Combined Inhibitory Effect against Postharvest Storage Rots and Their Effects on Postharvest Quality Parameters in Cherry Tomatoes by Cassia Oil and Calcium Chloride

WU FENG,1,2 XIAODONG ZHENG,2* AND JIAPING CHEN1

1College of Food Science and Technology, Huazhong Agricultural University, 430070 Wuhan, Hubei, People’s Republic of China; and 2Department of Food Science and Nutrition, Zhejiang University, 310029 Hangzhou, Zhejiang, People’s Republic of China

ABSTRACT

The inhibitory effect of cassia oil alone or in combination with calcium chloride (CaCl2) against Alternaria alternata in vitro and in vivo was assessed on cherry tomatoes. The results demonstrated that concentrations of CaCl2 ranging from 0.25 to 3% enhanced the inhibitory effects of 200 μl of cassia oil per liter on the growth of A. alternata in vitro. The combination of 0.25% CaCl2 and 500 μl of cassia oil per liter showed a significant inhibition effect on decay development in both wounded artificially infected and unwounded naturally infected fruit. Importantly, these treatments did not reduce the overall quality of tomatoes. Defense-related enzyme activities were also evaluated. The results indicated that cassia oil alone or in combination with CaCl2 significantly enhanced defense-related enzyme activities, such as peroxidase and polyphenol oxidase. Together, these data suggest that the combination of cassia oil and CaCl2 may be an efficient method to limit cherry tomato decay caused by fungi.

Fruit and vegetables are highly perishable products, especially during the postharvest phase, when considerable losses can occur (13, 27). Because of their high moisture content and rich nutrients, fresh fruit and vegetables are susceptible to postharvest diseases caused by various phytopathogenic fungi. Cherry tomatoes (Lycopersicon esculentum) are one of the most widely produced and consumed horticultural crops in the world, both for the fresh produce market and the processed food industries. Cherry tomatoes are susceptible to postharvest diseases caused by various pathogens. Postharvest rot of cherry tomatoes is caused mainly by fungal pathogens such as Alternaria alternata, Botrytis cinerea, Penicillium spp., and Alternaria solani (1, 8). Currently, synthetic fungicides are the primary means to control postharvest diseases of fruit and vegetables. But there is increasing concern over the level of synthetic chemical residues in food as well as the increasing resistance of many fungi to commonly used fungicides (6, 28). Thus, there is a considerable interest in exploring new alternatives in order to reduce the use of synthetic fungicides.

Essential oils are a promising alternative to synthetic fungicides. Essential oils are complex volatile compounds produced in different plants. Essential oils are known to have various functions in plants including conferring pest and disease resistance, and some essential oils have significant antifungal properties (3, 16). Many applications for controlling the growth of foodborne pathogens and food spoilage bacteria have been developed, using these essential oils as natural food preservatives (11, 31). In addition, application of essential oils has been shown to improve the shelf life, quality, and nutritional value of stored food commodities (29).

Inorganic salts have been reported to inhibit the growth of various postharvest fungi. In particular, postharvest treatments with calcium chloride (CaCl2) have been proposed as safe and effective means to control fruit rotting (30). There have been reports about essential oils in combination with other agents such as sodium citrate and monolaurin (4). Treatments of plants with essential oil have been shown to induce various responses. A previous study indicates that in plants, phenolic contents and defense-related enzymes may be affected by essential oil due to the antioxidant and antimutagenic phenolics in some essential oils such as cinnamon (14). However, reports about the combined inhibitory effect of essential oil and CaCl2 are still lacking. To the best of our knowledge, there are no reports on induction of defense responses by cassia oil and CaCl2 or on their inhibitory effects against fungal pathogens in cherry tomatoes during storage.

The main objectives of the present study were to determine the inhibitory activity in vitro and in vivo of cassia oil in combination with CaCl2 against A. alternata, a common pathogen known to cause postharvest disease in cherry tomatoes. The postharvest qualities of cherry tomatoes after storage and defense-related enzyme activities were determined, and the possible mechanism involved was provided.
MATERIALS AND METHODS

Chemicals. Pure-grade (without synthetic chemicals or nonnatural components) essential oils of cassia were supplied from International Flavors & Fragrances Inc, Shanghai, China. The essential oils were stored in bottles at 4°C. CaCl\(_2\) and other reagents were purchased from Shanghai Chemical Reagent Company, Shanghai, China.

Fungi and cultures. A. alternata was obtained from the Institute of Microbiology, Chinese Academy of Sciences. A. alternata was grown on potato dextrose agar medium at 28°C for 7 days. Spore suspensions were prepared by flooding 7-day-old sporulating cultures of A. alternata with sterile distilled water. The spore levels of the pathogens were determined with a hemocytometer.

In vitro antifungal assay. Antifungal activity was measured following the method described by Feng and Zheng (9). The experiments were conducted in potato dextrose agar petri plates (90-mm diameter) with or without CaCl\(_2\) (concentrations varied from 0.25 to 3%, wt/vol) and 200 μl of cassia oil per liter. Plates were inoculated with 6-mm plugs from 7-day-old A. alternata cultures. Plates were incubated for 7 days at 28°C, and fungal growth was assessed by measuring radial growth diameter. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control. All experiments were performed in triplicate.

Inhibitory effect of cassia oil alone or in combination with CaCl\(_2\) on artificially inoculated fruit. Experiments were conducted with commercially grown cherry tomatoes (Lycopersicon esculentum, Mill Miny Tomatoes) from Yunnan, China. Fruit at the pink stage of maturity were selected for freedom of injuries and diseases and were placed in 1.5-liter plastic boxes. Fruit were dipped in a solution of 1% sodium hypochlorite for 2 min, rinsed with tap water, and air dried before wounding.

Cherry tomatoes were wounded with a sterile puncher to make one uniform wound, 2 mm deep by 5 mm wide, on their peel at the equatorial zone. Aliquots of 20 μl of 0.25% CaCl\(_2\) and 500 μl of cassia oil per liter, 0.25% CaCl\(_2\), 1% CaCl\(_2\) and 500 μl of cassia oil per liter, 1% CaCl\(_2\), 3% CaCl\(_2\) and 500 μl of cassia oil per liter, 3% CaCl\(_2\), 500 μl of cassia oil per liter, and sterile distilled water (control) were pipetted into each wound site. After 0.5 h, 500 A. alternata conidia were pipetted into each wound. Treated tomatoes were stored at 20°C and 85% relative humidity for 3 days, and then the percentage of infected fruit was recorded. Each treatment was replicated three times with 20 fruits per replicate, and the experiment was repeated twice.

Inhibitory effect of cassia oil alone or in combination with CaCl\(_2\) on naturally infected fruit. Before its use, cassia oil was dissolved in 25 ml of 0.05% Tween 20 and then mixed with 475 ml of 0.25% CaCl\(_2\) solution. Tomatoes were dipped into the solution for 1 min at room temperature and air dried, whereas control fruit were dipped into sterilized water. Treated tomatoes were stored at 20°C for 30 days. The percentage of infected fruit was recorded when about 50% of the control fruits had decayed. Each treatment was replicated three times with 20 fruits per replicate, and the experiment was repeated twice.

Postharvest quality of fruit. To evaluate the effect of essential oil alone or in combination with CaCl\(_2\) on postharvest quality of tomatoes, harvested fruit were treated and then stored under the storage conditions as described above. Quality parameters were measured before treatment and after storage. Quality measurements were made on three replicates of five fruit each and performed at ambient temperature (about 20°C). The testing methods are described below.

Fruit firmness values of each fruit were measured at two points of the equatorial region by using the GY-1 hand sclerometer (Hangzhou Top Instruments Co., Ltd., Hangzhou, China). The probe descended toward the sample with a uniform force and stopped at a depth of 10 mm. The 2,6-dichloroindophenol titrimetric method (2) was used to determine the ascorbic acid content of pressed fruit juice. Results were expressed as milligrams of ascorbic acid per 100 g of fresh sample (25). Total soluble solids were determined by measuring the refractive index of the same juice with a hand refractometer, and the results were expressed as percentages on 100 g of fresh weight (18). Titratable acidity was measured by titration with 0.1 N NaOH (pH 8.1); 4 g of juice diluted with 20 ml of distilled water was used for each replicate. Titratable acidity was calculated as percentage of malic acid (32). Color readings of the fruit were performed with a chromameter.
FIGURE 3. Effects of cassia oil on naturally infected development in unwounded cherry tomatoes. The percentage of infected fruits was recorded after 30 days. (A) Control; (B) 500 μl of cassia oil per liter; (C) 0.25% CaCl₂; (D) 0.25% CaCl₂ and 500 μl of cassia oil per liter. Significant differences (P < 0.05) between means are indicated by letters above the histogram bars. Where the letters are the same, there is no significant difference between different treatments.

(WSC-S, Shanghai, China). Color changes were quantified in the L*, a*, b* color space. Chroma \( C^* = (a^*2 + b^*2)^{0.5} \) and hue angle \( h^* = \tan^{-1}(b^*/a^*) \) when \( a^* > 0 \) and \( b^* > 0 \) or \( h^* = 180° + \tan^{-1}(b^*/a^*) \) when \( a^* < 0 \) and \( b^* > 0 \) were calculated from \( a^* \) and \( b^* \) values \((17)\). L* refers to the lightness, ranging from 0 for black to 100 for white. Chroma represents the color saturation, which varies from dull color (low value) to vivid color (high value), and hue angle is defined as a position on the color wheel, with red-purple at an angle of 0°, yellow at 90°, bluish green at 180°, and blue at 270° \((17)\).

Measurement of enzymatic activity. Fruit samples were wounded as described above. Samples were stored at 20°C and taken for measurement of enzyme activities at 0, 24, 48, 72, and 96 h after treatment. After removing the wound tissue with a sterile borer (5-mm diameter and 2-mm depth), the fresh tissue near the wound was harvested by another sterile borer (9-mm diameter and 5-mm depth). One gram of the fresh tissue was ground in a mortar and pestle with 10 ml of cold (4°C) 50 mM sodium phosphate buffer (pH 7.8) containing 1.33 mM EDTA and 1% (wt/vol) polyvinyl pyrrolidone. The homogenates were centrifuged at 4°C for 15 min at 27,000 g; the supernatant was used in enzyme activity assays, and protein content was determined. Protein content was measured using the Bradford assay \( \text{(5)} \) with bovine serum albumin as a standard. Three replicates per treatment were examined with 10 fruits per replicate, and the experiments were done in triplicate.

Peroxidase (POD; EC 1.11.1.7) activity was measured by using guaiacol as a substrate following the method described by Lurie et al. \((20)\). Reaction mixtures contained 3 ml of 50 mM sodium phosphate buffer (pH 6.4), 220 μl of 0.3% (vol/vol) guaiacol, 60 μl of 0.3% (vol/vol) H₂O₂, and 20 μl of crude enzyme extract. The reaction was initiated immediately by adding H₂O₂ at 30°C followed by incubating in a shaking water bath for 5 min, during which \( A_{470} \) was measured once every 30 s. One unit of the POD activity was defined as the amount of the enzyme extract producing an increase of \( A_{470} \) by 0.01 in 1 min and expressed as units per milligram of protein.

Polyphenol oxidase (PPO; EC 1.14.18.1) activity was determined according to the method of Mohammadi and Kazemi \((23)\). Briefly, 0.5 ml of enzyme preparation was added to 3 ml of catechol (100 mM sodium phosphate, pH 6.4, and 500 mM catechol) as a substrate, and absorbance was measured at 398 nm on an MK3 microplate reader. The activity was expressed in units per milligram of protein. One unit was defined as \( \Delta A_{398} \) of 0.1/min.

Statistical analyses. Statistical analyses of the data were performed with SPSS statistical software (SPSS for Windows v.11.5). The statistical significance was set at \( P < 0.05 \). When the analysis was statistically significant, Duncan’s multiple range test was applied to separate means.

RESULTS

In vitro antifungal assay. The mycelial growth of \( A. \ alternata \) on potato dextrose agar amended with 0.25 to 3% CaCl₂ was not significantly inhibited. However, the fungus was inhibited 34.2% by cassia oil at 200 μl/liter. The inhibitory effect of cassia oil was enhanced by amending with 0.25 to 3% CaCl₂ by percentages ranging from 44.4 to 88.8% \((\text{Fig. 1})\). The combination of 200 μl of cassia oil per liter and 3% CaCl₂ resulted in 88.8% inhibition of the growth of \( A. \ alternata \).

Inhibitory effect of cassia oil alone or in combination with CaCl₂ on artificially inoculated fruit. The results showed that cassia oil at 500 μl/liter could significantly reduce the decay incidence of cherry tomatoes compared with the control. The cherry tomatoes treated with 3% CaCl₂ alone or in combination with 500 μl of cassia oil per liter showed a small reduction in decay incidence compared with the control \((\text{Fig. 2})\). The combination of 500 μl of cassia oil per liter and 0.25% CaCl₂ was the most effective treatment, and the percentage of decayed tomatoes was only 38.3% compared with 97.5% decayed tomatoes in the control.

Inhibitory effect of cassia oil alone or in combination with CaCl₂ on naturally infected fruit. The combination of CaCl₂ and cassia oil resulted in a synergistic

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( L^* )</th>
<th>( C^* )</th>
<th>Hue (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (before storage)</td>
<td>39.49 ± 0.13 A</td>
<td>48.65 ± 2.16 AB</td>
<td>41.30 ± 0.36 A</td>
</tr>
<tr>
<td>Cassia oil (500 μl/liter)</td>
<td>35.28 ± 0.65 B</td>
<td>46.24 ± 1.53 AB</td>
<td>45.25 ± 6.41 A</td>
</tr>
<tr>
<td>CaCl₂ (0.25%)</td>
<td>35.33 ± 0.61 B</td>
<td>45.07 ± 0.62 B</td>
<td>42.99 ± 7.57 A</td>
</tr>
<tr>
<td>Cassia oil (500 μl/liter) and 0.25% CaCl₂</td>
<td>37.99 ± 0.46 C</td>
<td>45.74 ± 0.87 AB</td>
<td>44.75 ± 1.06 A</td>
</tr>
</tbody>
</table>

\( L^* \), lightness; \( C^* \), chroma. Values (means ± standard deviations) followed by the same letters show no significant difference between treatments. The data were recorded after 30 days’ storage at 20°C.
Effect on inhibiting natural decay of cherry tomatoes compared with cassia oil and CaCl₂ application alone. The CaCl₂ concentration was most effective at 0.25% but less effective when increased up to 1 or 3% (Fig. 3). The concentration of decayed cherry tomatoes treated with 500 μl of cassia oil per liter and 0.25% CaCl₂ was reduced by about 35% compared with the control and about 20% compared with the cherry tomatoes treated with 500 μl of cassia oil per liter alone.

Postharvest quality of fruit. Analysis of variance for color attributes of cherry tomatoes showed that essential oil and CaCl₂ had no significant effect on hue angle and chroma, while lightness was affected (Table 1). Cherry tomatoes treated with 0.25% CaCl₂ alone or in combination with 500 μl of cassia oil per liter showed only a very slight decrease in lightness values. The essential oil and CaCl₂ had no significant effect on cherry tomatoes’ firmness, soluble solids, ascorbic acid, or titratable acidity after 30 days’ storage at 20°C (Table 2).

Measurement of enzymatic activity. The activity of POD in control fruit increased slightly between 24 and 72 h of storage and then decreased gradually during the remainder of storage (Fig. 4). The POD activity for all treatments decreased at 96 h compared with the values at 72 h. POD activity in fruit treated with 0.25% CaCl₂ in combination with 500 μl of cassia oil per liter increased gradually during the first 72 h of storage and reached the highest value at 72 h.

The PPO activity of control cherry tomatoes changed little within 96 h of storage at 20°C (Fig. 5). PPO activity reached its highest value at 48 h in fruit treated with 0.25% CaCl₂ in combination with 500 μl of cassia oil per liter, and the level was almost 12-fold more than that in control fruit treated with distilled water. CaCl₂ or 500 μl of cassia oil per liter stimulated the PPO activity of cherry tomatoes, resulting in approximately 3.75-fold and 7-fold increases within 72 h after inoculation compared with the water control.

**DISCUSSION**

This work highlights the combined inhibitory effect of cassia oil and CaCl₂ on *A. alternata* in vitro and in vivo. Under in vitro conditions, the percentage of inhibition was dependent on the CaCl₂ concentration: the higher the CaCl₂ concentration was in combinations with 200 μl of cassia oil per liter, the more significant the inhibition of mycelial growth was. However, the inhibitory effect of cassia oil and CaCl₂ was not as dramatic in vivo as in vitro. Cassia oil at 500 μl/liter in combination with 0.25% CaCl₂ showed a greater combined inhibitory effect on *A. alternata* on both wounded and unwounded cherry tomatoes. The need to use essential oils at higher concentrations in fruit than in laboratory media is likely due to the more complex growth environment in fruit, which provides microbial cells with greater protection from antifungal agents. The pulp of the tomatoes absorbs the antifungal agent, thus decreasing the concentration in situ and hindering its antifungal action (10, 24). The results proved that inhibitory effects in vitro do not always accurately represent the efficacy of those exerted in vivo (26). The results showed that using 0.25% CaCl₂ in combination with 500 μl of cassia oil per liter resulted in better control of *A. alternata* on tomatoes than use of cassia oil or CaCl₂ alone. CaCl₂ at 1 and 3% did not enhance the inhibitory effect of cassia oil in vivo. The concentration of CaCl₂ that could enhance the antifungal effect of cassia oil in vivo was lower than the 1% concentration combined with other antifungal agents reported in other studies (12). It seems difficult to explain that at the higher concentration of 1 or 3%, the enhanced effect of cassia oil and CaCl₂ was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Firmness (×10⁵ Pa)</th>
<th>Soluble solids (%)</th>
<th>Ascorbic acid (mg/100 g)</th>
<th>Titratable acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (before storage)</td>
<td>10.3 ± 0.16 a</td>
<td>8.7 ± 0.42 a</td>
<td>39.1 ± 4.73 a</td>
<td>0.50 ± 0.03 a</td>
</tr>
<tr>
<td>Control (after storage)</td>
<td>10.2 ± 1.16 a</td>
<td>8.7 ± 0.64 a</td>
<td>41.3 ± 0.59 a</td>
<td>0.42 ± 0.04 a</td>
</tr>
<tr>
<td>Cassia oil (500 μl/liter)</td>
<td>9.6 ± 2.22 a</td>
<td>8.4 ± 0.87 a</td>
<td>38.7 ± 0.78 a</td>
<td>0.42 ± 0.08 a</td>
</tr>
<tr>
<td>CaCl₂ (0.25%)</td>
<td>11.0 ± 1.31 a</td>
<td>8.3 ± 0.64 a</td>
<td>39.9 ± 2.58 a</td>
<td>0.46 ± 0.02 a</td>
</tr>
<tr>
<td>Cassia oil (500 μl/liter) and 0.25% CaCl₂</td>
<td>10.6 ± 1.42 a</td>
<td>8.9 ± 0.12 a</td>
<td>43.3 ± 0.97 a</td>
<td>0.51 ± 0.04 a</td>
</tr>
</tbody>
</table>

* Values (means ± standard deviations) followed by the same letters show no significant difference between treatments. The data were recorded after 30 days’ storage at 20°C.
lost. Some reports indicated that CaCl₂ increases resistance of host tissue (7, 22), which may help cassia oil in controlling fungal pathogens. But from the present studies, the mechanism(s) by which CaCl₂ can improve the efficacy of cassia oil is still not fully understood.

Some studies suggest that there is a direct relationship between increase of POD and PPO activity and natural resistance of fruit. Activation of POD and PPO is associated with triggering and accelerating infection and senescence processes in harvested fruit or other plant tissues. Liu et al. (19) found that the activities of PPO and POD and the level of phenolic compounds in chitosan-treated fruit increased significantly at 25 and 2°C. Yu et al. (33) also reported that the combination of Cryptococcus laurentii and plant hormone rapidly increased POD activity on pears. But Jin et al. (15) found that hot air and methyl jasmonate vapor treatment resulted in lower activities of PPO and POD than in the control in peach fruit. The underlying factors that result in the changes of PPO and POD activities by different treatments need further study.

Our data suggest that the combination of CaCl₂ and essential oils may be a successful strategy for controlling cherry tomato rot during postharvest storage. However, further study on the mechanism of essential oils in combination with salts against fungal pathogens is needed.

ACKNOWLEDGMENTS

This research was supported by grants from the National Natural Science Foundation of China (NNSFC-30170659) and the PhD Programs Foundation of Ministry of Education of China (PPFMRC-20040335025).

REFERENCES


