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Antibrowning and Antimicrobial Properties of Sodium Acid Sulfate in Apple Slices

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ABSTRACT: There are few available compounds that can both control browning and enhance microbial safety of fresh-cut fruits. In the present study, the antibrowning ability of sodium acid sulfate (SAS) on "Granny Smith" apple slices was first investigated in terms of optimum concentration and treatment time. In a separate experiment, the apple slices were treated with water or 3% of SAS, calcium ascorbate, citric acid, or acidified calcium sulfate for 5 min. Total plate count, color, firmness, and tissue damage were assessed during a 21-d storage at 4 °C. Results showed that the efficacy of SAS in inhibiting browning of apple slices increased with increasing concentration. A minimum 3% of SAS was needed to achieve 14 d of shelf life. Firmness was not significantly affected by SAS at 3% or lower concentrations. Antibrowning potential of SAS was similar for all treatment times ranging from 2 to 10 min. However, SAS caused some skin discoloration of apple slices. When cut surface of apple slices were stained with a fluorescein diacetate solution, tissue damage could be observed under a microscope even though visual damage was not evident. Among the antibrowning agents tested, SAS was the most effective in inhibiting browning and microbial growth for the first 14 d. Total plate count of samples treated with 3% SAS was significantly lower than those treated with calcium ascorbate, a commonly used antibrowning agent. Our results suggested that it is possible to use SAS to control browning while inhibiting the growth of microorganisms on the apple slices if the skin damage can be minimized.

Practical Application: Fresh-cut apples have emerged as one of the popular products in restaurants, schools, and food service establishments as more consumers demand fresh, convenient, and nutritious foods. Processing of fresh-cut apples induces mechanical damage to the fruit and exposes apple tissue to air, resulting in the development of undesirable tissue browning. The fresh-cut industry currently uses antibrowning agents to prevent discoloration. However, the antibrowning solutions can become contaminated with human pathogens such as *Listeria monocytogenes*, and washing of apple slices with the contaminated solutions can result in the transfer of pathogens to the product. It would be ideal if an antibrowning compound prevented the proliferation of human pathogens in solutions and minimized the growth of pathogens during storage. The study was conducted to investigate antibrowning and antimicrobial properties of sodium acid sulfate (SAS) in comparison with other common antibrowning agents on Granny Smith apples. Results showed that among the antimicrobial agents we tested, SAS was the most effective in inhibiting browning and microbial growth for 14 d at 4 °C. However, SAS caused some skin discoloration of apple slices. Overall, SAS can potentially be used to inhibit tissue browning while reducing the microbial growth on apple slices. The information is useful for the fresh-cut produce industry to enhance microbial safety of fresh-cut apples while minimizing browning, thus increasing the consumption of the health benefiting fresh fruit.

Keywords: antimicrobial, apple, browning, color, firmness, sodium acid sulfate

Introduction

Fresh-cut fruits represent a rapidly growing segment of the produce industry as more consumers demand fresh, convenient and nutritious foods. Fresh-cut apples have emerged as one of the popular products in restaurants, schools, and food service establishments (Gorny 2003). Processing of fresh-cut apples induces mechanical damage to the fruit and exposes apple tissues to air, resulting in the development of undesirable tissue browning due to enzymatic and nonenzymatic reactions (Walker 1995). The fresh-cut industry currently uses antibrowning agents such as calcium ascorbate and/or citric acid to prevent discoloration (Chen and others 1999; Sapers and others 2002; Fan and others 2005). However, the antibrowning solutions can become contaminated with *Listeria monocytogenes*, and washing of apple slices with the contaminated solutions can result in the transfer of pathogens to the product (FDA 2001). Innovative food safety technologies are needed to inactivate foodborne pathogens in antibrowning solutions and on the products. In addition, one of the largest costs in producing fresh-cut apples is the antibrowning solution. Finding alternative antibrowning compounds that have a lower cost will be beneficial to the fresh produce industry. Furthermore, it would be ideal if an antibrowning compound prevented the proliferation of human pathogens in solutions and minimized the growth of pathogens during storage. Such a compound has not been developed and/or employed by the produce industry.

Sodium acid sulfate ($NaHSO_4$, SAS), or sodium bisulfate, is a compound that has been used as a pH control agent, leavening agent and a processing aid (Anonymous 2008). Current uses for SAS are in dressings, sauces, marinades, fillings, beverages, cheeses, and syrups. SAS was designated "generally recognized as safe" (GRAS) by FDA in 1998 (FDA 2008). SAS should not be confused with sodium bisulfite. Unlike the sulfite compounds, SAS does not cause an allergic reaction. Sodium bisulfite has antibrowning and antimicrobial properties (Eissa and others 2006; Lu and others 2007), and

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was used on some fruits and vegetables (Sapers and others 2002). However, sodium bisulfite was banned by FDA on many fruits and vegetables because it may cause serious allergic reactions.

The antibrowning and antimicrobial properties of SAS have not been studied on fresh cut fruits. SAS solutions in concentrations between 0.5% and 3% have a pH of 1 to 2. Earlier research (Karaibrahimoglu and others 2004) has demonstrated that the pH of antibrowning solutions had a profound effect on the survival of *Listeria innocua* (a surrogate for *L. monocytogenes*) in solutions. At pH 2.5, *L. innocua* could be reduced by more than 4 logs compared with pH 7. Therefore, the objectives of the present study were to investigate the optimum concentration and treatment time of SAS for controlling browning of fresh-cut apples and to evaluate the antibrowning and antimicrobial properties of SAS in comparison with other common antibrowning agents on fresh-cut apples. In the present study, we used "Granny Smith" apples, an apple that has high potential of browning (Amiot and others 1992; Toivonen 2008) and is often used for fresh-cut apple slices (Abbott and others 2004).

Materials and Methods

Materials

"Granny Smith" apples were harvested at commercial maturity from an orchard in Central Pennsylvania. Fruit were stored at 4 °C before being used. SAS was provided by Jones-Hamilton Inc. (Walbridge, Ohio, U.S.A.). Acidified calcium sulfate (Safe$_2$O-ACS50) was from Mionix Inc. (Rocklin, Calif., U.S.A.). Calcium ascorbate and citric acid were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.).

Processing of apple slices

Processing of fresh-cut apples was performed in an 8 °C clean processing room as described earlier (Fan and others 2005). All cutting boards, holding vessels, and the fruit surfaces were sanitized with 200 ppm NaOCl for 3 min. Apple slicers were used to slice the apples into 8 equal pieces and to remove the core. The sliced apples were dipped into SAS or other antibrowning solutions. The temperature of solutions was maintained at around 8 °C during treatment. The slices were drained and placed into Zip-lok bags (15.2 × 12.7 cm) with 4 holes (0.6 cm in diameter), and stored for 21 d at 4 °C. There were 10 slices of apples in each bag. Each bag was regarded as a replicate that was processed from different batches of apples. There were 4 replicated bags for each experiment.

Effect of SAS concentration

Apple slices were dipped into solutions of 0 (water), 0.5%, 1%, 2%, 3%, 4%, and 5% SAS for 5 min, drained and packaged. Cut-surface color, texture, and skin discoloration of apple slices were measured at 1, 7, 14, and 21 d during storage at 4 °C.

Effect of treatment time

Apple slices were dipped into 3% SAS solution for 0, 2, 5, and 10 min. Color, texture, and skin discoloration of apple slices were measured at 1, 7, 14, and 21 d during storage at 4 °C.

Comparison with other common antibrowning agents

Apple slices were dipped into water, and 3% of each SAS, citric acid, Safe$_2$O-ACS50, and calcium ascorbate for 5 min. Total plate count, color, texture, and skin discoloration of apple slices were measured at 1, 7, 14, and 21 d during storage at 4 °C.

Microscopic assessment of damage

Possible damage to the cut surface of apple slices was assessed using separate groups of apples from those used in the previously mentioned experiments. A method similar to that of Galindo and others (2005) was used. Briefly, apple wedges, cut from the equator part of the apples, were dipped into different concentrations of SAS or different antibrowning agents (Figure 1). During 1, 7, 14, and 21 d of storage, the wedges were cut sagittally using a sharp razor blade into rectangular prisms with approximate dimensions of 0.5 cm in height, 1.5 cm in width, and 2 cm in length. The sections were incubated for 5 min in a solution containing 0.5 M sucrose and 1×10^{-6} M fluorescein diacetate at ambient temperature. The stained sections were then rinsed with deionized water and examined by epifluorescence using a MZFLIII stereofluorescence microscope system equipped with a model DC200 color charge coupled device camera (Leica Microsystems Inc., Bannockburn, Ill., U.S.A.). Undamaged cells fluoresced green while the damaged cells were autofluorescent red or nonfluorescent (dark brown) (see Figure 4). Digital images of each sample on the two 2 cm length sides were collected along with a millimeter scale. The width of the damaged area was measured at 4 separate locations (2 locations on each side) for each slice. Total of 6 slices from individual wedges were used for each treatment.

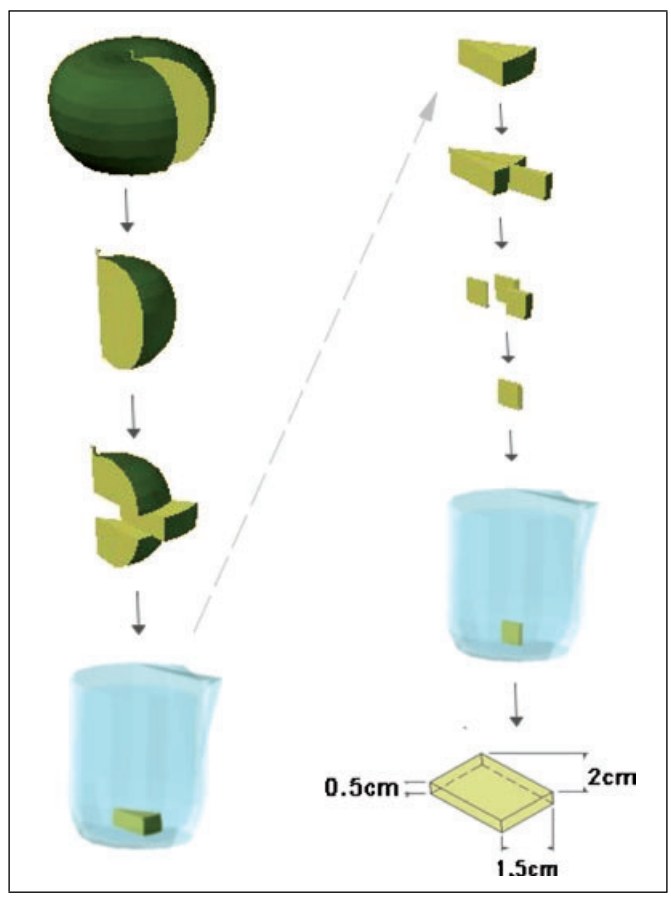

Figure 1—Preparation of apple tissues for microscopic assessment of damage. Apple wedges prepared from equator parts of the apples were dipped into antibrowning agents (left column). During storage, the wedges were further cut into rectangular prisms and then submerged in 1×10^{-6} M fluorescein diacetate solution for 5 min (right column) and rinsed with water. The prisms were then examined using a microscope.

Color measurement

The color of cut surfaces was measured with a Hunter Miniscan XE colorimeter (Hunter Associates Lab, Reston, Va., U.S.A.) using a 1.9 cm measuring aperture. The colorimeter was calibrated using the standard white and black tiles. D65/10° was used as the illuminant/viewing geometry. Surface color of 4 slices from each replicate of each treatment was measured; L^*, a^*, and b^* values were recorded at the 2 cut surfaces of each slice. Measurements were made at the middle point of the cut surface of apple slices.

Firmness measurement

Firmness was evaluated with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). The slices were first cut into wedges. Then, a 6-mm diameter probe was used to penetrate the centers of the wedges to 10 mm at 10 mm/s. Four slices from each bag were used for firmness measurements, and there were a total of 16 measurements for each treatment per experiment. Maximum force was recorded using the Texture Expert software (version 1.22, Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). In a preliminary study, firmness was measured both on the newly cut surface and on the surface that had directly been in contact with different concentrations (0% to 5%) of SAS. There was no difference in firmness between the 2 surfaces. Therefore, firmness of the newly cut surface was measured and presented. Furthermore, because the study was conducted during a period of several months, the initial firmness of the apples was different between the 2 trials. As a result, percentages of the changes in firmness (as compared with initial firmness) were used to evaluate effects of various treatments.

Microflora

Each sample consisted of a stomacher bag containing approximately 100 g of apple slices. A 150 aliquot of sterile buffered peptone water (BBL/Difco, Sparks, Md., U.S.A.) was added to each bag. The bag was then closed and agitated for 60 s to obtain a surface wash of the sample. Our preliminary results showed that hand agitation had a slightly higher (but not statistically significant) recovery rate compared with homogenization. Because of its easiness, hand agitation was used in the study to recover bacteria from the surface of apple slices. A 1 mL aliquot of the wash was serially diluted in Butterfield's phosphate buffer in 1 : 10 increments to 10^5. Dilutions were pour-plated with tryptic soy agar in triplicate. Plates were incubated at 37 °C for 2 d then enumerated. The microflora population was expressed as log CFU/g.

Statistical analysis

The experiment was designed as a randomized complete block with 3 to 4 replicates. Each experiment was repeated twice (2 trials), ending up with 6 to 8 replicates for each treatment. Analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$) among population means in response to concentration, treatment time, and various antibrowning agents. The Duncan's multiple range test was used for treatment effects within storage times, and the least significant difference (LSD) was used to test for storage effect within treatment. All statistical analyses and calculations of means and standard deviations were performed using Statistical Analytical System software (SAS Inst. Inc., Cary, N.C., U.S.A.).

Results and Discussion

Effect of SAS concentration

L^* values indicated the darkness of the cut surface of apple slices. The lower the L^* values were, the darker the apples were. One day after treatment, the L^* values of the cut surface of apple slices were increased (compared with the control) by SAS regardless of SAS concentration (Figure 2A), indicating the inhibition of browning by SAS. SAS at 0.5% had similar effect as other concentrations (1% to 5%). After 7 d of storage, the L^* values increased with increasing SAS concentration from 0% to 1%. Further increase in SAS concentration did not further increase the efficacy of SAS. However after 14 d of storage, SAS at 0.5% and 1% had no antibrowning activity compared with the control (0% SAS). After 21 d of storage, even 2% and 3% SAS did not prevent the decreases in L^* values.

The a^* values indicated the redness (browning) of cut surface of apple slices. The higher the a^* values were, the redder (browning) the apples slices were. After 1 and 7 d of storage, SAS at any concentration significantly decreased a^* values (Figure 2B). However after 14 and 21 d of storage, a^* values decreased (compared with control) only at higher SAS concentrations (3% to 5%). During storage, the cut surface of apple slices turned darker. Judged from the a^* values, it appeared that at least 3% SAS was needed to achieve significant antibrowning effect for 14 d shelf life compared with the control (0%) apple slices at day 14.

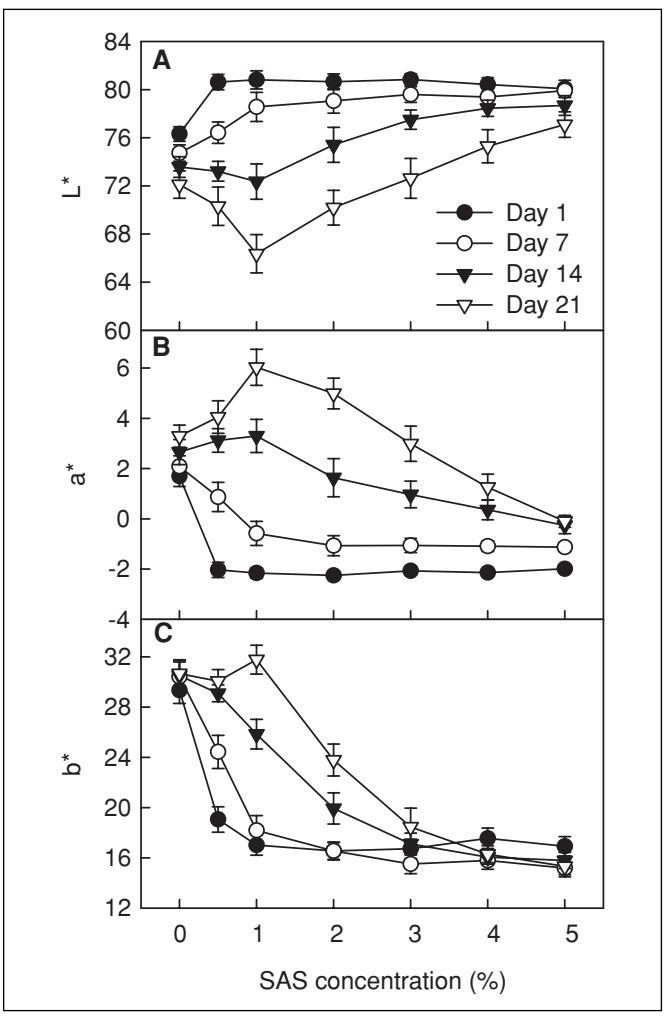

Figure 2—Effect of SAS concentration on color parameters, L^* (A), a^* (B), and b^* (C) values of cut surface of Granny Smith apple slices. Apple slices, dipped into 0%, 0.5%, 1%, 2%, 3%, 4%, and 5% SAS for 5 min, were packaged into perforated film bags. Each bag was regarded as a replicate that was processed from different batches of apples. Cut surface color of apple slices was measured at 1, 7, 14, and 21 d of storage. Vertical bars represent standard errors ($n = 8$).

The b^* values indicated the yellowness. SAS decreased b^* values, suggesting the loss of yellowness. At 1 and 7 d, SAS at a concentration of 1% or more achieved the maximum reduction in b^* values (Figure 2C). However, after 14 and 21 d of storage, a minimum of 3% SAS was needed to obtain the maximum reduction in b^* values.

The firmness of apple slices was increased by low concentrations of SAS (0.5% and 1%) during most of the sampling days (Figure 3A). After 1 and 7 d of storage, SAS at the concentrations we tested did not significantly reduce firmness. However, after 14 d of storage, firmness of apples slices decreased as SAS concentrations increased from 1% to 5%. At 4% and 5%, SAS caused significant decreases in firmness compared with that of nontreated (0%) samples.

A small portion of green skin of the apple slices turned to yellow after SAS treatments. The width of discoloration increased with increasing SAS concentration and increasing storage from day 1 to 7 (Figure 3B). However, skin discoloration did not further increase after additional storage. Compared with the control (water), significant skin discoloration was observed at concentrations above 1%.

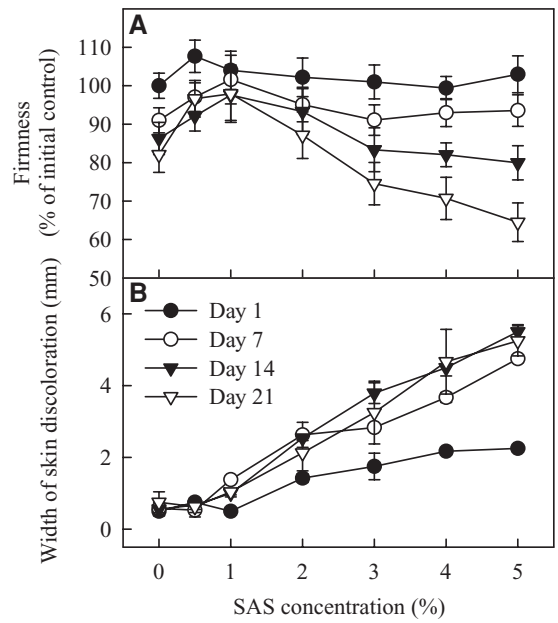

Figure 3—Effect of SAS concentration on firmness (A) and skin damage (B) of Granny Smith apple slices. Apple slices, dipped into 0%, 0.5%, 1%, 2%, 3%, 4%, and 5% SAS for 5 min, were packaged into perforated film bags. Each bag was regarded as a replicate that was processed from different batches of apples. Firmness and skin damage of apple slices were measured at 1, 7, 14, and 21 d of storage. Tissue injury was measured as the width of skin discoloration. Vertical bars represent standard errors ($n = 8$).

Even though SAS caused damage on the skin, no discoloration on the cut surface of the apple slices was observed. To assess possible injury on the cortex tissue (cut surface) of apple slices, we stained the samples with florescein diacetate followed by observation with a microscope. After being stained, the damaged tissue appeared darker compared with the nondamaged one (Figure 4). The injury was reported as the depth of injury into the cut surface. The depth of the damage increased linearly with SAS concentration (Figure 5). Unlike the damage on the skin of apple slices, the damage did not worsen during storage. The results indicated that the injury occurred immediately (within a day) after SAS treatment.

Effect of treatment time

Treating the apple slices with 3% SAS for 2 min significantly increased the L^* values and lowered a^* and b^* values 1 day after treatment (Figure 6). The effectiveness of 3% SAS in inhibiting the browning did not increase when treatment time was extended to 10 min. The results suggested that treatment time was not a critical factor in the effectiveness of the SAS. However, the effectiveness of SAS changed during storage. Significant increases in L^* values and reduction in a^* values were observed only at days 1 and 7. After 14 d storage, the L^* and a^* values of treated sample and nontreated (0%) samples became similar. However, b^* values of samples treated for 2 to 10 min were always lower than those of nontreated (0%) ones regardless of storage period.

Treatment time did not significantly affect the firmness of apple slices (Figure 7A), even though firmness tended to decrease over storage regardless of treatment time. The skin discoloration caused by 3% SAS was not significantly affected by treatment time either (Figure 7B). However, compared with day 1, the damage increased after 7 or more days of storage.

Comparison with other antibrowning agents

Compared with the control (water), all treatments increased L^* values and reduced a^* and b^* values at days 1 and 7 (Table 1). After 21 d of storage, citric acid and Safe$_2$O-ACS50 were not effective in controlling browning while SAS and calcium ascorbate were still effective. The best antibrowning treatment was 3% SAS especially for storage up to 14 d.

Compared with the control (water), none of the treatments significantly affected firmness of the cut apples during storage (Table 2). Skin discoloration was observed in samples treated with Safe$_2$O-ACS50 and SAS (Table 2). Other treatments did not cause any skin damage. Damage on the cut surface of the apple slices was primarily associated with the SAS treatment. SAS (3%) caused tissue damage penetrating up to 1. 5 mm in depth (Table 3). All

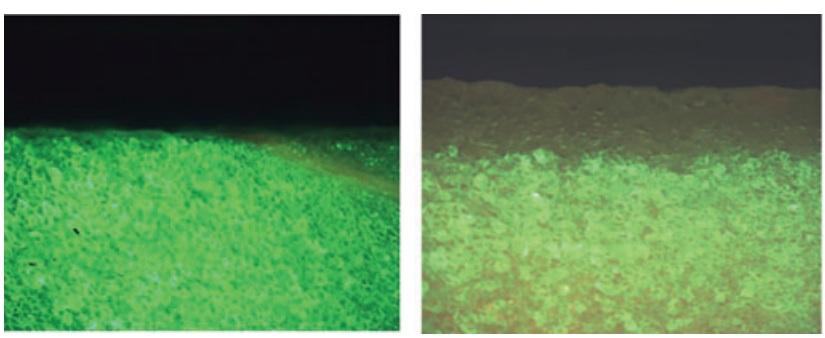

Figure 4—Typical result from microscopic observation of apple tissue treated with 0% (left) and 3% SAS (right) and stained with fluorescein diacetate. The dark zone marked by the parenthesis in the 3% SAS sample indicates a region of damaged cells. Apple tissues after being submerged in 1 × 10^{-6} M fluorescein diacetate solution for 5 min and rinsed with water were examined using a microscope system with 25× magnification.

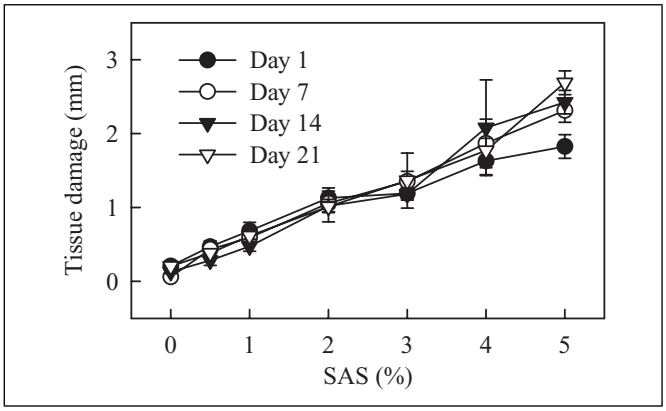

Figure 5—Effect of SAS concentration on tissue damage. Apple slices, dipped into 0%, 0.5%, 1%, 2%, 3%, 4%, and 5% SAS for 5 min, were stored for 21 d at 4 °C. Tissue injury of apple slices was assessed using a microscopic method at 1, 7, 14, and 21 d of storage. Vertical bars represent standard errors (*n* = 6).

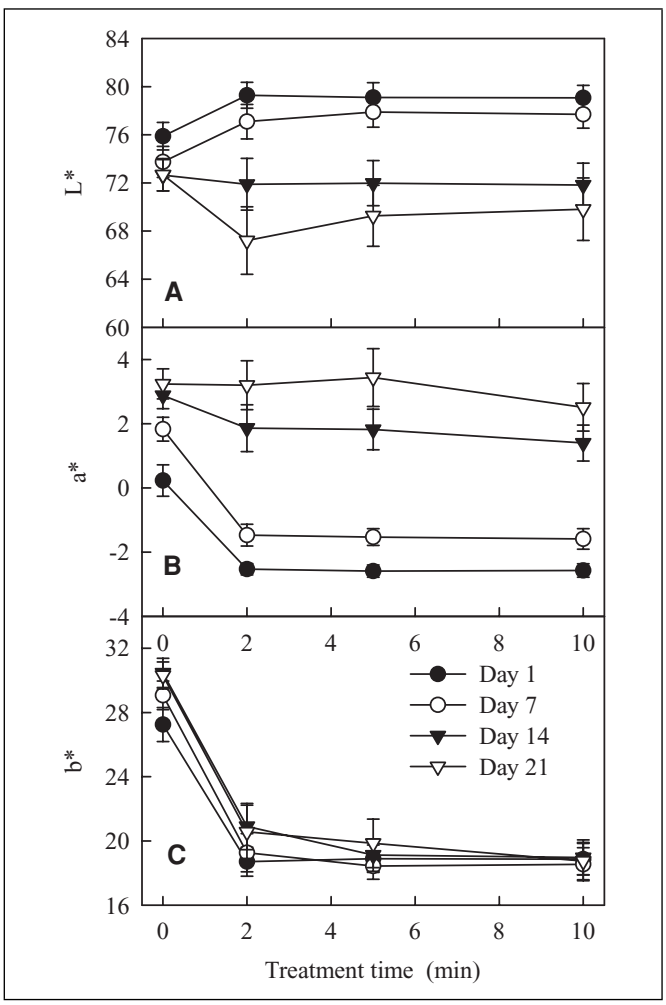

Figure 6—Effect of treatment time on color parameters, *L (A), *a** (B), and *b** (C) values of cut surfaces of Granny Smith apple slices. Apple slices, dipped into 3% SAS for 0, 2, 5, and 10 min, were packaged into perforated film bags. Each bag was regarded as a replicate that was processed from different batches of apples. Cut surface color of apple slices was measured at 1, 7, 14, and 21 d of storage. Vertical bars represent standard errors (*n* = 8).**

other treatments caused significantly less damage than the SAS treatment.

Total aerobic count (TPC) was below 1.5 log CFU/g for all samples 1 day after treatments (Table 2). SAS-treated samples had significantly lower TPC than that of calcium ascorbate-treated sample throughout the 21-d storage period. Samples treated with calcium ascorbate always had the highest TPC while SAS-treated samples had the lowest TPC through most of the storage period. After 7 to 21 d of storage, SAS-treated sample had at least log CFU/g lower TPC than that of calcium ascorbate-treated samples, which is the standard antibrowning agent used by the industry. It appears that calcium ascorbate promoted the growth of microorganisms during storage compared with the samples treated with water. There was no significant difference in TPC among samples treated with control (water), Safe2O-ACS50, citric acid or SAS during storage even though among the 4 treatments, control samples always had the highest TPC while samples treated with SAS had the lowest TPC during most of the sampling days.

One of our concerns in working with strong acidified compounds was carryover of the acidified compounds into the recovery medium and recovery of acid-injured cells. Lowering the pH of medium would reduce or inhibit the growth of bacteria. Our preliminary results showed that using buffered peptone water maintained the pH of the buffer to levels above pH 6. On the contrary, neutralizing buffer was not strong enough to neutralize the acids, which resulted in lower pH. Earlier studies also demonstrated that cells that were injured by acid treatment may be recovered using a buffered peptone water (Baylis and others 2000; Liao and Fett 2005; Gray and others 2008).

Acids have been widely used for the preservation of various foods (Davidson and others 2002; Doores 2002). Lu and others (2007) found that sodium chlorite in combination with citric acid

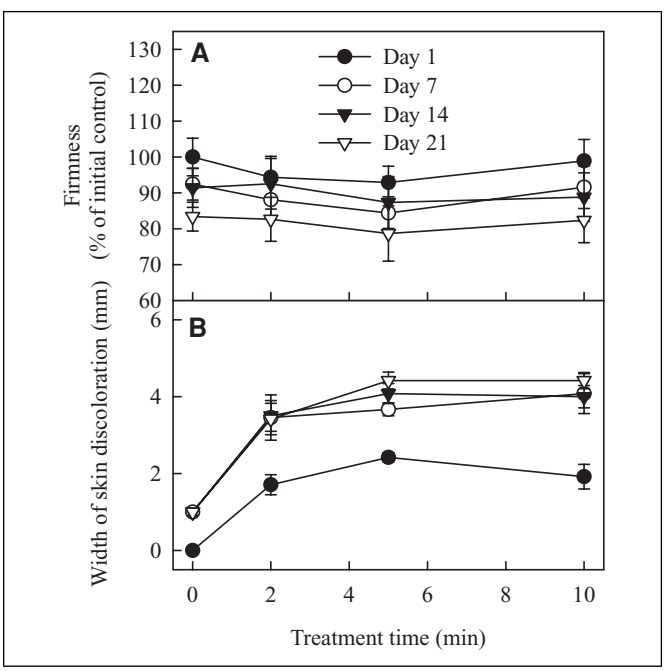

Figure 7—Effect of treatment time on firmness (A) and skin damage (B) of Granny Smith apple slices. Apple slices, dipped into 3% SAS for 0, 2, 5, and 10 min, were packaged into perforated film bags. Each bag was regarded as a replicate that was processed from different batches of apples. Firmness and skin damage of apple slices were measured at 1, 7, 14, and 21 d of storage. Vertical bars represent standard errors (*n* = 8).

and other organic acids significantly reduced the browning of apple slices. The most commonly used acids in food application are organic acids. It is believed that the mode of action for antimicrobial properties of organic acids is more related to the undissociated portion of the acids than the reduction in pH brought by the addition of acids (Doores 2002). SAS is an inorganic compound. The action of SAS may be due to hydrogen sulfate ion (HSO_4^-) and lower pH. The pH of 0.5% to 5% SAS solutions is between 1 and 2, which is much lower than most organic acid solutions at the same concentration.

The mode of action in inhibiting browning of apples slices is probably due to inhibition of polyphenol oxidase (PPO) activity. The optimum pH for PPO activity is between 5 and 7 (Sapers and others 2002). At pH values below 3, the enzyme activity was effectively inhibited (McEvily and Lyengar 1992). Lowering pH inhibited activity of PPO because hydrogen ion can be a noncompetitive inhibitor of apples PPO at pH lower than 5 (Janovitz-Klapp and others 1990). Even though 3% SAS inhibited the browning of apple slices, it appears that the inhibition lasted only for 2 wk. The PPO enzyme seems to be tolerant to acid pH and the residue

Table 1—Effect of antibrowning agents on color parameters of "Granny Smith" apple slices during storage at 4 °C. Apple slices were dipped into water (control), or 3% of Safe$_2$O-ACS50, SAS, calcium ascorbate, citric acid, or acidified calcium sulfate for 5 min. Cut surface color (L^*, a^*, and b^*) of apple slices packaged in perforated plastic film bags was measured at 1, 7, 14, and 21 d of storage. Each bag was regarded as a replicate that was processed from different batches of apples. The numbers are means followed by standard error deviations (n = 8).

Treatments	Storage time (d) 1	7	14	21	LSD[A]
			L^*		
Control	75.8 ± 0.9b[B]	74.2 ± 0.7c	72.2 ± 0.8d	70.8 ± 1.0c	0.8
Safe$_2$O-ACS50	80.5 ± 0.8a	80.1 ± 0.9a	76.3 ± 1.6b	66.3 ± 1.9d	1.3
SAS	80.2 ± 1.1a	80.2 ± 1.0a	78.3 ± 1.2a	74.7 ± 2.5b	1.5
Citric acid	79.6 ± 1.2a	78.6 ± 1.1b	74.4 ± 1.2c	70.5 ± 1.4c	1.1
Calcium ascorbate	80.3 ± 1.1a	79.4 ± 1.3ab	77.6 ± 0.9a	77.0 ± 0.9a	1.0
			a^*		
Control	0.7 ± 0.4a	1.5 ± 0.3a	2.7 ± 0.5a	3.7 ± 0.6b	0.4
Safe$_2$O-ACS50	−2.8 ± 0.1c	−2.7 ± 0.1d	0.1 ± 0.7c	5.3 ± 0.7a	0.5
SAS	−2.6 ± 0.2c	−2.4 ± 0.2c	−1.6 ± 0.3e	1.4 ± 1.1c	0.6
Citric acid	−2.3 ± 0.3b	−0.9 ± 0.3b	1.8 ± 0.5b	4.4 ± 0.7b	0.4
Calcium ascorbate	−3.3 ± 0.2d	−2.5 ± 0.4cd	−1.0 ± 0.4d	−0.1 ± 0.4d	0.3
			b^*		
Control	28.0 ± 0.8a	28.9 ± 0.8a	29.8 ± 0.9a	31.1 ± 1.1a	0.8
Safe$_2$O-ACS50	19.4 ± 1.3c	20.1 ± 1.1d	21.8 ± 1.7d	29.4 ± 1.6b	1.3
SAS	19.2 ± 1.3c	19.2 ± 1.1d	19.8 ± 1.2e	21.5 ± 1.7d	1.3
Citric acid	22.0 ± 1.7b	24.4 ± 1.3b	28.0 ± 1.0b	30.2 ± 1.2ab	1.2
Calcium ascorbate	19.8 ± 1.1c	21.8 ± 1.4c	24.9 ± 1.0c	26.2 ± 1.0c	1.1

[A]The least significant difference at $P < 0.05$ levels for the storage effect.
[B]Means with the same letters are not significantly different (Duncan's multiple range test $P < 0.05$). Comparison was made within same storage period.

Table 2—Effect of antibrowning agents on texture, skin injury, and TPC of "Granny Smith" apple slices during storage at 4 °C. Apple slices were dipped into water (control), or 3% of Safe$_2$O-ACS50, SAS, calcium ascorbate, citric acid, or acidified calcium sulfate for 5 min. Texture, skin injury, and TPC of apple slices packaged in perforated plastic film bags were measured at 1, 7, 14, and 21 d of storage. Each bag was regarded as a replicate that was processed from different batches of apples. The numbers are means followed by standard error deviations (n = 8).

Treatments	Storage time (d) 1	7	14	21	LSD[A]
			Firmness (% of initial control)		
Control	100.0 ± 4.4a[B]	96.4 ± 4.5a	94.9 ± 4.3ab	90.1 ± 4.8a	5.9
Safe$_2$O-ACS50	103.4 ± 4.9a	97.0 ± 6.4a	98.9 ± 5.2ab	98.0 ± 6.8a	7.7
SAS	101.8 ± 4.0a	100.6 ± 5.1a	101.4 ± 4.6a	97.9 ± 5.9a	6.5
Citric acid	103.9 ± 4.7a	97.5 ± 3.8a	92.7 ± 4.6b	92.7 ± 5.1a	6.1
Calcium ascorbate	102.4 ± 4.6a	97.8 ± 7.5a	99.5 ± 6.2ab	94.8 ± 4.7a	7.7
			Skin injury (mm)		
Control	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	
Safe$_2$O-ACS50	1.0 ± 0.0b	1.9 ± 0.4a	2.0 ± 0.3a	2.8 ± 0.4b	0.6
SAS	1.6 ± 0.3a	1.9 ± 0.3a	1.9 ± 0.3a	3.7 ± 0.7a	0.8
Citric acid	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	
Calcium ascorbate	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	
			TPC (log CFU/g)		
Control	1.1 ± 0.6ab	0.9 ± 0.5b	3.1 ± 1.1ab	3.1 ± 1.1ab	2.2
Safe$_2$O-ACS50	0.1 ± 0.1ab	0.8 ± 0.6b	2.3 ± 1.0ab	2.7 ± 1.3ab	2.7
SAS	0.0 ± 0.0b	0.3 ± 0.3b	1.5 ± 0.7b	2.0 ± 0.9a	1.9
Citric acid	0.7 ± 0.3ab	0.0 ± 0.0b	1.8 ± 0.7b	2.4 ± 0.9ab	1.9
Calcium ascorbate	1.4 ± 0.7a	3.4 ± 0.7a	5.0 ± 1.0a	5.0 ± 1.1b	2.5

[A]The least significant difference at $P < 0.05$ levels for the storage effect.
[B]Means with the same letters are not significantly different (Duncan's multiple range test $P < 0.05$). Comparison was made within same storage period.

Table 3 — Effect of antibrowning agents on tissue damage of "Granny Smith" apple slices during storage at 4 °C. Apple slices were dipped into water (control), or 3% of Safe$_2$O-ACS50, SAS, calcium ascorbate, citric acid, or acidified calcium sulfate for 5 min. Tissue damage of apple slices was measured at 1, 7, 14, and 21 d of storage under a microscope after being dipped in a fluorescein diacetate solution. The numbers are means followed by standard error deviations (n = 6).

Treatments	Storage time (d)				
	1	7	14	21	LSD[A]
	Tissue damage (mm)				
Control	0.1 ± 0.1c[B]	0.3 ± 0.1b	0.4 ± 0.2b	0.2 ± 0.0b	0.3
Safe$_2$O-ACS50	0.5 ± 0.1b	0.6 ± 0.0b	0.5 ± 0.1b	0.5 ± 0.2b	0.3
SAS	1.2 ± 0.2a	1.4 ± 0.2a	1.3 ± 0.1a	1.5 ± 0.2a	0.4
Citric acid	0.3 ± 0.1bc	0.4 ± 0.2b	0.4 ± 0.1b	0.3 ± 0.1b	0.4
Calcium ascorbate	0.0 ± 0.0c	0.4 ± 0.3b	0.7 ± 0.4b	0.4 ± 0.2b	0.7

[A]The least significant difference at $P < 0.05$ levels for the storage effect.
[B]Means with the same letters are not significantly different (Duncan's multiple range test $P < 0.05$). Comparison was made within same storage period.

activity of the enzyme may be present (Janovitz-Klapp and others 1990). Longer storage of "Granny Smith" apple slices may require combination of SAS with other antibrowning agents. The browning of "Granny Smith" apples is one of the hardest ones to control (Amiot and others 1992; Toivonen 2008). To control the browning of apple slices of other cultivars, lower SAS concentrations may be employed to avoid tissue damage and/or to extend longer shelf life.

An earlier study (Karaibrahimoglu and others 2004) suggested that reduction of the pH to below 5 reduced the risk of *L. monocytogenes* associated with consumption of fresh-cut apples. When the pH of antibrowning solution was below 4.5, *L. innocua* was completely inactivated. Furthermore, at pH 2.5, the browning of apple slices was also reduced. However, treating apples with the low pH calcium ascorbate solution softened apple slices. The results from the present study showed that firmness was not reduced by the treatments of SAS solutions at 0.5% to 3%, which had pHs of 1 to 2. It is unclear why the results between the 2 studies were different. In the previous study (Karaibrahimoglu and others 2004), acetic acid was used as the acidulant in calcium ascorbate solution.

In addition to the skin discoloration caused by high concentrations of SAS, our preliminary experiment suggested that SAS might increase the sourness of "Granny Smith" apples. "Granny Smith" is a tart apple, containing high levels of organic acids. SAS will increase the sourness of these organic acids by keeping them in the undissociated state. It is possible that SAS may not increase the sourness of "sweet" apple cultivars because they contain lower levels of organic acids and higher sugar contents than "Granny Smith" apples. In addition, an increase in sourness may balance out the sweetness of "sweet apple."

Even though SAS at concentrations of 0.5% and 1% did not cause skin discoloration, SAS at those concentrations could not achieve 14 d of shelf life. SAS at higher concentrations (3 and above) achieved 14 d of shelf life but caused skin discoloration. Skinless fresh-cut apples are marketed in some restaurants and establishments, such as those sold in the Burger King restaurant. SAS at 3% may be used to produce skin-less fresh-cut apples. To produce apple slices with skin, SAS may have to be combined with other antibrowning agents to minimize the skin damage. In the present study, the apple slices were packaged in air. Use of modified atmosphere packaging (MAP) may prolong the effectiveness of SAS. Studies are planned to study the effect of SAS combined with other antibrowning agents and MAP.

Acidified calcium sulfate (Safe$_2$O-ACS50), a compound similar to SAS, has been developed to reduce the total number of aerobic bacteria and pathogens in meat products (Zhao and others 2004). Our results showed that Safe$_2$O-ACS50 was not as effective as SAS in inhibiting browning of apple slices or the growth of microorganisms, particularly after 7 d of storage. Due to the proprietary nature of Safe2O-ACS50, the concentration of acidified calcium sulfate in the commercial formula was unknown.

Conclusions

The antibrowning ability of SAS on apple slices was affected by the concentration. To inhibit tissue browning of "Granny Smith" apple slices for 14 d, 3% SAS was required. Treatment time from 2 to 10 min did not have much influence on the efficacy of SAS. SAS caused a discoloration of apple skin, which might limit its use on apple slices with skin. Apple slices treated with SAS had a significantly lower TPC compared with those samples treated with calcium ascorbate, a common antibrowning agent used by the industry, throughout the 21 d storage period. It appears that SAS could be used to inhibit browning while reducing the microbial growth on apple slices. Research is needed to minimize the SAS damage on apple skin and possible adverse effects on the taste and firmness.

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