**ABSTRACT**

Zucchini squash slices dipped in solutions of CaCl₂ alone or with chlorine were stored at 0°C, 5°C, and 10°C. Slices developed water-soaked areas (chilling injury) at 5°C and brown discoloration at 5°C and 10°C, which increased with storage. The amount and severity of chilling injury/browning/decay of water-dipped controls were least at 5°C. Calcium treatments helped in reducing development of decay, rate of total microbial count increase of carrot shreds (Izumi and Watada, 1994). Quality of zucchini squash slices is difficult to maintain because of perishability and injury caused by mechanical actions of slicing, which hastens quality deterioration of lightly processed vegetables (Rolle and Chism, 1987; Watada et al., 1990).

Calcium (Ca) has an important role in maintaining quality of fruits and vegetables in respect to structural integrity of membranes and cell walls (Bangerth, 1979; Poovaiah, 1986). Ca binds anionic groups of all membranes to form bridges between structural components, thereby maintaining cell permeability and compartmentation and structural integrity (Bangerth, 1979; Conway et al., 1992). Conway et al. (1992) reported that Ca bridges also resist accessibility to enzymes produced by the fruit and material pathogens that cause decay. This results in calcium-induced resistance to decay in apples and potatoes. Ca treatments retain firmness and/or reduce browning with cut produce such as slices of apple (Ponting et al., 1972), strawberry (Morris et al., 1985; Rosen and Kader, 1989), and pear (Rosen and Kader, 1989). Such treatments helped in retaining textural quality and reducing the rate of total microbial count increase of carrot shreds (Izumi and Watada, 1994).

Our objective was to determine the effects of Ca treatments on quality of zucchini squash slices stored at 0°C, 5°C, and 10°C. The 0°C was used to determine if Ca treatments were beneficial in reducing chilling injury. Chlorine is widely used to reduce postharvest decay of fruits and vegetables as a disinfectant (Smith, 1962), while Ca enhances tissue resistance to fungal or bacterial attack by stabilizing or strengthening cell walls (Conway and Sams, 1984). Addition of chlorine to CaCl₂ seemed to have some benefits at 0°C or 10°C.

**Key Words:** zucchini squash, lightly processed, calcium, ascorbic acid, texture

**INTRODUCTION**

Zucchini squash is highly perishable (Lorenz, 1951) and sensitive to chilling temperatures (Mencarelli et al., 1983; Wang and Ji, 1989). The storage life of whole fruits is 1 to 2 weeks, even when held at recommended temperatures, 5°C to 10°C (Hardenburg et al., 1986). Quality of zucchini squash slices is difficult to maintain because of perishability and injury caused by mechanical actions of slicing, which hastens quality deterioration of lightly processed vegetables (Rolle and Chism, 1987; Watada et al., 1990).

Calcium (Ca) has an important role in maintaining quality of fruits and vegetables in respect to structural integrity of membranes and cell walls (Bangerth, 1979; Poovaiah, 1986). Ca binds anionic groups of all membranes to form bridges between structural components, thereby maintaining cell permeability and compartmentation and structural integrity (Bangerth, 1979; Conway et al., 1992). Conway et al. (1992) reported that Ca bridges also resist accessibility to enzymes produced by the fruit that cause softening and to enzymes produced by fungal or bacterial pathogens that cause decay. This results in calcium-induced resistance to decay in apples and potatoes. Ca treatments retain firmness and/or reduce browning with cut produce such as slices of apple (Ponting et al., 1972), strawberry (Morris et al., 1985; Rosen and Kader, 1989), and pear (Rosen and Kader, 1989). Such treatments helped in retaining textural quality and reducing the rate of total microbial count increase of carrot shreds (Izumi and Watada, 1994).

Our objective was to determine the effects of Ca treatments on quality of zucchini squash slices stored at 0°C, 5°C, and 10°C. The 0°C was used to determine if Ca treatments were beneficial in reducing chilling injury. Chlorine is widely used to reduce postharvest decay of fruits and vegetables as a disinfectant (Smith, 1962), while Ca enhances tissue resistance to fungal or bacterial attack by stabilizing or strengthening cell walls (Conway and Sams, 1984). Addition of chlorine to the Ca solution was evaluated to determine if it reacted synergistically with Ca.

**MATERIALS & METHODS**

**Sample preparation**

Zucchini squash (Cucurbita pepo L. cv. Elite, 156 ± 34g) were freshly harvested at a farm in Beltsville, MD. They were washed with water and after 2 cm of blossom and stem ends were sliced away, the midsection was cut transversely into 5 mm thick slices with a Cuisinart Food Processor (Model DLC-10; Cuisinarts Corp., East Windsor, NJ). About 600g of slices were dipped in 2L solution of 0.5% CaCl₂, 0.5% CaCl₂ + 100 ppm sodium hypochlorite (ca 5 ppm available chlorine), or distilled water for 2 min at room temperature (23°C). Treated slices were washed for 10 sec. and air-dried for 5 min under a stream of air. A Beltsville washing machine to remove surface solution. A 150-g sample (~30 slices) was placed in a 1L tray and three trays were placed in a 10L flat container with 2L of distilled water at the base. The 10L container was inserted into a polyethylene bag and a stream of humidified air was provided such that the CO₂ level did not exceed 0.5%. Three replicates of each treatment were stored at 0°C, 5°C, and 10°C for 17, 16, and 12 days, respectively.

**Ca concentration**

Following dip treatment and centrifugation, about 20g of flesh (mesocarp) tissues from each treatment were immediately frozen in liquid nitrogen, freeze-dried, and ground for Ca analysis. Ca concentration was determined in triplicate with an atomic absorption spectrophotometer and recorded on a dry-weight basis (Conway and Sams, 1984).

**Visible appearance**

From each treatment 30 slices were evaluated daily for percentage and severity of injury and subsequent decay throughout storage. Samples were scored on a scale of 0 to 4, with 0 = no abnormality, 1 = slight, 2 = moderate, 3 = severe, and 4 = extreme chilling injury/browning/decay, and the severity index (SI) was expressed as: SI = [(No. of slices ranked slight × 1) + (No. of slices ranked moderate × 2) + (No. of slices ranked severe × 3) + (No. of slices ranked extreme × 4)]/30 slices × 4.

**Analytical determinations**

A 150-g sample (~30 slices) from each lot was removed at scheduled dates for analyses of weight, L-ascorbic acid content, texture, total microbial count, and color.

L-ascorbic acid in the flesh was determined using HPLC according to a modified method of Kissingler and Pachla (1987) and Vanderslice and Higgs (1990). A 3-g sample was finely sliced into pieces and homogenized with 10–20 mL 0.1M citric acid solution containing 1 μM EDTA and 0.1 μM diethyldithiocarbamic acid under N₂, with a Polytron homogenizer (Model Pt 10–35). The extract was filtered through Whatman #1 filter paper, through a glass fiber filter, and then through a 0.45 μM nylan/glass disposable membrane. An aliquot (20 μL) of filtrate was injected onto PLRP-S 100A column (25 cm × 4.6 mm, 5 μm) (Polymer Laboratories, Co.) installed in a Waters Model 510 HPLC pump and Waters Model 712 WISP auto injector, and F/CAEC Model 480 electrochemical detector (glassy carbon at 0.7V vs Ag/AgCl). The mobile phase was 0.02M KH₂PO₄ (pH 2.2) and the flow rate was 1 mL/min. L-ascorbic acid was identified by comparison of retention times (5 min) with standard solution of L-ascorbic acid and the amount was determined by comparing measured peak areas to standard curves. Texture was based on force required to shear a 40g sample, which was cut in half and placed in the cell box, and was expressed in newtons (N) as previously described (Izumi and Watada, 1994). Total microbial count on the surface of 10g tissues was determined and was expressed as log₁₀ count/g sample as outlined earlier (Izumi and Watada, 1994).

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Table 1—Percentage and severity of chilling injury/browning/decay of zucchini squash slices treated with calcium solution as related to storage temperature

<table>
<thead>
<tr>
<th>Storage temp (°C)</th>
<th>Days in storage</th>
<th>% of slices with injury/decay</th>
<th>Severity index of injury/decay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.5% CaCl₂</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
<td>0.0</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>13.3</td>
<td>6.7</td>
<td>10.0</td>
</tr>
<tr>
<td>10</td>
<td>100.0</td>
<td>90.0</td>
<td>13.3</td>
</tr>
</tbody>
</table>

*Chilling injury denoted by water-soaked tissue.*

RESULTS & DISCUSSION

CA CONCENTRATION of the zucchini squash slices increased by 1.7- and 1.6-fold (P ≤ 0.05) when treated with 0.5% CaCl₂ with and without chlorine, respectively, as compared with 1.0 mg Ca/g dry weight of control slices. The slices developed chilling injury and/or natural deterioration symptoms with storage (Table 1). At 0°C, water soaked areas developed on the cut surface by day 13, which probably was caused by the chilling temperature. The symptoms became very severe when slices were transferred to 20°C for 2 days (data not shown). Pitting on the skin, the typical symptoms of chilling injury with zucchini (Mencarelli et al., 1983; Wang and Ji, 1989), did not develop with slices held at 0°C. At 5°C and 10°C, water soaked areas did not develop; however, the cut surface became discolored (brown) by day 13 and 9, respectively, which probably was due to natural deterioration. Tissues began to deteriorate and decay shortly after development of water soaked areas or brown discoloration. The data on water-soaked areas, brown discoloration, and decay were combined in determining percentage of slices with the undesirable condition or in calculating the severity index of the undesirable condition.

Among the control samples, the percentage and severity index of chilling injury/browning/decay were the least with samples held at 5°C. The disorders were less at 5°C than at 10°C, because the natural deterioration was delayed more and the amount of chilling injury was less at 5°C. Whereas at 0°C, although the natural deterioration was delayed to a greater extent, the severity of chilling injury was much more severe than that at 5°C. This resulted in less disorder at 5°C, than at 0°C or 10°C.

Ca treatment was beneficial at 0°C and 10°C as indicated by the lower severity index with the Ca treated samples than that of control. Ca probably maintained the integrity of the tissue and hence, the tissue had increased resistance to injury by chill-
ing at 0°C or to increased rate of deterioration at 10°C. At 5°C, Ca did not have any effect on percentage or severity index of chilling injury/browning/decay. Ca reduced browning of apple slices stored at 1°C (Ponting et al., 1972) and pears stored at 2.5°C (Rosen and Kader, 1989). Addition of chlorine to CaCl₂ did not have any effect at 5°C or 10°C, but was beneficial in reducing decay following chilling injury at 0°C.

The zucchini slices lost weight during storage and the total loss of control samples approached 2% at 0°C and 5°C and 2.7% at 10°C. Ca treatment reduced weight loss by 15% and 40% at 0°C and 10°C respectively, but not at 5°C (data not shown).

L-ascorbic acid content began to decrease after development of chilling injury symptoms on day 12 at 0°C and before occurrence of browning on day 2 at 10°C (Fig. 1). The content did not decrease at 5°C. Ca treatments reduced the rate of decrease at 0°C and 10°C at the end of storage. By day 17, control samples at 0°C lost all L-ascorbic acid, while the Ca-treated samples still had 1/3 of the initial contents. Ca-treated samples with and without chlorine had two and three times higher L-ascorbic acid content respectively than controls on day 12 at 10°C. Chlorine seemed to enhance the effect of CaCl₂ in reducing L-ascorbic acid loss at 0°C or 10°C, which may relate to the effect on reducing chilling injury/browning, rather than a direct effect of chlorine. No consistent changes or differences were noted between controls and Ca-treated samples at 5°C.

Occurrence of chilling injury accompanied by browning and the simultaneous destruction of L-ascorbic acid content have been reported with chill sensitive fruits and vegetables such as pineapple (Miller, 1951; Miller and Heilman, 1952), sweet potato (Lieberman et al., 1959; Izumi et al., 1984), and sweet pepper (Yamauchi et al., 1978). It appeared that loss of L-ascorbic acid was not due to oxidative destruction but related to a non-enzymatic decrease associated with reduction of quinones to phenols in the presence of phenol groups (Miller and Heilman, 1952). Yamauchi et al. (1978) reported that the oxidized ascorbate reductase activity decreased with increased conversion of L-ascorbic acid to dehydroascorbic acid (DHA) by non-enzymatic reduction of quinones. With zucchini squash slices, DHA and phenol contents were not measured; however, the desirable effects of CaCl₂ with or without chlorine on L-ascorbic acid content may be involved in reduction of chilling injury/browning in slices at 0°C and 10°C with Ca treatments (Table 1).

The shear force of control samples decreased 34% and 16% after occurrence of chilling injury/browning at 0°C and 10°C respectively, but not at 5°C (Fig. 2). Ca treatments were beneficial in maintaining shear force of slices stored at 0°C and 10°C, probably by stabilizing membranes and cell walls by cross-linkage of pectin components (Bangerter, 1979; Poovaiah, 1986). Ca treatments were reported to maintain firmness of strawberry slices (Morris et al., 1985; Rosen and Kader, 1989), pear slices (Rosen and Kader, 1989), and carrot shreds (Izumi and Watada, 1994). Ca was beneficial at 0°C and 10°C, but not at 5°C, probably because the integrity of membranes and cell walls were not affected at 5°C by either chilling injury or senescence.

The total microbial count on the surface of the tissue increased with all samples during storage and was higher at 0°C than 5°C because of greater chilling injury at 0°C (Fig. 3). The count was lower with Ca-treated samples than controls held at 0°C and 10°C on the last day, but not at 5°C. This trend was similar to that of the development of chilling injury/browning/decay in slices stored at each temperature (Table 1). The inhibitory effect of Ca was noted only later in storage, possibly due to the effects of Ca on stabilizing or strengthening cell walls giving the tissue resistance to bacterial infection rather than to a bactericidal action (Conway and Sams; 1984). The addition of chlorine to CaCl₂ did not affect growth of microbes, although chlorine is believed to reduce the initial microbial load of product as a disinfectant (Smith, 1962). This may result from the reduced initial level of microflora of controls by the dip or the limited improvement in microbicidal effect of chlorine due to the lack of pH adjustment and surfactant (Adams et al., 1989). Appropriate pH would lie between 6.5 and 7.5 for an effective sanitizing chlorine solution (Boyette et al., 1993), while the solution we used was about pH 5 because of addition of 0.5% CaCl₂.
Fig. 3—Total microbial count on surface tissue of zucchini squash slices treated with calcium solutions and stored at 0°C, 5°C, and 10°C. Vertical lines represent SE. SE bars were not shown when masked by the graph symbol.

Fig. 4—L* and hue angle (tan⁻¹ b/a) values of zucchini squash slices treated with calcium solutions and stored at 0°C, 5°C, and 10°C. Vertical lines represent SE. SE bars were not shown when masked by the graph symbol.
Color, as expressed as the \( L^* \) and hue angle (\( b^*/a^* \)) values, did not change with slices held at 0°C and 5°C. However, it decreased after day 6 only in the control slices at 10°C, and the decrease in \( L^* \) and hue angle values coincided with the onset of browning and decay (Fig. 4 and Table 1). Water soaked areas from chilling injury were noted visually in slices held at 0°C, but they were not measurable with the instrument. These results indicate that color deterioration was characterized mainly by browning and was inhibited by Ca.

**CONCLUSIONS**

Ca concentration of zucchini squash slices was increased by a 0.5% CaCl\(_2\) dip. Benefit of CaCl\(_2\) treatment on storage quality was noted at 0°C, where chilling injury prevailed, and at 10°C, where chilling injury was minimal. The desirable effect of Ca was somewhat enhanced by addition of chlorine. Holding at 5°C was recommended for preservation of zucchini squash slices, however, if zucchini slices are stored at higher or lower than 5°C, Ca treatments would help in reducing quality deterioration.

**REFERENCES**


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