Research Article

Citral Delays Postharvest Senescence of Kiwifruit by Enhancing Antioxidant Capacity under Cold Storage

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Citral is an aliphatic aldehyde extracted from citrus essential oil. The aim of the study was to examine how citral treatment affects the weight loss, firmness, respiration, and ripening index, as well as the antioxidant capacity of kiwifruit (Actinidia chinensis cv. ‘Jinkui’). The citral treatment was seen to reduce the weight loss, softening, and fruit respiration compared to control fruits. Citral treatment also had an inhibitory effect on ripening index, O$_2^-$ production rate, and malondialdehyde (MDA) accumulation. The degradations of ascorbic acid (AsA) content, total flavonoids content (TFC), and total phenolics content (TPC) were also suppressed by citral. In contrast, citral treatment induces the activation of antioxidant enzyme system such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Collectively, the results indicated that citral treatment delays postharvest senescence and prolongs storage life by enhancing antioxidant capacity in harvested kiwifruits. These findings suggest that citral has the potential to be used as a promising natural preservative for the extension of postharvest quality in harvested kiwifruit.

1. Introduction

Kiwifruit (Actinidia deliciosa, Actinidiaceae) is quite popular because of its taste and having high vitamin C and antioxidant capacities [1]. Kiwifruit is highly perishable and susceptible to pathogenic fungi causing significant economic losses due to the rotting of fruits during storage [2, 3]. Till date, various postharvest approaches such as edible coatings (alginate oligosaccharide, chitosan, and lacquer wax) [2, 4, 5], 24-epibrassinolide [6], oxalic acid [7, 8], aminoethoxyvinylglycine [9], methyl jasmonate [10], hydrogen sulfide [11, 12], and ozone treatment [13] have been implied and found to prolong the postharvest storability of kiwifruit. Postharvest application of natural phytochemicals, particularly plant essential oils, has been proved to be effective in controlling diseases and preservation of fruit quality [14, 15].

Recently, essential oils (EO) and their various constituents are used as natural fungicides for controlling postharvest fruits diseases and have been extensively studied. Essential oils from Artemisia herba-alba, Mentha pulegium, and Syzygium aromaticum had certain antimicrobial effects on Penicillium expansum population and blue mold development of apples [15]. Moreover, cinnamon essential oil displayed a strong antifungal inhibition on Colletotrichum acutatum of 'Hongyang' kiwifruit [16], and cassia oil has been used against Alternaria alternata (black spot rot) in tomatoes [17]. It is also shown that some essential oils could enhance antioxidant capacities of many horticultural products such as strawberries and blueberries [18, 19]. Citral, a terpenoid isolated from Litsea cubeba and Cymbopogon citratus (lemongrass), is widely used for food, beverages, and cosmetics industry [20]. Recently, citral has been reported to have significant antifungal effects on postharvest pathogens such as P. italicum, P. digitatum, and Geotrichum citri aurantii [21, 22], which effectively enhances the antioxidant capacities and disease resistance in some fruits [23, 24].
addition to these, citral has been used a promising plant-derived pesticide, which seems to be a surprising application to protect horticultural products from fungal attacks [21, 24]. Therefore, it remains a mighty possibility that citral could be used commercially for controlling postharvest fungal decay and enhancing disease resistance of horticultural products.

To develop a postharvest preservation using more applicable form of citral for kiwifruit, the effects of citral on the postharvest quality and on the response of reactive oxygen species (ROS) and antioxidant capacity shall also be studied in the present study.

2. Materials and Methods

2.1. Materials and Treatment. Healthy “Jinkui” kiwifruits (Actinidia chinensis) were harvested from a commercial orchard in Fengxin County (Jiangxi Province, China) with a commercial maturity [mean flesh firmness: 7.19 N; soluble solids content (SSC): 7.61 ± 0.16%]. The picked fruits with uniform size, color, and shape, free from any mechanical damage or pests were randomly divided into 39 groups comprising 10 fruits each. Eighteen groups were dipped in 0.6 µLmL−1 citral (prepared in 0.1% Tween 80 solution) for 15 min, and another eighteen groups immersed in 0.1% Tween 80 solution only, to serve as the controls. The citral concentration of 0.6 µLmL−1 was chosen based on the evaluation in a preliminary experiment using 0, 0.2, 0.4, 0.6, and 0.8 µLmL−1 citral solutions (data not shown). The remaining three groups were also sampled before treatment to determine physicochemical quality parameters at harvest time (0 day).

Both citral-treated and control kiwifruits were air-dried (at 20 ± 1°C and 90 ± 5% relative humidity), packed in low-density polyethylene bags (39 × 30 cm, 0.03 mm thickness), and then stored at 0–1°C and 90 ± 5% RH for 90 d. Three replicates of 30 fruits for each treatment were taken at intervals of 15, 30, 45, 60, 75, and 90 d and sampled the flesh tissue for determination of flesh firmness, quality parameters, membrane permeability, and antioxidant enzyme activities.

2.2. Evaluation of Weight Loss, Fresh Firmness, Total Soluble Solids (TSS) and Titratable Acid (TA) Content, and Ripening Index. The weight of the same 10 kiwifruits from the control and citral-treated groups was measured immediately after days 0 and at the different sampling times (15, 30, 45, 60, 75, and 90 d). Weight loss is defined as the ratio of final sample weights to the harvested sample weights and expressed as percentage (%).

Fresh firmness of kiwifruit was evaluated on two opposite positions around the equator of 10 fruits (skin removed) using a texture analyzer (TAXT Plus, Stable Micro Systems, Surrey, UK) equipped with a 2 mm diameter probe and expressed as N.

The SSC in kiwifruit juice was recorded on a digital Brixmeter (RA-250WE, KYOTO, Tokyo, Japan) and specified in °Brix. TA content was analyzed by the titration with 0.1 M NaOH and expressed in terms of the percentage of citric acid following the method described by Horak et al. [25].

Ripening index was calculated by simply taking the ratio of SSC and TA (SSC:TA).

2.3. Respiration Rate, O2− Production Rate, and Malondialdehyde (MDA) Content. The respiration rate was determined according to a method described by Hu et al. [5]. The respiration rate was analyzed by an infrared CO2 fruit breathing instrument (GHX-3051H, Shanghai, China) and recorded as mg CO2 kg−1 h−1.

For O2− production rate assay, 2.0 g frozen tissue powder was homogenized with 5 mL ice-cooled 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA, 0.3% (v/v) Triton X-100, and 2% (w/v) polyvinyl pyrrolidone (PVP) and then centrifuged at 10,000 × g for 20 min at 4°C. 1 mL of the supernatant was mixed with 1 mL of 50 mM phosphate buffer (pH 7.8) and 1 mL of 1 mM hydroxylamine hydrochloride and incubated for 1 h at 25°C. 2 mL of the mixed solution comprised of 17 mM 4-aminobenzenesulfonic acid and 7 mM naphthylamine (1:1) was then added into the mixture, followed by incubation for 20 min at 25°C. The absorbance of the mixture was recorded at 530 nm. The unit of O2− production rate was expressed as nmol g−1 min−1.

MDA content was measured using the thiobarbituric acid colorimetric method according to the procedure of Nie et al. [26]. Frozen flesh tissue was milled to a powder with liquid nitrogen, and 1.0 g of powder was homogenized in 5 mL 10% (m/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 × g for 15 min. 2 mL of the obtained supernatant was mixed with 2 mL of 0.67% thiobarbituric acid, followed by boiling water bath for 15 min. The mixture was then cooled and the absorbance of the supernatant was determined at 450, 532, and 600 nm. Each treatment was conducted in triplicate.

2.4. Assays for Ascorbic Acid (AsA) Content, Total Phenolics Content (TPC), and Total Flavonoids Content (TFC). Ascorbic acid (AsA) content of kiwifruit flesh was acquired by the titration with a standard solution of 2,6-dichlorophenol indophenol [26] and expressed in terms of milligram on a fresh weight basis (g kg−1).

The levels of TPC and TFC were measured following the method described by Jiang et al. [27]. Both TPC and TFC were represented as milligram of gallic acid and rutin equivalent per gram of frozen sample (mg kg−1), respectively.

2.5. Assay of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Capacity and Ferric Reducing Antioxidant Power (FRAP). The DPPH scavenging capacity was carried out according to a previous method described by Horak et al. [25]. 100 µL of extracted juice was added to 1.9 mL of 0.1 mM DPPH solution (dissolved in ethanol) and then left in the dark at 25°C for 30 min. The absorbance of the tested sample was monitored at 515 nm. DPPH free radical scavenging capacity was calculated and expressed as a percentage using the following equation:
Scavenging capacity (%) = \frac{A_0 - A_t}{A_0} \times 100,  \tag{1}

where $A_0$ and $A_t$ are the absorbance of the control (without extracted juice) and kiwifruit juice added after 30 min of reaction incubation, respectively. The unit of DPPH scavenging capacity was expressed as %.

The FRAP assay was carried out according to the method of Benzie and Strain [28] with a slight modification. 0.5 mL of extracted juice was added to 3 mL of fresh FRAP reagent [0.3 M acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (dissolved in 40 mM HCl), and 20 mM FeCl3 \* 6H2O (10:1:1, v/v/v)] and then left in the dark at 37 °C for 30 min. The absorbance of the mixture was determined at 700 nm using distilled H2O as the blank solution.

2.6. Measurement of Antioxidant Enzyme Activities. Fresh tissue (2.0 g) from 10 fruits was homogenized with 8 mL of ice-cold 50 mM phosphate buffer solution (pH 7.0) containing 2% polyvinyl pyrrolidone and 5 mM dithiothreitol and then centrifuged at 12,000 × g for 20 min at 4 °C. The obtained supernatant was used to determine the activity of the antioxidant enzymes.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured using a SOD test kit (No: A001-1-2; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with the hydroxylamine method. The absorbance of the tested sample was monitored at 550 nm. The quantity of enzyme that controlled 50% of nitroblue tetrazolium (NBT) photoreduction per hour was defined as one unit of SOD activity.

Catalase (CAT, EC 1.11.1.6) activity was determined by measuring the decline of absorbance at 240 nm (Shimadzu UV-2600, Tokyo, Japan) due to the decomposition of H2O2. The collected supernatant (200 μL) was mixed with 2.8 mL of 20 mM H2O2 (prepared in 50 mM phosphate buffer solution). The quantity of CAT that changed the absorbance of the reaction solution by 0.01 at 240 nm per minute was defined as one unit of CAT activity.

Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol method reported by our previous studies [3, 26]. The reaction system consisted of 50 μL of enzyme solution, 3.0 mL of 25 mM guaiacol (prepared in 50 mM phosphate buffer solution), and 200 μL of 0.5 mM H2O2. The increase in absorbance at 470 nm was determined at 25 °C for 5 min. The quantity of POD that changed the absorbance of the reaction solution by 1 at 470 nm per minute was defined as one unit of POD activity.

2.7. Statistical Analysis. All data were described as the means of three replicated samples ± standard error (S.E.), and analyses were carried out by using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). P value less than 0.05 ($p < 0.05$) was considered as significant. Origin version 8.5 (Microcal Software, Northampton, MA) was applied to create figures.

3. Results

3.1. Change of Fruit Weight Loss and Firmness after Citral Treatment. Fruit weight loss in both control and citral-treated fruits depicted a clear increase during storage (Figure 1). However, the weight loss in the fruit treated with citral maintained a low level compared with the control fruits. By the end of cold storage period of 90 d, the weight loss in the citral-treated kiwifruit was lower (18.9%, $p < 0.05$) than that in the control fruits.

Flesh firmness in the control and citral-treated kiwifruit decreased throughout the storage period (Figure 2). Further comparison indicated that there was a higher level of flesh firmness in the citral-treated kiwifruit than that in the control kiwifruit under storage, with a remarkable ($p < 0.05$) difference during the entire storage period.

3.2. Effects of Citral Treatment on Postharvest Senescence Related Parameters of Harvested Kiwifruit. The rate of fruit respiration in both control and citral-treated fruits sharply increased in the first 15 d after treatment, followed by a sharp drop during the rest of storage periods (Figure 3(a)). The peak of respiration rate in the control kiwifruit was 1.56-folds higher than that of the citral-treated kiwifruits. Respiration rate in the citral-treated kiwifruit was significantly ($p < 0.05$) lower than that in the controls during the entire storage periods (except for 75 d).

Ripening index of the control and citral-treated kiwifruits increased over the period of cold storage (Figure 3(b)). Citral treatment delayed the increase of ripening index in kiwifruits. There was a lower level of ripening index in the citral-treated kiwifruit than that in the control fruits during storage period. The ripening index in the citral-treated kiwifruit was lower (12.7%) than that of the control fruit at day 90.

The alterations of O2•− production rate in the control and citral-treated kiwifruit flesh were increased at the early storage (0 d to 45 d) but afterwards declined (Figure 3(c)). Compared to the control fruits, the O2•− production rate in the citral-treated kiwifruit retained lower levels during storage, with a significant ($p < 0.05$) difference during the middle and later periods of postharvest storage (45 d to 90 d).

MDA is the final product in the peroxidation of membrane lipids, which can produce the Schiff alkali, destroying membrane integrity to promote membrane leakage. The MDA content in both control and citral-treated fruits increased with the prolongation of the storage time (Figure 3(d)). Citral treatment significantly ($p < 0.05$) delayed the accumulation of MDA content. The MDA content in the citral-treated kiwifruits was lower than that in the control fruits. There was a decrease (15.7%) in the citral-treated kiwifruit at the end of storage period. It is worth mentioning that there was a prominent difference ($p < 0.05$ or $p < 0.01$) between the control and citral-treated kiwifruits during the entire storage period (Figure 3(d)).
The TFC depicted the same trend as TPC, where the former in both control and citral-treated kiwifruits decreased over the period of cold storage (Figure 4(c)). Citral treatment prevented the decline of TFC in kiwifruit flesh, which maintained higher level of TFC in comparison with the control sample (Figure 4(c)). The TFC in the citral-treated fruits was significantly \( p < 0.05 \) higher than that of the control fruits during the whole of storage period.

The DPPH scavenging capacity was decreased gradually during storage time in both control and citral-treated fruits (Figure 4(d)). Citral treatment significantly \( p < 0.05 \) inhibited the decrease of DPPH scavenging capacity during the middle and later period of storage (45 d to 90 d). Kiwifruits treated with citral exhibited a remarkable higher levels of DPPH scavenging capacity than the control fruits.

The FRAP showed a similar pattern with DPPH scavenging capacity in both control and citral-treated fruits during storage time (Figure 4(e)). In addition, the FRAP in the citral-treated fruits was significantly higher \( p < 0.05 \) than that in the control fruits. Moreover, there was a prominent difference between the control and citral-treated kiwifruits during the entire storage period (except for 60 d).

### 3.4. Effects of Citral Treatment on SOD, CAT, and POD Activities of Harvested Kiwifruit

Antioxidant enzymes such as SOD, CAT, and POD serve an indispensable role in scavenging ROS in horticultural fruits. Both SOD and CAT activities exhibited similar tendency; i.e., they gradually increased during the initial storage of 45 d and then dropped rapidly. The activities of SOD and CAT in the citral-treated fruits were consistently higher \( p < 0.05 \) than those in the control fruits, and there was a prominent difference between the control and citral-treated kiwifruits during the entire storage period (Figures 5(a) and 5(b)). The POD activity in both control and citral-treated fruits increased for 0–75 d and then declined in the following storage period. The POD activity significantly increased by citral treatment after treatment, and even at 75 d the POD levels still remained 1.15 times higher than those in the control fruits (Figure 5(c)). It is worth mentioning that an obvious significance was detected between the control and citral-treated groups during the whole storage period up to 90 d (except for 45 d).

### 4. Discussion

The application of plant essential oils could effectively control postharvest fruit diseases by enhancing the antioxidant ability of many horticultural products that have extensively been reported recently [29, 30]. It is widely accepted that the important fruit quality parameters evaluating kiwifruit acceptability by consumers include fruit weight, firmness, color, and fragrance [31, 32]. However, fruit quality of kiwifruit declined rapidly in both storage period and shelf life. Consequently, help to develop an effective preservations method for maintaining postharvest fruit quality of kiwifruits is of great interest [6, 32]. However, there is hardly any study that describes the effect of citral...
treatment on kiwifruit postharvest quality during cold storage or analyzes different quality parameters related to senescence stress. The current study examined that kiwifruits treated with citral exhibited lower ripening index while maintaining the antioxidant capacity in comparison with the control fruits.

For the senescence related parameters, citral treatment reduced changes on weight loss, firmness, respiration rate, and ripening index throughout the storage period of kiwifruits. In the current study, an increase in ripening index showed the process of kiwifruit ripening and senescence after harvest, which was significantly delayed in the citral-treated kiwifruits (Figure 3). A similar response has been reported following 5 μmol L⁻¹ 24-epibrassinolide treatment during storage in kiwifruit, by Wang et al. [6].

Ascorbic acid (AsA) could protect kiwifruits from the oxidative damage due to higher scavenging capacity for various ROS through the ascorbate peroxidase reaction [26]. A huge loss of AsA content occurred in the control treatment followed by citral treatment quite similar to a recent study where citral was applied during postharvest storage that declined AsA content in tomato during cold storage [24]. The results in the present study indicated that citral treatment could delay the oxidation process in kiwifruits by maintaining higher levels of AsA content than the control fruits after the harvest.

Both phenolics and flavonoids compounds are effective antioxidants that contribute to eliminating ROS. The current study revealed that citral treatment maintained the higher levels of both TPC and TFC in comparison with control kiwifruits. This is further accompanied with higher levels of DPPH scavenging capacity and FRAP, thereby reducing the damage caused by the ROS. These results indicated that both TPC and TFC were positively correlated with DPPH scavenging capacity and FRAP and negatively correlated with the ROS damage in kiwifruits. Several studies have also reported the inalienable relationship between phenolics content and their antioxidant capacity in postharvest fruits. Jin et al. [33] demonstrated that higher antioxidant capacity in Chinese bayberries treated with linalool and cinnamaldehyde was mainly linked to the higher phenolics contents. The high levels of antioxidant contents may delay
Figure 4: Effect of citral treatment on AsA content, (a) total phenolics content (TPC), (b) total flavonoids content (TFC), (c) 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, (d) and ferric reducing antioxidant power (FRAP) (e) of harvested kiwifruit stored at 0-1°C for 90 d. Vertical bars represent the mean ± standard error (S.E., n = 3). Letters indicate the statistical differences according to the independent samples t-test (p < 0.05) on each storage day.
The physiological deterioration and hence postpone the fruit senescence.

Respiration rate is an important parameter for postharvest fruit metabolic activity and quality assessment. The findings herein showed that citral-treated fruits exhibit lower respiration rates and hence offer better storage quality. In addition, MDA content is another indicator for cellular oxidative damages. Lipid peroxidation is a major reason for membrane integrity damages during fruits senescence [34]. High MDA content could cause the damage in cellular membrane systems, which further led to the softening of kiwifruits. The current results indicated that citral treatment could reduce the MDA accumulation in kiwifruits to resist the senescence induced ROS damage, thereby delaying postharvest senescence in kiwifruits. Furthermore, similar findings in kiwifruits have been observed when treated with 24-epibrassinolide [6].

The ripening process in plant has been seen as an oxidative phenomenon accompanied by burdens of ROS (H$_2$O$_2$ and O$_2$$^{-}\text{•}$) [35]. The ROS burst may result in oxidative stress mediated damages to the plant tissue specifically the membranes. Herein, the citral-treated kiwifruits had significantly lower level of O$_2$$^{-}\text{•}$ production rate, which suggested that citral treatment was better for protecting kiwifruit against the ROS-mediated oxidative damages. The elimination of ROS is linked with the activities of antioxidant enzymes such as SOD, CAT, and POD in fruits and vegetables [6, 13, 36]. SOD plays a key role in the dismutation of O$_2$$^{-}\text{•}$ into H$_2$O$_2$, while H$_2$O$_2$ is obliterated by CAT which is indispensable for the detoxification of ROS [11]. POD is the key enzyme in the biosynthesis and oxidation of phenolics compounds [37]. Therefore, higher antioxidant enzyme activities and their coordinated actions might be the underlying mechanism for reducing lipid peroxidation and delaying senescence in horticultural fruits [35, 36]. The exogenous treatment of methyl jasmonate (MeJA) help induce the increased activities of POD and SOD, thereby reducing oxidative damage and enhancing disease resistance in kiwifruit [3].
Meanwhile, alginate oligosaccharide (AOS) treatment elevated SOD and CAT activities, which contributed to enhancing abiotic stress resistance and delaying postharvest senescence in kiwifruits [2]. Herein, citral treatment induces higher levels of SOD, CAT, and POD activities compared with the respective controls. Thus, the findings suggested that citral treatment effectively induces the increases in antioxidant enzymes activities and helps coordinate the balance of ROS level in kiwifruits. The link between these antioxidant enzymes might be the major factor that triggers the oxidation resistance, thereby maintaining postharvest quality and prolonging storage time of kiwifruits.

5. Conclusions
The beneficial effects of citral treatment on postharvest quality and antioxidant capacity of 'Jinkui' kiwifruit were investigated in the present study. The study revealed that citral treatment has significantly decreased fruit weight loss, delayed softening, alleviated senescence progress, and maintained higher levels of antioxidant contents that help boost antioxidant enzyme activities in comparison with the respective control fruits during storage. The alleviation of postharvest senescence in kiwifruits treated with citral might be attributed to maintaining antioxidant capacity, higher levels of AsA content, TPC, and TFC, contributing to enhanced DPPH scavenging capacity and FRAP. Furthermore, weight loss, respiration rate, and ripening index were lower in the citral-treated kiwifruits than those in the control fruits, which have been dedicated to less consumptions of nutrients and have got extended storage time. Therefore, it is concluded that citral treatment could serve as a potential and feasible preservation method for delaying postharvest senescence decay and maintaining antioxidant capacity in 'Jinkui' kiwifruit.

Data Availability
All the statistical data used to support the findings of this study are included within the paper.

Conflicts of Interest
The authors declare no conflicts of interest.

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References