

Efficacy of sodium chlorite as an inhibitor of enzymatic browning in apple slices

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Abstract

Sodium chlorite (SC) is an effective sanitizer for inhibiting microbial growth. This investigation was conducted to determine the efficacy of SC as a browning control agent for use on fresh-cut apple slices, applied alone, or in conjunction with organic acids. Additionally, the authors compared the efficacy of SC to that of acidified sodium chlorite (ASC) and to several other salts and examined the effect of pH and several different organic acids on efficacy of SC. The fresh-cut apple slices were dipped in treatment solutions for 1 min, then drained and placed in plastic containers at 20 °C for 24 h, and finally stored in polyethylene bags at 5 °C for 2 weeks. Color was measured periodically during storage. Lightness (L) values for all treated and control samples measured at 4 h, 24 h, and 2 weeks of storage were compared to L value for untreated samples measured immediately after cutting. Percent decrease in L -values was calculated for each sample at each time interval. Apple slices treated in ASC or SC solution had a significantly smaller decrease in L value indicating less browning than those treated in citric acid or water control at 4 h ($P < 0.01$), and with the exception of 1 g L⁻¹ ASC and 0.1 g L⁻¹ SC, all other ASC and SC treated slices still had significantly less browning than those for the water control ($P < 0.01$) at 24 h. After 2 weeks of storage, only SC (0.5–1.0 g L⁻¹), sodium bisulfite (0.5 g L⁻¹) and calcium L-ascorbate (10 g L⁻¹) continued to inhibit browning. Treatment with 0.5 g L⁻¹ SC and pH adjusted in the range from 3.9 to 6.2 using citric acid (CA) reduced browning more effectively than 0.5 g L⁻¹ SC without pH adjustment. Two organic acids, salicylic acid and cinnamic acid, when added to SC solution, were found to achieve even better inhibition of browning than CA at the same pH value.

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1. Introduction

Fresh-cut apples have recently emerged as popular snacks in food service establishments, school lunch programs, and for family consumption (Gorny, 2003). The market for fresh-cut apples is projected to continue growing as more consumers demand fresh, convenient and nutritious foods. However, the fresh-cut industry is still thwarted by product quality deterioration caused by physiological disorders induced by cut-wounding, microbial

growth in the exudates from the cut surface of produce, and unsatisfactory processing conditions.

Browning is the main physiological disorder that impairs sensory properties and discourages consumer purchase of fresh-cut apples. Enzymatic browning reactions in fruits are primarily catalyzed by polyphenol oxidase (PPO) in the presence of oxygen (Martinez & Whitaker, 1995). Extensive research has been focused on control of browning in fresh-cut apples and several approaches to browning inhibition have been explored.

Inhibitors of enzymatic browning fall into six categories based on their mode of action as reviewed by McEvily, Iyengar, and Otwell (1992). These six groups

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comprise reducing agents, chelating agents, complexing agents, acidulants, enzyme inhibitors and enzyme treatments. Most inhibitors are reducing agents and acidulants; there are no reports of the inhibitory effects of oxidizing agents and alkaline substances on browning of apple slices.

Sodium chlorite (SC) is an oxidizing agent, which is able to generate chlorine dioxide gas in an acidic environment. Chlorine dioxide, also a powerful oxidizing agent, may be used in applications such as disinfecting food (Mullerat, Klapes, & Sheldon, 1994; Zhang, Lu, & Levin, 2003) and purifying drinking water (Sussman, 1983). In our earlier study, we found that SC inhibited the browning reaction in addition to the control of microbial growth. The acidified form of SC is a highly effective antimicrobial agent, which is produced by lowering the pH (2.5–3.2) of a solution of SC with any GRAS acid (Warf, 2001). The FDA has approved its use on raw fruits and vegetables in the range of 0.5–1.2 g L⁻¹ followed by a potable water rinse (Anonymous, 2000).

The control of cut-surface browning and growth of disease-causing microorganisms is critical to maintaining the quality and safety of fresh-cut produce. However, the incompatibility between the use of commercially available browning inhibitors (reducing agent-based) and conventional anti-microbial treatments (oxidant-based) presents a major technical challenge to the industry. Our preliminary experiment found that sodium chlorite, an anti-microbial agent, had a strong inhibitory effect on the browning reaction of fresh-cut apples. Further studies on its mechanism of browning inhibition indicated that SC is a mixed type of inhibitor for polyphenol oxidase activity, the key enzyme responsible for browning reaction (Lu, Luo, & Feng, 2006). In this study, we evaluated the efficacy of SC with or without organic acid on browning control of freshly prepared apple slices. The main purposes of this study were to compare the effectiveness of browning inhibition by SC and other known browning and PPO inhibitors and to identify the most effective SC-based formula for browning inhibition of apple slices. The experiment comprised four parts. In the first part, apple slices were treated with solutions of the two components of SANOVA (Alcide Corporation's trade name for acidified sodium chlorite), SC and citric acid (CA), separately, and in combination (ASC), at a series of different concentrations and with a water control. In the second segment, slices treated with a variety of different saline solutions were compared to the SC treatment and to the water control. In the third part, the pH of a 0.5 g L⁻¹ SC solution was adjusted, using different concentrations of citric acid. In the final phase, several different organic acids were used to adjust the pH of a 0.5 g L⁻¹ SC solution to the same value of 6.2. In each part of the experiment, color measurements were taken for treated and control apple slices after different storage durations to evaluate the efficacy of treatments on browning control.

2. Materials and methods

2.1. Apples

Red Delicious apple (*Malus domestica* Borkh.) was chosen for this study for its high susceptibility to browning. Apples grown in Washington state were purchased from a wholesale market in Jessup, Maryland, stored at 0 °C and used within 3 months of harvest.

2.2. Treatment solutions

The two components of SANOVA (acidified sodium chlorite), sodium chlorite, 250 g L⁻¹ and citric acid 500 g L⁻¹ solutions were provided by Alcide Corporation (Redmond, WA, USA). For preparation of an acidified sodium chlorite solution in the first part of experiment, 500 g L⁻¹ CA solution was added to 250 g L⁻¹ SC solution in a ratio of 3/1 (w/w) according to the formula provided by the supplier. The concentrations of SC or ASC solutions used were 0.1, 0.25, 0.5 and 1.0 g L⁻¹, and those of CA solution were 0.6, 1.5, 3.0 and 6.0 g L⁻¹. All other chemicals, including solid sodium chlorite, citric acid, sodium hypochloride, sodium chlorate, sodium bisulfite, sodium ascorbate, sodium carbonate, sodium bicarbonate, sodium chloride, oxalic acid, salicylic acid, and cinnamic acid, were obtained from the Sigma-Aldrich company (St. Louis, MO, USA).

2.3. Apple slice preparation and treatment

For each part of the experiment, apples of uniform size and color were washed in tap water and cut into eight equal slices using a sharp stainless steel knife. One slice from each of eight different apples was pooled together and treated in one of the dipping solutions for 1 min, drained on a clean paper towel, and then stored at 20 °C in a covered plastic tray for 24 h. Paper towels wet with an equal volume of water for each box were used to minimize moisture loss. Samples were then packaged in 16 cm × 16 cm polyethylene bags for subsequent storage at 5 °C for 2 weeks for the shelf life study. Experiments were done in triplicate.

2.4. Color measurement

The color of apple slices was measured using a Minolta colorimeter (Minolta Co. Ltd, Japan) at 4 h, 24 h and 2 weeks. The *L* values reported were means of 16 readings; one reading from each side of each of the eight slices composing a replicate. The degree of browning was expressed by the percent decrease in the *L* value calculated by subtracting the *L* value of treated and control samples measured at evaluation times from the time zero *L* value obtained from untreated samples measured immediately after cutting, divided by the time zero value and multiplied by 100.

Table 1
Changes in pH values of ASC, SC and CA solutions before and after dipping apple slices

Treatment	ASC (g L ⁻¹)				SC (g L ⁻¹)				CA (g L ⁻¹)			
	0.1	0.25	0.5	1.0	0.1	0.25	0.5	1.0	0.6	1.5	3.0	6.0
Before dipping	2.9	2.6	2.4	2.2	7.6b	8.5b	9.3b	10.0b	2.7	2.5	2.3	2.1
After dipping	2.8	2.6	2.4	2.2	4.3a	4.4a	4.6a	5.0a	2.6	2.4	2.2	2.1

Mean value of three replicates within a column followed by a different letter are significantly different ($P < 0.01$).

2.5. Experimental design and data analysis

All quality evaluations were performed in a temperature controlled room at 5 °C to minimize the effect of temperature variation during testing. Data were analyzed as a two-factor linear model using the Proc Mixed procedure of SAS (SAS Inst., Cary, NC) with storage time and treatment as the factors.

3. Results and discussion

3.1. The effect of SC, CA and ASC concentrations on apple slice quality

The pH of solutions before and after dipping apple slices is shown in Table 1. The pH values of SC solutions ranged from 7.6 to 10.0 and increased with increasing concentration of SC; the pH ranges for ASC and CA solutions, were 2.2–2.9 and 2.1–2.7, respectively, and decreased with increasing concentration of ASC or CA. Only in the SC group was a significant reduction in pH seen after dipping apple slices, while there were only minor changes in the CA and ASC groups. The dramatic change in pH of SC solutions, shown in Table 1, may be attributed to the reaction between SC and the acidic apple juices. Fig. 1 shows the percent decrease in *L* values for the cut surfaces of apple slices with different treatments during storage. There were no significant differences in the % change in *L* values among the different CA treated apple groups ($P > 0.05$) at 4 h, but decreases in *L* values of the 1.5 g L⁻¹ and 3.0 g L⁻¹ CA treatments were significantly greater than that of the control ($P < 0.05$), indicating that 1.5–3.0 g L⁻¹ CA aggravated the browning of apple slices. However, the decrease in *L* values for ASC and SC treatment groups were significantly smaller than those for the control at 4 h ($P < 0.01$). These results indicate that both ASC and SC can inhibit the browning of apple slices and that the inhibitory role of ASC may be attributable to SC, a main component of ASC. The only significant difference among the different groups of ASC and SC treated apple slices, was significantly more browning for the 1.0 g L⁻¹ ASC treatment, possibly due to tissue damage caused by the high concentration. At 24 h, percent change in *L* values in the three treatment groups showed the same tendencies as at 4 h. However, significantly more browning occurred in slices treated with 3.0 g L⁻¹ CA than in those treated with 0.6 g L⁻¹ CA and significantly more browning occurred in 0.1 g L⁻¹ SC treated slices than in other SC treatment groups ($P < 0.05$). The percent

decreases in *L* values for ASC and SC treated slices were still significantly smaller than those for the control ($P < 0.01$) at 24 h, except for 1.0 g L⁻¹ ASC and 0.1 g L⁻¹ SC treatments. Only apple slices treated with 1.0 g L⁻¹ SC maintained a significantly smaller percent decrease in *L* value than control slices ($P < 0.01$) on day 14. Treatments with 0.1 or 0.25 g L⁻¹ ASC browned only slightly less than SC treatments, while 0.5 g L⁻¹ and 1.0 g L⁻¹ ASC caused

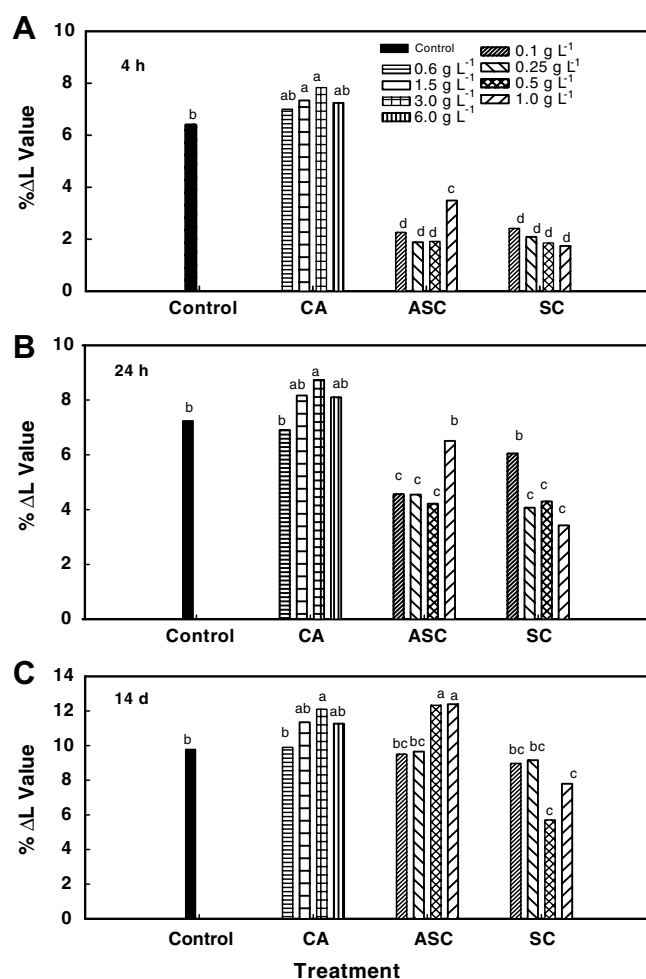


Fig. 1. Effect of concentration of sodium chlorite (SC), acidified sodium chlorite (ASC) or citric acid (CA) on % reduction in *L* values of fresh-cut apple slices after different storage durations: (A) 4 h; (B) 24 h and (C) 14 d. The concentrations of SC, and ASC were 0.1, 0.25, 0.5 and 1.0 g L⁻¹ and concentrations of CA were 0.6, 1.5, 3.0 and 6.0 g L⁻¹. The control was tap water. The vertical bars represent the standard errors of the means for triplicate experiments. Treatments with no significant difference from one another are illustrated as the same letter on the bar graph ($P > 0.05$).

severe tissue damage. Treatment with 0.5–1.0 g L⁻¹ ASC or 1.5–3.0 g L⁻¹ CA caused heavy browning after 2 weeks of storage, and thus a significant increase in the % change in *L* values ($P < 0.05$) compared with the control.

Citric acid has been widely used commercially as an antibrowning agent and studied extensively for its inhibitory activity on polyphenol oxidase (Pizzocaro, Torreggini, & Gilardi, 1993) and its antibrowning activity on minimally processed fruits and vegetables (Rocha, Brochado, & Morais, 1998). In our test, 1.5–3.0 g L⁻¹ CA treatment appeared to aggravate the extent of browning on apple slices. Jiang, Pen, and Li (2004) reported that citric acid at a concentration lower than 0.02 M (3.84 g L⁻¹) stimulated PPO activity of fresh-cut Chinese water chestnut, but at 0.1 M (19.2 g L⁻¹) or higher markedly inhibited the activity. Treatment with 0.1–1.0 g L⁻¹ SC had almost the same browning inhibitory effect on apple slices, with or without CA, except for 1.0 g L⁻¹ ASC at 4 h. After storage for 24 h, 0.1 g L⁻¹ SC and 1.0 g L⁻¹ ASC showed the greatest decrease in *L* value, indicating that 0.1 g L⁻¹ SC is too low a concentration for optimal browning inhibition, and that 1.0 g L⁻¹ ASC is too high a concentration and may actually aggravate browning by causing tissue damage.

3.2. Comparative inhibitory effect of different saline solutions

Since SC is a saline alkali and also a potent oxidizer, we selected a series of saline chemicals either with oxidative (sodium chlorite, sodium hypochlorite, sodium chlorate, group I), reductive (sodium bisulfite, sodium ascorbate, group II) or alkaline (sodium carbonate, sodium bicarbonate, sodium chloride, group III) properties to compare their efficacy to inhibit browning of apple slices.

The percent decrease in *L* values for slices treated with 0.5 g L⁻¹ SC and 0.5 g L⁻¹ sodium hypochlorite in group I, and 0.5 g L⁻¹ sodium bisulfite and 10 g L⁻¹ sodium ascorbate in group II, was significantly lower than for that of the water control and group III at 4 h ($P < 0.05$, Fig. 2A). However, only 0.5 g L⁻¹ SC, 0.5 g L⁻¹ sodium bisulfite, and 10 g L⁻¹ sodium ascorbate maintained significantly less browning of slices at 24 h ($P < 0.05$, Fig. 2B) and 14 days ($P < 0.05$, Fig. 2C). The best inhibition on browning was found to be 0.5 g L⁻¹ sodium bisulfite, followed by 0.5 g L⁻¹ SC at 4 h and 24 h and 0.5 g L⁻¹ SC ranked third at 2 weeks. Treatment with 0.5 g L⁻¹ sodium hypochlorite caused significant browning of slices after 24 h. Treatments in group III did not contribute to browning inhibition compared with the water control and 10 g L⁻¹ sodium carbonate caused significantly more browning than the water control by the end of storage. Sodium bisulfite, a sulfating agent, was once widely used as a preservative in prepared foods, and in the fresh fruit and vegetable industry, because of its effectiveness for controlling both browning and microbial activity, as well as being economical. However, the FDA banned its use on fresh fruits and vegetables because it poses a health hazard to allergic individuals. Ascorbic acid and its derivatives, are

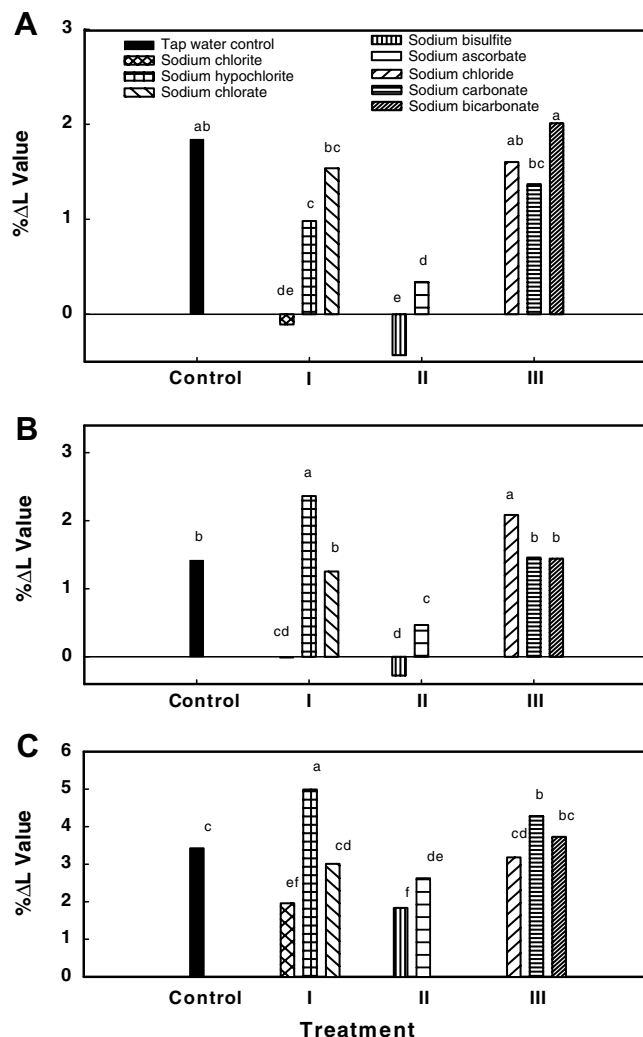


Fig. 2. Effect of different saline solutions on % reduction in *L* values of fresh-cut apple slices after different storage durations: (A) 4 h; (B) 24 h, and (C) 14 d. Saline solutions were classified into three groups: (I) 0.5 g L⁻¹ sodium chlorite, 0.5 g L⁻¹ sodium hypochlorite, 0.5 g L⁻¹ sodium chlorate; (II) 0.5 g L⁻¹ sodium bisulfite, 10 g L⁻¹ sodium ascorbate; (III) 10 g L⁻¹ sodium chloride, 10 g L⁻¹ sodium carbonate, 10 g L⁻¹ sodium bicarbonate. The control was tap water. The vertical bars represent the standard errors of triplicate experiments. Treatments with no significant difference from one another are illustrated as the same letter on the bar graph ($P > 0.05$).

frequently added to acidic dips used for the pretreatment of peeled or sliced fruits to prevent oxidative browning of fruit juice prior to pasteurization (Walker, 1995, chap. 2). However, its efficacy decreases with time as ascorbic acid is depleted, because the dehydro ascorbic acid formed during the oxidation reaction does not participate in the reduction process and thus large quantities of ascorbic acid must be added to effectively inhibit browning (McEvily et al., 1992). Furthermore, ascorbic acid and its derivatives, unlike sulfite, do not have antimicrobial activity and should therefore be used in conjunction with a sanitizer to reduce the potential for pathogen contamination and spoilage microorganism development during storage of produce. However, sodium chlorite at a concentration of

0.5 g L⁻¹, as shown in this experiment, can inhibit browning as well as sodium bisulfite and sodium ascorbate for 4 h and 24 h and still provides some browning inhibition after 2 weeks of storage.

3.3. Sodium chlorite combined with different concentrations of citric acid

In the first part, we found that, when the pH of ASC solution was below 2.4, the browning inhibitory effect was greatly reduced and much tissue injury occurred. In order to determine how the pH of SC solution above 2.4 would contribute to browning control, we added 0, 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 g L⁻¹ CA to 0.5 g L⁻¹ SC to achieve a series of solutions with pH values: 9.3, 6.2, 4.7, 3.9, 3.3, 3.0, and 2.8, respectively. Percent decrease in *L* values of apple slices after treatment are shown for three different storage durations in Fig. 3. At 4 h, there was no significant difference in *L* values among treatments and control except for a significantly smaller decrease in *L* value at pH 6.2 than at pH 2.8 ($P < 0.05$). The similarity of *L* values for solutions of all pHs indicated that 4 h was too early to observe much effect of pH on browning control, however a trend was beginning to emerge in that a relatively higher pH of SC solution inhibited browning more than that of a lower pH, except for pH 9.3 (SC control). This tendency increased with storage time. At 24 h, SC solutions with pH in the range from 3.3 to 6.2 caused less browning than SC control (pH 9.3) and solution with pH 2.8. Overall, relatively less browning occurred after 2 weeks of storage on apple slices treated in 0.5 g L⁻¹ SC with pH adjusted in the range from 3.9 to 6.2 using CA. This range of pH is close to the pH of apple tissue and might therefore cause less tissue damage.

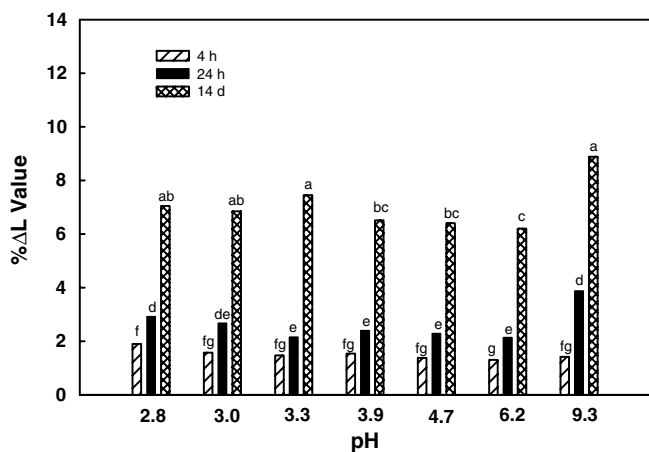


Fig. 3. Effect of pH of sodium chlorite (SC) solutions on relative *L* value of fresh-cut apple slices after different time of storage: 4 h; 24 h; and 14 d. The pH of 0.5 g L⁻¹ SC solution was adjusted to 9.3, 6.2, 4.7, 3.9, 3.3, 3.0, or 2.8 with different concentrations of citric acid. The vertical bars represent the standard errors of triplicate experiments. Treatments with no significant difference from one another are illustrated as the same letter on the bar graph ($P > 0.05$).

3.4. Sodium chlorite combined with different organic acids

In the last part, we used four different organic acids to adjust the pH of SC solution to 6.2 and compared the efficacy of SC solution as a browning inhibitor on apple slices when combined with different organic acids. To attain a solution pH of 6.2, organic acid was added to 0.5 g L⁻¹ SC to achieve an acid concentration of 0.02–0.04 g L⁻¹. At this concentration, the organic acids when used alone produced little effect on browning of slices. Percent change in *L* values measured on the surface of treated apple slices at three storage durations are shown in Fig. 4. There were no differences among organic acid adjusted SC treatments (pH 6.2) and SC control (pH 9.3) at 4 h. Significantly less browning ($P < 0.05$) was observed in cinnamic acid (CinA) and salicylic acid (Sal) adjusted SC treatments than those of CA and OA adjusted SC treatments and SC control at 24 h and after 2 weeks of storage. CA adjusted SC treatment did reduce browning over SC control and OA adjusted SC treatment at 2 weeks of storage, although not as much as cinnamic or salicylic acid. The inhibitory effect of oxalic acid on browning of apple was investigated by Son, Moon, and Lee (2001) with a minimal concentration above 0.05% (or 0.5 g L⁻¹) and on browning of litchi by Zheng and Tian (2006) at a concentration of 2 mM (0.18 g L⁻¹). Browning inhibition was most prominent on banana and apple slices treated with oxalic acid solutions at a concentration of 60 mM (5.4 g L⁻¹) and 5 mM (0.45 g L⁻¹), respectively (Yoruk, Yoruk, Balaban, & Marshall, 2004). However, in this study, when oxalic acid was combined with SC, we saw no synergistic effect on browning control. Salicylic acid, a phenolic found in many plants, including many used as foods (e.g., strawberries, almonds, tomatoes), is a precursor of aspirin, which has many benefits for human health, including reduction of fever and inflammation and relief of headache, and other pain (Bax-

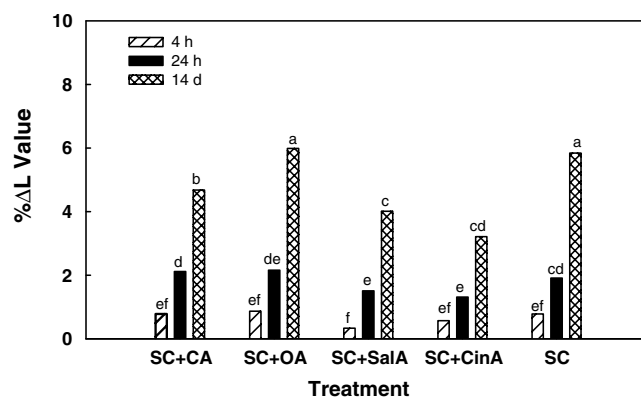


Fig. 4. Effect of sodium chlorite (SC) combined with organic acid on *L* value of fresh-cut apple slices after different storage durations: 4 h; 24 h; and 14 d. Solutions of 0.5 g L⁻¹ SC were adjusted to a pH of 6.2 using different organic acids: CA, citric acid; OA, oxalic acid; Sal, salicylic acid; CinA, cinnamic acid. The vertical bars represent the standard errors of the means for triplicate experiments. Treatments with no significant difference from one another are illustrated as the same letter on the bar graph ($P > 0.05$).

ber, Graham, Lawrence, Wiles, & Paterson, 2001; Scheier, 2001). Salicylic acid has also been shown to have an inhibitory effect on browning of some fruits and vegetables (Peng & Jiang, 2006). A kinetic study by Zhang, Chen, Song, and Xie (2006) indicated that salicylic acid is a competitive inhibitor of mushroom tyrosinase. Shi, Chen, Wang, Song, and Qiu (2005) found that cinnamic acid strongly inhibited the diphenolase activity of mushroom tyrosinase by a noncompetitive mechanism. In this experiment, we found that these two organic acids, cinnamic and salicylic, could be added to the SC solution to achieve greater inhibition of browning than obtained with citric acid. Further testing is necessary to determine whether organoleptic attributes of apple slices other than visual characteristics are affected by the addition of these two acids. For industrial use, CA is still a good choice for its economy, stable chemistry and consumer acceptance.

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