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Effect of lemon essential oil-enriched coating on the postharvest storage quality of citrus fruits

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Abstract

The objective of this study was to evaluate the use of lemon essential oil and its mixtures with chitosan, calcium chloride, and natamycin on the postharvest storage quality of citrus. The analysis of the physiological and biochemical index changes of citrus stored at 20 ± 2 °C and 75 ± 5% relative humidity for 60 days showed that lemon essential oil and its mixtures could help to maintain nutritional and sensory quality of citrus. In addition, the lemon essential oil and its mixtures increased the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). The citrus treated with 1.0% lemon essential oil, 1.0% chitosan, 1.0% calcium chloride, and 0.03% natamycin presented the highest contents of total soluble solids(TSS), titratable acid (TA), and vitamin C (Vc), and the lowest decay rate, weight loss, relative conductivity, and malondialdehyde (MDA) content, and better SOD, CAT, and POD activities, all of which suggested that this mixture had the best effect on keeping citrus fresh. This work could provide a theoretical basis for using a lemon essential oil mixture as a green preservative to improve the storage quality of citrus.

Keywords: citrus; lemon essential oil; storage quality; antioxidant enzymes.

Practical Application: Lemon essential oil and its mixtures reduced fruit decay and maintained the citrus storage quality.

1 Introduction

Citrus fruits are the preferred fruits in China and are favored by consumers (Saini et al., 2019). Citrus has excellent color, flavor, and high nutritional value with sugars, organic acids, vitamins, dietary fiber, and with active substances such as carotenoids, polyphenols, and total flavonoids known for their antioxidant properties that have beneficial effects on the human health (Chen et al., 2017). However, citrus fruits rot due to factors such as mechanical damage, tissue senescence, and pathogenic microorganism infection during harvest, storage, and transportation, resulting in significant economic losses. The diseases caused by microbes are the main cause of citrus fruit rot during the postharvest storage and transportation (Zhang & Timmer, 2007). The most economically important postharvest citrus diseases are caused by *Penicillium digitatum* and *P. italicum* (Montesinos-Herrero et al., 2011). Currently, citrus postharvest disease control is achieved using chemical fungicides. However, the long-term use of chemical fungicides presents problems such as resistance, food safety, and environmental pollution (Wuryatmo et al., 2014). In addition, chemical fungicides are not ideal for reducing the weight loss and preventing the decline in the appearance and nutritional value that generally occur after citrus harvest. Therefore, it is necessary to develop efficient, safe, and natural antistaling agents to reduce citrus diseases and maintain the fruit quality.

Chitosan is a natural high molecular weight polysaccharide polymer with good film-forming properties, biocompatibility, and antibacterial properties (Verlee et al., 2017; Zhang et al., 2021), and it is widely used in the preservation of fruits and vegetables such as strawberry and pineapple (Shahbazi, 2018; Nguyen et al., 2020; Basaglia et al., 2021; Shankar et al., 2021). However, the application of chitosan as a fresh-keeping treatment is limited because of its poor antibacterial and antioxidant properties. Addition of essential oils to chitosan may greatly improve the overall performance. The incorporation of antimicrobial agents, such as essential oils, in edible coatings may extend the shelf life of fruits and vegetables and maintain quality. Essential oils are natural liquid oil with strong antibacterial activity and antioxidant properties (Niu et al., 2018; Seydim et al., 2020). Essential oils are composed of aliphatic and aromatic compounds, and terpenoids (Bakkali et al., 2008) and their components are affected by many factors, such as the source and extraction method. Lemon essential oil is mainly composed of limonene, which inhibits the growth of common microorganisms, such as *Penicillium italicum* and black mold, after fruit harvesting (Sánchez-González et al., 2011). In addition, lemon essential oil can remove $\cdot O^2$ and $\cdot OH$ and is considered safe by the Food and Drug Administration (USA); therefore, it can be added as an antibacterial agent to food. Research on chitosan-essential oil composite coating has recently attracted attention. Demircan

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& Özdestan-Ocak (2021) reported that the addition of lemon essential oil to a chitosan film can effectively improve the fresh-keeping performance of the film. Tügen et al. (2020) studied the effect of adding different concentrations of lemon essential oil to a chitosan-gelatin film for the preservation of fresh-cut apples, and found that adding 0.75% lemon essential oil can effectively improve the film antioxidant properties, reduce the total number of bacteria, and extend the shelf life of fresh-cut apples. Chitosan-essential oil film is widely used in strawberry preservation (Shahbazi, 2018) and its effect is better than that of chitosan alone. The chitosan-lemon essential oil film may effectively improve the antibacterial performance of the film, extend the shelf life of cold-stored strawberries, and maintain the quality (Perdones et al., 2012). In addition, to expand the broad spectrum of essential oils and reduce their dosage, the combined use of essential oils with other antibacterial agents has become a research hotspot. Studies have confirmed that the natural biological antibacterial agent natamycin and essential oils have synergistic effects. The combination of natamycin and essential oils to treat cheese inhibits the growth of cheese microorganisms, extends its shelf life, and maintains sensory quality (Yangilar, 2017). Natamycin can effectively reduce the occurrence of common black rot after citrus harvest and reduce citrus rot (Wang et al., 2021a). However, there are few studies on the combination of an essential oil and natamycin in an edible film to keep citrus fresh at room temperature.

Satsuma mandarin fruit is the main citrus variety used for canned food. To meet the production requirements, processing facilities need to keep the citrus fresh. However, cold storage preservation requires a considerable amount of energy; in addition, foreign countries have barriers to the detection limit of pesticide residues in canned products. Therefore, research and development of green citrus preservatives have an important practical significance. In this study, Satsuma mandarin fruits were used as samples for evaluating the effect of lemon essential oil and its combination with chitosan, calcium chloride, and natamycin on the postharvest storage quality and physiological changes of the fruit. This study could provide a theoretical basis for replacing chemical fungicides with a lemon essential oil mixture for keeping citrus fruits fresh.

2 Materials and methods

2.1 Materials

Satsuma mandarin fruits were harvested from the Zhejiang Institute of Citrus Research (picked on November 3rd 2019) and transported to the laboratory within two hours. The fruits uniform in size, color, and maturity, and free of mechanical damage and diseases were selected.

The fungal pathogens *Penicillium digitatum* and *P. italicum* used in this study were obtained from the plant protection laboratory Zhejiang Institute of Citrus Research and preserved on potato dextrose agar (PDA) at 28 ± 2 °C.

2.2 Bacterial growth inhibition

The effect of lemon essential oil on the mycelial growth of *P. digitatum* and *P. italicum* was evaluated according to Wu et al. (2017) with slight modifications. The lemon essential oil was fully emulsified with tween 80, and then filtered and sterilized through a 0.22-μM membrane. An aliquot of 100 μL was added to each sterilized PDA medium to generate a final concentration of 0 (control), 0.25%, 0.5%, 0.75%, 1.0%, and 1.25%. The media were homogenized and poured into sterilized 90 mm diameter Petri dishes. Then, a 5-mm diameter disc of inoculum taken from 5-day-old cultures of *P. digitatum* or *P. italicum* was placed at the center of each Petri dish, respectively. The plates were cultured at 28 ± 2 ^{0}C and the colony diameters were measured with a caliper on days 3 and 10. All the treatments were performed in triplicates.

2.3 Edible coatings

The coating solution was prepared according to Shahbazi (2018) with some modifications. Chitosan (1.0 g) was dissolved in 100 mL of distilled water with 1.0 mL of glacial acetic acid and stirred overnight. Then, 1.0% (v/v) lemon essential oil mixed with 0.2 mL tween 80 as emulsifier was added to the coating solution together with 1.0% (m/v) calcium chloride ($CaCl_2$) and 0.03% (m/v) natamycin dissolved in distilled water. The final solution was homogenized.

Satsuma mandarin fruits were washed with distilled water, dried overnight at room temperature, and then divided into four groups. All the treatments were performed in triplicates. The treatments were: (CK), control, fruit treated with sterile distilled water; (A), 1.0% lemon essential oil; (B), 1.0% lemon essential oil + 1.0% chitosan + 1.0% $CaCl_2$; (C), 1.0% lemon essential oil + 1.0% chitosan + 1.0% $CaCl_2$ + 0.03% natamycin. Fruits were dipped into the solution for two minutes. Samples were dried under natural ventilation, and then placed in a sterile plastic basket and stored at 20 ± 2 °C and 75 ± 5% relative humidity (RH).

2.4 Determination of decay and weight loss rates

The decay rate was determined for all the treatments by counting the number of rot fruit and expressed as a percentage of the total number. The weight loss rate was expressed as the reduction in weight as a percentage of initial weight.

2.5 Respiration rate determination

The respiration rate was determined using a titration method.

2.6 Evaluation of fruit quality parameters

The titratable acid (TA) was determined with the method of Kilburn (1965). The results were expressed as the percentage of the citric acid determined. The total soluble solids (TSS) were measured with a hand refractometer (Brix Meter, model PAL-1, ATAGO Company, Japan). Vitamin C (Vc) was determined according to Assis et al. (2009) using the 2, 6-dichloroindophenol titration method.

2.7 Relative conductivity and Malondialdehyde (MDA) determinations

The relative conductivity was determined as described in Ali et al. (2019) with some modifications. The fruit peel was sliced into 0.09 cm small discs. Fifteen discs were added to 50 mL deionized water and shaken at 25 °C on a rotary shaker for 30 min. The electrical conductivity of the solution was measured using a conductivity meter (Model DDS-307, Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China). Thereafter, samples were boiled for 10 min in a water bath and the total electrical conductivity was determined. The relative conductivity was expressed as a percentage of the total electrical conductivity. MDA concentration was measured according to the method of Sun et al. (2011).

2.8 Determination of the Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD) activities

The CAT, SOD, and POD activities were determined using a kit (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China).

2.9 Sensory analysis

The sensory evaluation was analyzed according to Guerreiro et al. (2015). Ten trained panelists evaluated the sensory quality of citrus on day 60 of storage based on a 7-point hedonic scale: 1, dislike definitely; 2, dislike; 3, dislike mildly; 4, neither like nor dislike; 5, like mildly; 6, like; 7, like definitely. Appearance, texture, aroma, taste, and overall liking were the sensory parameters evaluated. Overall liking was calculated as a mean of the sensory parameters evaluated.

The data were processed by analysis of variance (ANOVA). Statistical significances were performed by Duncan's test at $P < 0.05$ using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Lemon essential oil inhibits bacterial growth in a concentration-dependent manner

Table 1 shows that the lemon essential oil (0.5-1.25%) significantly inhibited the growth of *P. digitatum* and *P. italicum*. The lemon essential oil had stronger antimicrobial activity against *P. italicum* than against *P. digitatum*. Moreover, in the presence of 1.00% and 1.25% lemon essential oil, no *P. italicum* growth was observed 3 days after inoculation. The relative inhibition rates of 1.00% and 1.25% lemon essential oil against *P. italicum* reduced to 60.37% and 65.61%, respectively, 10 days after inoculation. The relative inhibitory efficacy of 1.25% lemon essential oil on *P. digitatum* was 35.16% 3 days after inoculation and decreased to 20.14% 10 days after inoculation. In a preliminary test, more than 1.25% of lemon essential oil caused brown scars on the Satsuma mandarin fruit peel, which led to fruit decay. Therefore, 1.0% lemon essential oil was selected as the concentration for the test.

3.2 Lemon essential oil and its mixtures decrease the decay and weight loss rates of citrus

Figure 1A shows that the fruit decay rate in all the groups gradually increased during storage. In CK and B groups decayed after 10 days and in A and C groups decayed after 20 days. The fruit decay rate in the experimental groups was lower than that in the CK group during the whole storage period. The decay rate in the CK group was 14.0%, which was 2.0 times higher than that in the C group on day 60 of storage. The decay rate in the C group was significantly lower than that in the other groups from day 30 of storage indicating that the C group had a better effect on reducing citrus decay ($P < 0.05$).

During storage, water is lost due to transpiration and respiration, which reduces the quality of fruits (Petriccione et al., 2015). Figure 1B shows that the weight loss rate in all the experimental groups was lower than that in CK group. Weight loss rates in CK, A, B, and C groups were 20.56%, 19.65%, 17.20%, and 14.33%, respectively, after 60 days of storage. The weight loss rate was the lowest in the C group, which had the best water retention.

3.3 Lemon essential oil and its mixtures modify the nutritional quality of citrus

The TSS is an important indicator for evaluating fruit nutrition (Wang et al., 2019). Figure 2A shows that the TSS content in all the treatments gradually increased up to the peak on day 30 and then decreased. This may be due to the hydrolysis of starch and other polysaccharides to carbohydrates during fruit storage (Xin et al., 2017) and because respiration continues to consume soluble solids in the later storage. The TSS content in the B and C groups was significantly higher than that in the CK group on day 60 ($P < 0.05$). There was no significant difference in TSS content between A and CK groups except on days 20 and 30 ($P > 0.05$).

Table 1. Inhibitory effect of lemon essential oil on the growth of the pathogens *P. italicum* and *P. digitatum.*

Lemon essential oil concentration (%)	Relative inhibition rate against *P. italicum* (%)		Relative inhibition rate against *P. digitatum* (%)	
	3-d culture	10-d culture	3-d culture	10-d culture
0.25	35.26 ± 2.86d	30.12 ± 0.90e	-15.63 ± 2.06e	-4.67 ± 1.34e
0.50	64.75 ± 3.30c	42.63 ± 3.57d	6.03 ± 1.00d	2.09 ± 0.81d
0.75	81.16 ± 1.92b	53.15 ± 1.15c	15.36 ± 1.76c	10.53 ± 2.00c
1.00	100.00 ± 0a	60.37 ± 0.99b	28.54 ± 1.72b	15.69 ± 1.19b
1.25	100.00 ± 0a	65.61 ± 2.45a	35.16 ± 1.50a	20.14 ± 1.40a

Results are presented as the mean ± SD. Different lowercase letters in the same column indicate significant differences at $P < 0.05$.

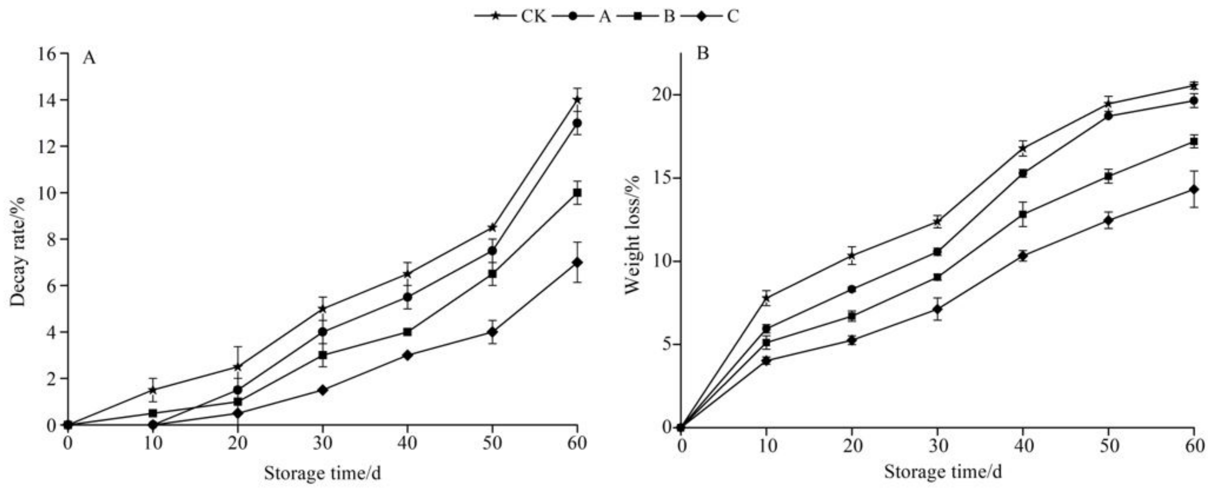

Figure 1. Effect of lemon essential oil and its mixtures on the decay and weight loss rates of citrus. CK, control; A, 1.0% lemon essential oil; B, 1.0% lemon essential oil + 1.0% chitosan + 1.0% $CaCl_2$; C: 1.0% lemon essential oil + 1.0% chitosan + 1.0% $CaCl_2$ + 0.03% natamycin. The same as following.

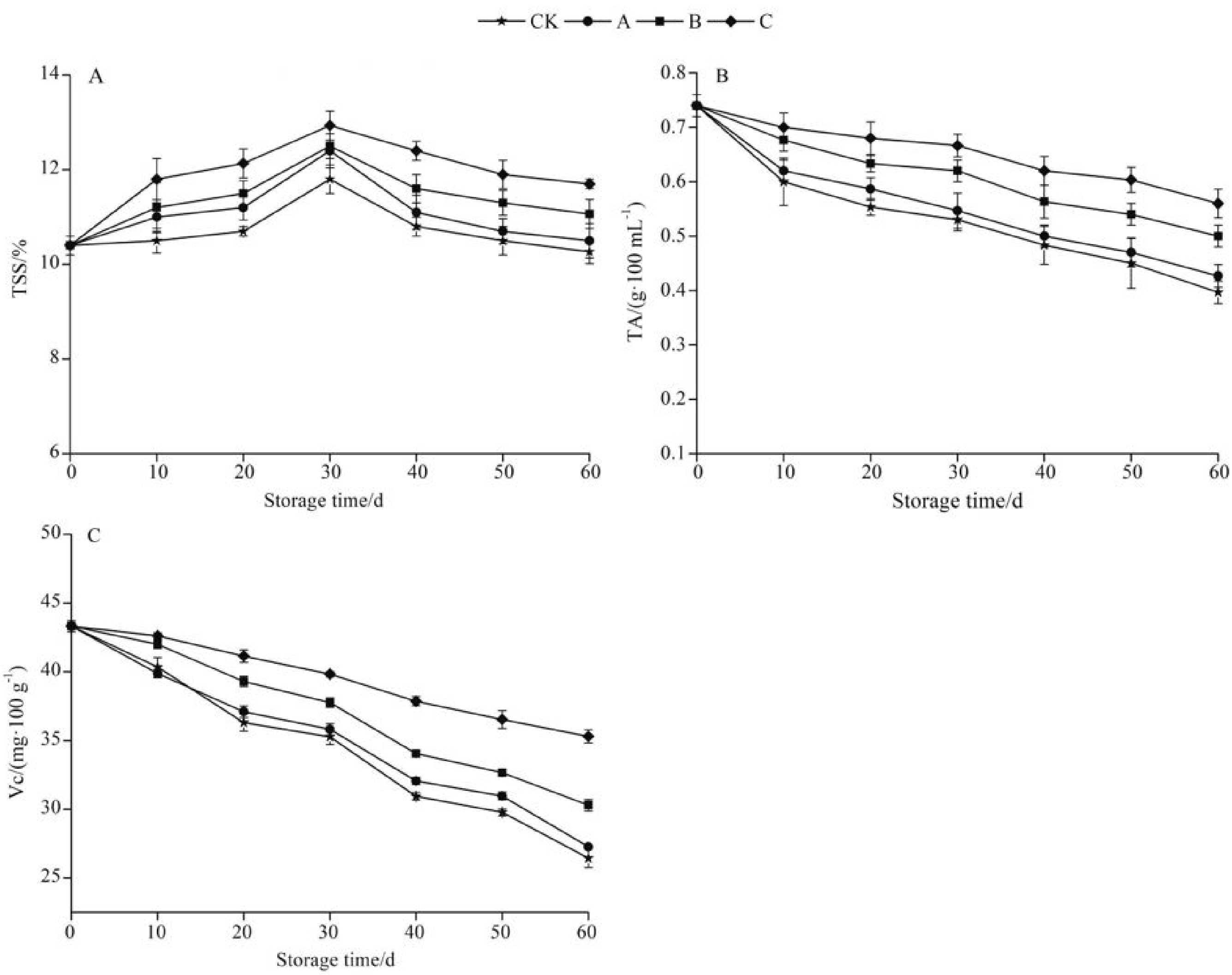

Figure 2. Effect of lemon essential oil and its mixtures on the nutritional quality of citrus.

TA is the main substrate of fruit respiration (Souza et al., 2015) and is an important indicator of fruit preservation. Figure 2B shows that the TA content in all the groups gradually decreased during storage. The TA content in the C group decreased the most slowly with a loss of 0.18 g·100 mL^{-1} after 60 days of storage, and the TA content in the CK group lost 0.34 g·100 mL^{-1}. The TA content in the C group was 1.4 times higher than that in the CK group on day 60 of storage. The TA content in the B and C groups was significantly higher than that in the CK ($P < 0.05$). There was no significant difference in TA content between the A and CK groups ($P > 0.05$) and there was significant difference between the B and C groups after 20 days of storage ($P < 0.05$).

Vc is an important citrus nutrient that scavenges free radicals and delays the senescence of fruits (Quintana et al., 2021). Figure 2C shows that the Vc content in the CK and treatment groups gradually declined during storage. Vc content in the C group was significantly higher than that in the other groups after 20 days of storage ($P < 0.05$). The Vc contents in CK, A, B, and C groups were 26.44, 27.26, 30.29 and 35.31 mg·100 g^{-1}, respectively, on day 60 of storage. The Vc content decreased the most in the CK group and the least in the C group.

3.4 Determination of the citrus respiration rate in the presence of lemon essential oil and its mixtures

Table 2 shows that the respiration rate in all groups decreased first and then increased with storage time. The respiration rate in the CK group was higher than that in the experimental groups. Respiration rates in CK, A, B and C groups were 17.53, 16.98, 15.22, and 12.52 $mg.kg^{-1}.h^{-1}$, respectively, after 60 days of storage. The respiration rate in the C group was the lowest during storage.

3.5 Determination of antioxidant enzyme activities in citrus

Figure 3A shows that the CAT activity in all groups showed an overall downward trend with storage time. The CAT activity was 2.74–8.35 $U.g^{-1}$. Within 30 days of storage, a significant difference in CAT activity between the CK and A groups was observed ($P < 0.05$) and there was no significant difference from day 40 ($P > 0.05$). Except on day 20 of storage, there were significant differences in the CAT activity among B, C, and CK groups ($P < 0.05$). The CAT activity in the C group was significantly higher than that in the other groups ($P < 0.05$). Figure 3B shows that the POD activity in all the groups increased first and then decreased during storage. The POD activity was between 33.7 and 78.4 $U.g^{-1}$ and was significantly higher than that in the CK group on day 60 ($P < 0.05$). The C group presented the best POD activity, which was 53.7 $U.g^{-1}$ at the end of storage. Figure 3C shows that the SOD activity in all the groups increased first and then decreased, and was significantly higher than that in the CK except on day 20 ($P < 0.05$). SOD activity in the C group was the highest, and decreased to 89.33 $U.g^{-1}$, which was 1.7 times higher than that in the CK group on day 60 of storage.

3.6 Relative conductivity and MDA concentration in citrus

Figure 4A shows that the MDA content in the CK and experimental groups gradually increased during storage. The MDA content changed the most slowly in the C group and increased the fastest in the CK group. Moreover, the MDA content in the C and CK groups increased to 0.909 $\mu mol.g^{-1}$ and 1.393 $\mu mol.g^{-1}$, respectively, on day 60 of storage. The MDA content in the CK group was 1.5 times higher than that in the C group on day 60 of storage. There were significant differences in the MDA content among the CK, B, and C groups from day 20 of storage ($P < 0.05$).

Membrane permeability is usually expressed through relative conductivity. Figure 4B shows that the relative conductivity of citrus peel in all groups increased to varying degrees during storage. The relative conductivity increased the least in the C group and increased the fastest in the CK group. The relative conductivity in the CK group was 34.5% on day 60 of storage, which was higher than that in the other groups.

3.7 Sensory analysis of processed citrus

Figure 5 shows that the appearance, taste, texture, aroma, and overall liking scores in the A group were not significantly different from those in the CK group after 60 days of storage ($P > 0.05$). The sensory scores in the B and C groups were significantly higher than those in the CK group ($P < 0.05$). The appearance, texture, aroma, taste, and overall liking scores in the C group were 6.3, 5.9, 6.3, 6.1, and 6.2, respectively, which were significantly higher than those in the other groups ($P < 0.05$).

4 Discussion

During storage, fruits decline in quality parameters such as weight loss, softening, and off flavor due to respiration and transpiration (Liu et al., 2018). The fruit decay rate is an important parameter used for measuring preservation effects. Moreover, TSS, TA, and Vc are important indicators for evaluating fruit flavor and nutritional quality. The results in this study showed that the treatment with lemon essential oil and its mixtures resulted in lower weight loss and decay rate, and higher TSS, TA, and Vc contents of citrus. This may be because the lemon essential oil and its mixtures produce a film with an appropriate

Table 2. Effect of lemon essential oil and its mixtures on the respiration rate of citrus.

Group	Storage time /d						
	0	10	20	30	40	50	60
CK	13.26 ± 0.46a	12.90 ± 0.22a	11.95 ± 0.40a	9.64 ± 0.61a	13.36 ± 0.28a	14.89 ± 0.30a	17.53 ± 0.31a
A	13.26 ± 0.46a	12.13 ± 0.55b	10.89 ± 0.55b	8.31 ± 0.65b	11.68 ± 0.18b	12.67 ± 0.57b	16.98 ± 0.75a
B	13.26 ± 0.46a	11.47 ± 0.32b	8.96 ± 0.12c	7.40 ± 0.40b	11.02 ± 0.25c	11.71 ± 0.21c	15.22 ± 0.14b
C	13.26 ± 0.46a	9.35 ± 0.26c	6.97 ± 0.40d	5.58 ± 0.37c	8.56 ± 0.28d	10.02 ± 0.26d	12.52 ± 0.43c

Results are presented as the mean ± SD. Different lowercase letters in the same column indicate significant differences at $P < 0.05$.

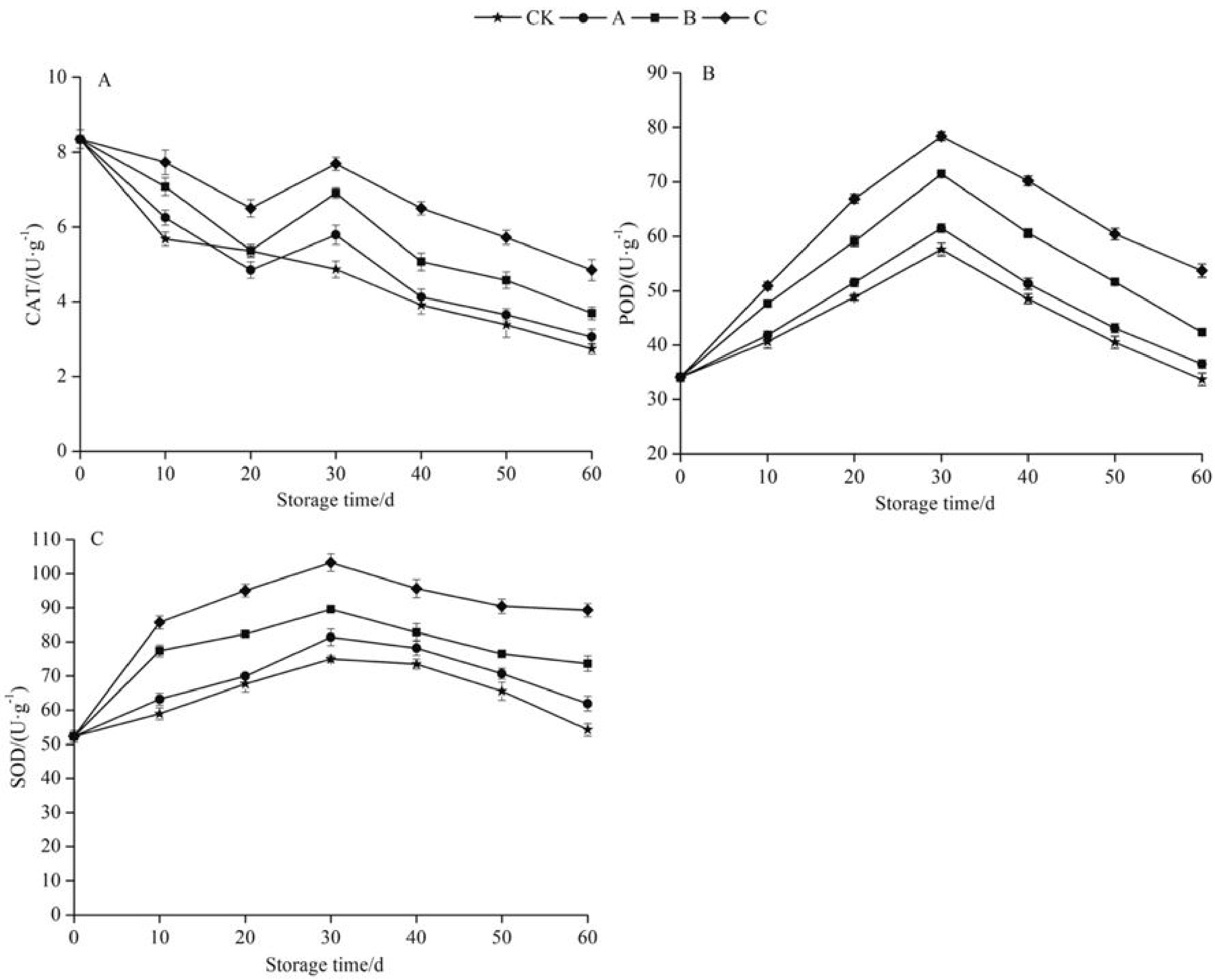

Figure 3. Effect of the lemon essential oil and its mixtures on the activities of antioxidant enzymes in citrus.

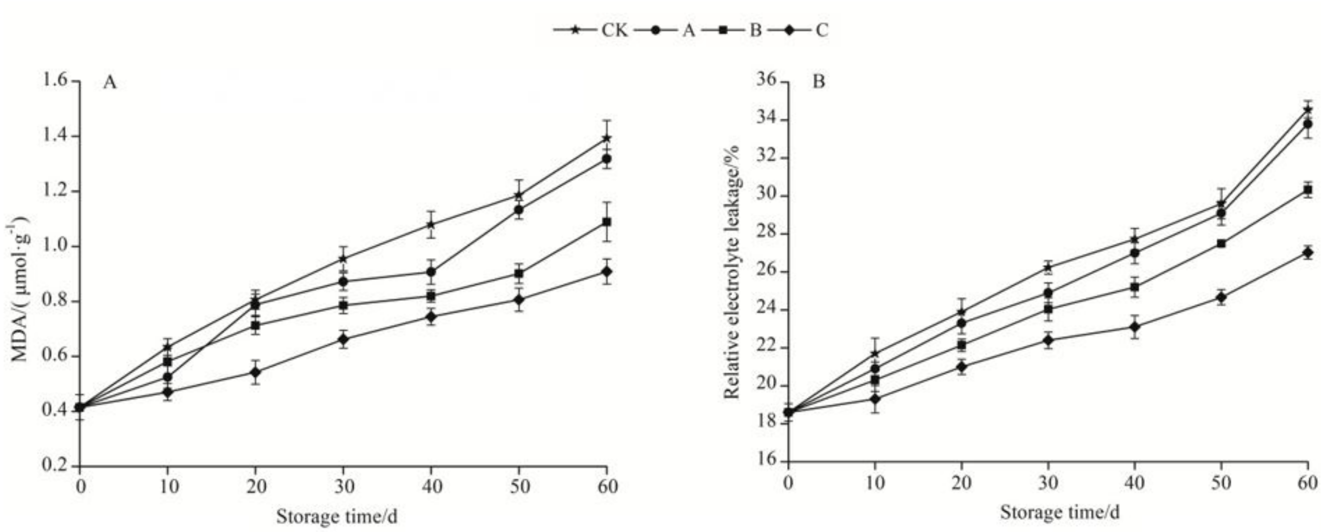

Figure 4. Effect of lemon essential oil and its mixtures on the MDA content and relative conductivity of citrus.

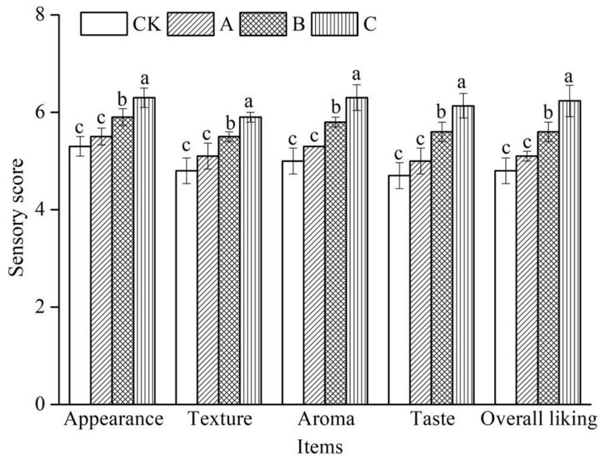

Figure 5. Effect of lemon essential oil and its mixtures on the sensory score of citrus.

thickness and air-permeability on the surface of the fruit to decrease respiration rate, water desorption, and nutrient degradation. In addition, this study showed that the C group had the highest TSS, TA, and Vc contents, as well as the lowest decay and weight loss rates, indicating that the treatment in the C group had the best fresh-keeping effect. These results are in agreement with a previous study, which reported that chitosan-Mentha spicata essential oil has a better effect on the preservation of the quality of strawberries than that of chitosan alone (Shahbazi, 2018). Similarly, Wang et al. (2021b) reported that the addition of nisin and essential oil to chitosan has the best effect on delaying the decay of strawberries during storage. Chien & Chou (2006) reported that chitosan inhibits *P. italicum* and *P. digitatum* growth and reduces the decay rate of Tankan fruit. In addition, lemon essential oil contains active substances, such as limonene, terpenes, and citral, which have a good inhibitory effect on *P. italicum* and *P. digitatum* (Caccioni et al., 1998). Fu et al. (2021) reported that the treatment of grape berries with calcium salt after harvest increases resistance infection by *Botrytis cinerea*. These results suggested that lemon essential oil, chitosan, natamycin, and calcium chloride had synergistic effects on keeping citrus fresh.

Three antioxidant enzymes, SOD, POD, and CAT, are closely related to the deterioration of citrus quality and together reduce the damage of free radicals and H_2O_2 to cell membranes, which help to reduce the accumulation of the lipid peroxidation product MDA and delay fruit senescence. Previous studies have confirmed that chitosan promotes high antioxidant enzyme activity in fruits (Zhang et al., 2019; Saleem et al., 2021) and maintains the fruit quality. In addition, essential oils and their volatile components enhance the activities of SOD, POD, and CAT in fruits. Xing et al. (2011) found that the chitosan-cinnamon oil treatment can increase the activities of CAT, POD, and SOD in sweet pepper and delay the increase of the MDA content. Similar results were obtained in this study, where experimental group treatments reduced MDA content by increasing the activities of three antioxidant enzymes, thereby maintaining fruit quality. Moreover, the SOD and POD activities in all the groups showed a downward trend after reaching a peak, accompanied by deterioration of the citrus quality, which is in agreement with Wang et al. (2019).

5 Conclusions

The results showed that the lemon essential oil and its mixtures maintained the nutritional and sensory quality of the fruit after coating. The combination of lemon essential oil, chitosan, calcium chloride, and natamycin could effectively reduce decay rate of citrus and delay the degradation of TSS, TA and Vc during storage. This may be due to the chitosan and essential oil treatment. Calcium chloride and natamycin have good antibacterial ability. This study showed that lemon essential oil could improve fruit storage quality. Due to the complex composition of the lemon essential oil, further research needs to be conducted to reveal the oil components and understand the mechanism of the lemon essential oil in citrus preservation.

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