with CO-CMC coating was significantly higher \((P < 0.05)\) than the CMC-coated and control groups. The results showed that the coatings provided a beneficial semi-permeable film around the fruit, modifying the internal atmosphere by elevating CO\(_2\) and/or reducing O\(_2\) and delaying the degradation rate of nutrients in the fruit [19]. Meanwhile, the TSS content in CO-CMC-coated fruits was significantly higher \((P < 0.05)\) than in the CMC-coated fruits during the late stage of storage, which points to the possible effect of CMC coating combined with clove oil on the metabolic activity of ‘Xinyu’ mandarin oranges. These results are in accordance with those observed by Perdones [20], who reported that the use of chitosan edible coating containing lemon essential oil on strawberry deferred the degradation rate of nutrients during the middle and later periods of storage.

The TA concentration of all samples fell greatly after 120 days of storage (figure 2B) and the value was significantly higher \((P < 0.05)\) in CO-CMC-coated fruit compared with the control group. Since organic acids in fresh fruit, such as citric or malic acid, are primary substrates for respiration, the high respiration rate leads to the reduction in acidity [21].

The vitamin C concentration (VC) of coated and control fruits increased to a maximum after 20 days of storage and declined in the subsequent storage period (figure 4C). The highest levels of the VC were observed in the CO-CMC-coated fruits, closely followed by fruit treated with CMC coating. During the later storage stage, the VC of CO-CMC-coated fruits was significantly higher \((P < 0.05)\) than the control fruits. In citrus fruit, the VC increases with maturity and the stage of ripening; however, once fruits reach the stage of full ripening, the VC starts to decline [22].

### 3.5 Effect of CMC enriched with clove oil on the MDA content and respiration rate

Maleic dialdehyde (MDA) is the final product of lipid peroxidation, and its content has been used as one of the direct indices of cell oxidative damage [23]. As shown in figure 5A, the MDA content increased, and a significant difference was
3.6 Effect of CMC enriched with clove oil on SOD and CAT activity

To assess if the progress of senescence was associated with the induction of the antioxidant enzymes, SOD and CAT, reactive oxygen species (ROS)-metabolizing enzymes, have been proved to be important oxyradical detoxification enzymes in response to stress [16, 24, 25]. During cold storage at 5 °C, the pattern of changes in SOD and CAT activities increased first, and then decreased gradually. The activities of SOD and CAT in the control group increased to reach the peak at 40 and 60 days after treatment, decreasing rapidly thereafter (figures 6A and 6B). The maximum enzyme activities of the coated fruits were delayed for 20 and 40 days, when compared with the control group. The peak of the CO-CMC-coated fruits was significantly higher than that of the control group. During the late stages of storage, the activities of SOD and CAT in the CO-CMC-coated fruits were significantly higher (P < 0.05) than in the CMC-coated and control fruits.

3.7 Effect of CMC enriched with clove oil on PPO and PAL activity

PPO and PAL have been confirmed to play crucial roles in plant tissues infected by pathogens. A steady increase for PPO and PAL activities was found in the first 40 and 80 days of storage in all treatments, and afterward the PPO and PAL activities decreased up to the end of the storage period. The fruits treated with CO-CMC coating had significantly higher (P < 0.05) PPO activity compared with the control group,
while PAL activity in the CO-CMC-coated fruits was significantly higher \( (P < 0.05) \) than in those of the other two groups (figures 6C and 6D).

PPO has been confirmed to play a vitally important role in quinone synthesis, which is one of the first symptoms in response to fungal infection [34]. PAL is the key enzyme of the phenylpropanoid metabolism which is involved in the defense response of plant cells against pathogen invasion and enhancing disease resistance [16]. In the present study, we found that SOD, CAT, PPO and PAL in CO-CMC-coated fruits were higher than in the control group or fruits with CMC coating alone, for most of the fruit during the storage period. The reason was probably that clove oil added to CMC coating can induce activities of these enzymes, which play important roles in disease resistance in ‘Xinyu’ mandarin oranges [35]. Our results were in agreement with Zeng et al. [36], who found that carboxymethyl cellulose coating enriched with extract of *Impatiens balsamina* L. stems could effectively enhance the activities of defense enzymes in navel orange.

**4 Conclusion**

The CO-CMC coating is potentially a natural and safe alternative treatment for enhancing fruit quality of ‘Xinyu’ mandarin oranges. The coated treatments significantly decreased the decay rate and weight loss, postponed fruit quality deterioration and enhanced the activities of SOD, CAT, PPO and PAL enzymes. The addition of clove oil as an antifungal fungicide to the CMC coating had an inhibitory effect on mold growth, when compared with the CMC coating alone. In addition, the CO-CMC coating had a significantly beneficial effect on the respiration rate, commercial quality and storage properties of ‘Xinyu’ mandarin oranges. This coating alleviated the degradation of TSS, TA and VC, when compared with the other treatments. Meanwhile, the results showed that the activities of SOD, CAT, PPO and PAL in fruits treated with the CO-CMC coating were higher than those in CMC-coated and control samples. Therefore, our results indicated that the CO-CMC coating was considered beneficial for efficient application for ‘Xinyu’ mandarin orange postharvest preservation.

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**References**


