Control of Browning and Microbial Growth on Fresh-Cut Apples by Sequential Treatment of Sanitizers and Calcium Ascorbate

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ABSTRACT: This study investigated the efficacy of different sanitizers, including acidic electrolyzed water (AEW), peroxyacetic acid (POAA), and chlorine, on the inactivation of Escherichia coli O157:H7 on fresh-cut apples. The effects of the sanitizers and sequential treatments of AEW or POAA followed by calcium ascorbate (CaA) on browning inhibition and organoleptic qualities of fresh-cut apples stored under different package atmospheres at 4 °C were also evaluated. Changes in package atmosphere composition, product color, firmness, total aerobic bacterial counts, yeast and mold counts, and sensory qualities were examined at 0, 4, 8, 11, and 21 d. Among all sanitizer treatments, POA and AEW achieved the highest reduction on E. coli O157:H7 populations. The sequential treatment of AEW followed by CaA (AEW-CaA) achieved the best overall dual control of browning and bacterial growth on fresh-cut apple wedges. Package atmospheres changed significantly over time and among package materials. Packages prepared with films having an oxygen transmission rate (OTR) of 158 had significantly lower O₂ and higher CO₂ partial pressures than those prepared with 225 OTR films and the Ziploc™ bags. The effect of package atmospheres on the browning of apples is more pronounced on AEW, POAA, and POAA-CaA-treated apple wedges than on AEW-CaA-treated samples.

Keywords: browning, calcium ascorbate, electrolyzed water, fresh-cut apple, peroxyacetic acid

Introduction

Fresh-cut apples have emerged as popular food snacks at both retail and food service sectors, and are frequently included in school lunch programs (Warren 2004). The production and consumption of fresh-cut apples are projected to continue growing as more consumers demand fresh, convenient, and nutritious foods. A challenge to the fresh-cut apple industry is the darkening of apple flesh after cutting caused by enzymatic-browning reactions. Currently, wash solutions containing calcium ascorbate (CaA) as the principal ingredient are used in fresh-cut apple production to inhibit enzymatic-browning reactions and to maintain quality and prolong shelf life (Chen and others 1999). CaA is a relatively expensive chemical and hence the washing solution is usually reused to reduce production costs. The reuse of CaA solution, however, results in an accumulation of nutrients, microorganisms, and even human pathogens, if present, which renders the washing solution a source of contamination. The detection of Listeria monocytogenes on sliced apples (US FDA 2001) was thus considered as a possible consequence of such contamination (Karaibrahimoglu and others 2004). Recently, in a washing experiment with a commercial CaA-based solution, Bhagwat and others (2004) concluded that the washing solution should not be reused on multiple batches of sliced apples because of a lack of antibacterial activity.

Efforts have been made in recent years to find an effective method to control possible microbial contamination during fresh-cut apple washing operations. Karaibrahimoglu and others (2004) found that planktonic L. innocua population was reduced by 4 to 5 and 2 log CFU/mL after 96 h treatment in CaA solutions with pH of 4.5 and 5.0, respectively. Pilizota and Sapers (2004) developed an acidic browning inhibition formulation that did not support Listeria survival or growth because of low pH (2.9). The survival of 5 enteric pathogens, including L. monocytogenes and E. coli O157:H7, in 4 washing solutions with different pH was examined by Bhagwat and others (2004). For the washing solution with pH of 2, a greater than 5-log reduction was observed after treating selected planktonic pathogenic microorganisms with the solution for 2 h at 23 °C. The emphasis of previous studies was mainly placed on inhibiting pathogen growth in treatment solutions rather than on disinfecting fresh-cut apples that could have been contaminated by human pathogens during peeling, coring, and slicing. Few studies evaluated techniques for the simultaneous inactivation of foodborne pathogens and control of browning in fresh-cut apples. Fan and others (2005b) used irradiation to treat sliced apples inoculated with L. monocytogenes suspension in a CaA solution. The dose of 1.6 kGy that can achieve a 5-log reduction in L. monocytogenes population had no significant effect on quality, except a loss in firmness, during 14 d storage at 4 °C. Another approach to achieving both inactivation of foodborne pathogens and inhibition of browning is the use of washing solutions with both sanitizing and antibrowning capacities.

Chlorination of wash water at a concentration of up to 200 mg/L is routinely applied to reduce microbial populations in produce processing lines (Wei and others 1985). However, the use of chlorine is of concern due to the formation of harmful by-products (Richardson and others 1998), rapid depletion under high organic loading, requirement for pH adjustment of chlorine, and chlorine gas off during processing. There is hence much interest in the development of safer and more effective sanitizers. Acidic electrolyzed water (AEW) and peroxyacetic acid (POAA, also called Tsunami as produced by Ecolab) are 2 new sanitizers that have been tested in recent studies. AEW
has a strong bactericidal effect against pathogens and spoilage microorganisms due to a combined action of free chlorine (~80 mg/L), low pH (2.3 to 2.7) and high oxidation–reduction potential (ORP) (1130–1160 mV) (Wang and others 2004). AEW has been reported to work better than chlorinated water for inactivating *E. coli* O157:H7, *Salmonella enteritidis*, and *L. monocytogenes* on selected fresh produce and meat products (Park and others 2001; Bari and others 2002). POAA is a mixture of acetic acid (CH₃COOH) and hydrogen peroxide (H₂O₂) in an aqueous solution. The sanitizing efficacy of POAA is not affected by organic load, solution pH, and temperature and it can rapidly break down after use into water, oxygen, and acetic acid (Anon. 2004). Beuchat and others (2004) reported that 80 mg/L POAA was more effective in reducing populations of *L. monocytogenes* on lettuce than a chlorine solution of 100 mg/L.

Outbreaks associated with apple cider and apple juice indicated that *E. coli* O157:H7 was tolerant to low pH conditions (Yokogawa and others 2003). *E. coli* O157:H7 has been found to grow exponentially in wounds on Golden Delicious apples with pH of about 3.6 (Janisiewicz and others 1999). Survival of *E. coli* O157:H7 on cut surfaces of apples has also been observed by Sapers and others (2000), Riordan and others (2001), and Gunes and Hotchkiss (2002). It is therefore necessary to examine the survival of this organism on cut surfaces of apples when treated with antibrowning and antimicrobial solutions.

The main objectives of this study were to investigate bactericidal ability of AEW, POAA, and CaA solutions on *E. coli* O157:H7 on fresh-cut apple surfaces and to explore the feasibility of combining selected sanitizers with CaA solution to control both browning reactions and microbial growth on the surfaces of fresh-cut apples under modified atmosphere conditions.

**Materials and Methods**

**Preparation of treatment solutions**

AEW was generated using an AEW generator (ROX-20TA, Hoshizaki, Nagoya, Japan) and collected from the anode of the generator in sanitized beakers. The pH and ORP of the AEW were measured with an AR15 pH and ORP meter (Accumet Research, Pittsburgh, Pa., U.S.A.) and the residual chlorine concentration was determined using an EPA-approved chlorine colorometric test kit (Model PCT-DR, LaMotte Co., Chestertown, Md., U.S.A.). POAA (80 mg/L; Tsunami 100; Ecolab, St Paul, Minn., U.S.A.) was prepared according to the manufacturer’s instruction. The concentration of POAA was measured with a POAA test kit provided by the manufacturer.

Chlorine solution was prepared with a concentrated food grade bleach and the pH was adjusted to 6.5 with 1.0 N HCl. The available chlorine was determined with a chlorine test kit. Chlorine concentration was controlled at 80 mg/L.

CaA solution (5%) was prepared by dissolving solid CaA (CaA; Spectrum chemical & laboratory products, New Brunswick, N.J., U.S.A.) in sterilized deionized water (4°C) and used within 30 min. AEW and POAA were prepared 12 h prior to the test and stored at 4°C until use. Sterilized deionized water was used as the control.

**Inoculum preparation**

*E. coli* O157:H7 (DD642) cultures were transferred 3 times to tryptic soy broth (pH 7.3, Difco Laboratories, Detroit, Mich., U.S.A.) by loop inoculation at successive 24-h intervals and incubated at 37°C. Bacterial cells were harvested, after 24 h of growth, by centrifugation (10000 × g) at 4°C for 10 min. The cell pellets were washed twice in salt peptone (0.85% NaCl, 0.1% Bacto Peptone), and resuspended in 10 mL of 0.1% peptone water. The final concentration of *E. coli* O157:H7 in the inoculum, determined by plating serial dilutions on Sorbitol MacConkey Agar (SMAC, Difco Laboratories) and incubating at 37°C for 24 h, was approximately 10⁶ CFU/mL.

**Sample preparation and treatment procedures**

Golden Delicious apples (*Malus domestica* Borkh) were obtained from a local produce provider, kept at 4°C, and used within 3 d. The sound, defect-free apples of uniform size and shape (75-mm dia) were cored and then cut into wedges with sterilized knives. For each treatment, 90 wedges (14.4 g ± 2.1 g per wedge) were prepared. Fresh-cut apples were washed with a 1:10 (w/v) ratio of samples to one of the following 5 treatment solutions: sterilized deionized water, AEW (pH 2.7, ORP 1150 mV, and free chlorine 45 mg/L), POAA (80 mg/L), and sequential wash with POAA or AEW followed by CaA (5%, pH 5.0, POAA-CaA, AEW-CaA). The washing solution was circulated by a submerged pump (2E-38 N, Little Giant Pump Co., Oklahoma City, Okla., U.S.A.) for 5 min for all the treatments except the sequential wash in which a 3 min AEW or POAA was washed by a 2 min CaA wash. In the sequential treatment, the residual POAA or AEW on produce surface was removed by a manually operated salad spinner (OXO and Good Grips, Elmira, N.Y., U.S.A.) for 1 min before CaA wash. Three pieces of dewatered samples (average 43.3 g ± 2.1 g) were packaged in one of the 3 kinds of bags: polypropylene bags (11 cm × 17 cm) with film oxygen transmission rate (OTR) of 158 and 225 mL/d (m² · m²), respectively, and Ziploc™ Sandwich & Snack bags (16.5 × 15.5 cm). Samples were then stored at 4°C for up to 21 d. Quality evaluations of samples were performed on 0, 4, 8, 11, and 21 d. For quality evaluation, 3 packages (or a total of 9 wedges) per treatment were used. In the analysis of samples from each of the 3 packages, the sensory and color evaluation were made on all 3 wedges before a bag was opened. Then, 1 wedge from the bag was used for microbiological test and the other 2 for texture measurement.

In *E. coli* O157:H7 inactivation tests, 360 g of Golden Delicious wedges were submerged in 2.5 L of bacterial inoculum containing 10⁶ CFU/mL *E. coli* O157:H7 for 15 min with gentle agitation. The inoculated apple slices were drained and air-dried for 30 min in a laminar flow biological hood (Labconco Purifier PCR Enclosure, Kansas City, Mich., U.S.A.) before treatments. Inoculated apple samples, about 43.3 g each, were then submerged in one of the 5 treatment solutions: sterilized deionized water, AEW (pH 2.7, ORP 1150 mV, and free chlorine 70 mg/L), POAA (80 mg/L), chlorine (pH 6.5, free chlorine 80 mg/L), and CaA (5%, pH 5.0) with an apple to solution ratio of 1:20 at 4°C for 5 min with agitation at 60 rpm with an agitator (Thermolyne Cimarec, Dubuque, Iowa, U.S.A.). One wedge (about 14.4 g) was removed from the solutions at the 5th min and immediately drained. The drained sample was transferred to a sterile stomacher bag for microbiological analysis.

**Analysis of gas composition**

The partial pressures of O₂ and CO₂ in the packages were determined using an O₂ and CO₂ analyzer (PBI Dansensor, Checkmate 9900, Ringsted, Denmark). Prior to opening packages for analysis, a rubber septum was attached to each package and a gas sample taken using a 0.5-in 26 G needle.

**Color measurement**

A Minolta Chroma Meter CR-300 (Minolta Corp., Osaka, Japan) was used to assess the color of apple wedges. Considering color variations among cut surfaces of apples within the same bag, 6 readings per wedge (3 readings on each side) and 18 readings per bag were taken to ensure that the data obtained truly represented the color
of the samples. The means of $L^*$, $a^*$, and $b^*$ from 18 readings were recorded on each sampling day.

**Texture measurement**

The firmness of the apple wedges was determined using a TA-XT1 Texture analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.) with a TA-212-cylinder probe (11 mm ID). The middle portion of each wedge was cut into a 2.5 mm $\times$ 2.5 mm $\times$ 0.8 mm (W $\times$ L $\times$ H) cube to avoid the slant wedge slipping when the probe pressed it. The peak force of the measurement was used to determine the firmness of apple wedges. Three measurements were conducted for each package.

**Microbiological analysis**

Apple wedges (14.4 g ± 2.1 g per wedge) from each package were macerated in 85 mL 0.1% (w/v) sterile salt peptone water for 2 min with a stomacher blender (Lab-Blender 400, Cooke Laboratory Products, Alexandria, Va., U.S.A.). The homogenate was filtered through sterile glass-wool, serially diluted in peptone water, and plated (100 μL in triplicate) on an appropriate medium. The total aerobic plate count (TAPC) was determined by plating the samples on trypticase soy agar (TSA, Difco Laboratories) and incubating at 37°C for 24 h. Yeast and mold enumeration was performed by culturing with potato dextrose agar (PDA, Difco Laboratories) and incubating at 25°C for 4 to 5 d. The final *E. coli O157:H7* population from inoculated samples treated with sanitizers or CaA was determined by plating serial dilutions of the homogenate on Sorbitol MacConkey Agar (SMAC; Difco Laboratories) and incubating at 37°C for 24 h.

**Sensory evaluation**

The extent of decay, browning, and acceptability of apple wedges was evaluated by a 7-member trained panel following a modified procedure from Loaiza and Cantwell (1997). Decay was scored on a 1 to 5 scale, where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5% to 20% surface affected), 4 = moderately severe (20% to 50% surface affected), and 5 = extreme (> 50% surface affected). Browning was evaluated on a scale of 1 to 5, where 1 = none, 2 = slight, 3 = moderate, 4 = severe, and 5 = extreme browning. Acceptability was evaluated on a 9 to 1 scale, where 9 = excellent, no defects, 7 = very good, minor defects, 5 = fair, moderate defects, 3 = poor, major defects, and 1 = extremely poor.

**Statistical analysis**

Three replicate samples of each treatment were taken from a single experiment. Data were analyzed using SAS (SAS institute, Cary, N.C., U.S.A.). The effect of treatments and package types, as well as the interaction between treatments and package types, was examined using the GLM procedure. The correlation between gas composition and colorimetric values was analyzed using the CORR procedure. The Fisher’s LSD test was used to determine differences among means at $\alpha = 0.05$.

**Results and Discussion**

**Effect of treatments on reduction of *E. coli* O157:H7 population**

Preliminary tests showed that washing fresh-cut apples for 5 min achieved more bacteria population reduction than 1 min or 3-min washing (for 1-min and 3-min washes, the reduction was in the range of 0.8 to 1.8 and 0.9 to 1.9 log, respectively). To better distinguish the differences caused by different treatments, a 5-min washing time was used in both the *E. coli* inactivation tests and the quality comparison tests. The initial count of *E. coli* O157:H7 inoculated onto fresh-cut apple surfaces was 6.4 ± 0.1 log CFU/g tissue. Washing with AEW, POAA, or chlorine removed significantly ($P < 0.05$) higher numbers of *E. coli* O157:H7 cells than did water or CaA treatments (Table 1). There was no significant difference ($P > 0.05$) on *E. coli* O157:H7 reduction between the water and CaA treatment. With a 5-min wash, POAA and AEW reduced *E. coli* O157:H7 population by 2.7 and 2.1 log CFU/g, respectively, while CaA and water treatments removed no more than 1.1 log CFU/g. The AEW (free chlorine 70 mg/L) achieved greater reduction of microbial populations than did chlorine (free chlorine 80 mg/L). The POAA was the most effective sanitizer among the 3 sanitizing agents tested.

**Package atmosphere**

The CO$_2$ partial pressure increased gradually while O$_2$ partial pressure decreased during storage among all treatments (Figure 1). The packages prepared with 158 and 225 OTR films had significantly ($P < 0.05$) higher CO$_2$ partial pressure and lower O$_2$ partial pressure than the Ziploc bags within the same treatment during the storage. The lower CO$_2$ accumulation and high O$_2$ partial pressure in the Ziploc bags are mainly caused by the fast gas exchange due to the lose seal of the packages. Generally, O$_2$ levels in 158 OTR bags were slightly lower than those in the 225 OTR bags due to the lower O$_2$ transmission rate. Apple wedges in 158 OTR and 225 OTR bags maintained O$_2$ partial pressure above the recommended optimum O$_2$ partial pressure (1%) for fresh-cut apples (Fan and others 2005a).

The responses of fresh-cut apples to different treatments during storage were slightly different. It can be seen from Figure 1(a–c) that the CO$_2$ production from the POAA-CaA-treated samples started to slow down from 8th day, and at 11th day, the CO$_2$ partial pressure of POAA-CaA treatment were significantly lower than that of other treatments in all the bags except the control in packages with the 225 OTR film ($P < 0.05$). It seems POAA-CaA treatment reduced the respiration activity of apple slices after 8 d, which may have an impact on the quality of the samples. For the AEW-CaA treatment, CO$_2$ partial pressure experienced a fast increase until 11 d, which was accompanied by a fast depletion of O$_2$ during the same period. Except the POAA-CaA treatment, other treated samples exhibited gas composition similar to AEW-CaA-treated samples during storage. This pattern may suggest that the increased respiration was caused more by tissue wounding from cutting operations than by damage resulting from washing with the oxidizing agents. Baur and others (2004) washed fresh-cut lettuce with ozonated and chlorinated water and observed that there were no significant differences between samples treated with the sanitizers and the control (water) for the changes of O$_2$ and CO$_2$ in packages during storage at 4°C. They suggested that the gas composition in package was determined more by the degree of wounding through cutting than by the individual treatments. Fan and others (2005a) also reported that the changes in headspace composition of fresh-cut apples induced by irradiation at 0.5 kGy and 1.5 kGy were not significantly different from the control (0 kGy).

### Table 1 — *E. coli* O157:H7 population reduction on inoculated apple samples after 5-min treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Population reduction (Log CFU/g tissue)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.93 ± 0.06 a*</td>
</tr>
<tr>
<td>CaA</td>
<td>1.12 ± 0.24 a</td>
</tr>
<tr>
<td>Chlorine</td>
<td>1.69 ± 0.06 b</td>
</tr>
<tr>
<td>POAA</td>
<td>2.71 ± 0.33 c</td>
</tr>
<tr>
<td>AEW</td>
<td>2.07 ± 0.28 b</td>
</tr>
</tbody>
</table>

— Initial counts of *E. coli* O157:H7 on the apple pieces were 6.41 ± 0.08 log CFU/g tissue (mean ± standard deviation).
— Data followed by different letters in the same column are significantly ($P < 0.05$) different among the treatments.
Color
The colorimetric readings $a^*$ and $L^*$ for apple slices during storage are presented in Figure 2. A high $a^*$ and low $L^*$ value indicated more browning of fresh-cut apple surface (Sapers and Douglas 1987). The color readings of apple tissues from the same treatment packed in 3 kinds of bags did not show significant differences ($P > 0.05$) at day 0 and other storage days, which indicated that the permeability of the packaging materials was not a key factor determining the browning reactions. This is in agreement with the observation of Soliva-Fortuny and others (2001) in a study to examine the effect of modified atmosphere packaging on browning reactions of apple cubes in 2 packaging materials. For all 3 kinds of packages, POAA treatment resulted in significantly higher $a^*$ ($P < 0.05$) and significantly lower $L^*$ values compared to other treatments. This trend may result from more severe apple-tissue damage caused by POAA wash so that more polyphenol oxidase was released out of the apple cells to promote oxidation of phenolic substrates of apple tissues. The inhibitory effect of CaA on browning reactions was evidenced by low $a^*$ and high $L^*$ values in AEW-CaA and POAA-CaA-treated samples in comparison with the AEW- and POAA-alone treatments. For the POAA-CaA sequential treatment, the effect of CaA on browning control seemed to be depressed after 11 d. Although the CO$_2$ and O$_2$ partial pressures for POAA-CaA-treated apple wedges were significantly different from other treatments after 11 d (Figure 1), the lower CO$_2$ and higher O$_2$ in this treatment after 11 d did not correspond with reduced browning, indicating that the gas composition during storage does not have a direct impact on browning reactions.

A correlation analysis was conducted to determine the role played by the gas composition in color changes (Table 2). For $a^*$, 32% to 46% of the color changes were caused by changes in CO$_2$ and O$_2$ partial pressure, whereas for $L^*$, only 12% to 31% of the changes in lightness could be attributed to gas composition.

Texture
Figure 3 shows the firmness of fresh-cut apples stored for 8 d and 21 d. There were no significant differences in firmness loss ($P > 0.05$) for the same treatment among 3 packaging bags on either storage day. On the 8th day, the AEW treatment in 225 OTR bags caused the most firmness loss, significantly lower ($P < 0.05$) than the control and POAA-CaA treatments. Tests on the 8th day did not find significant differences in firmness reduction among samples packed in 158 OTR bags and Ziploc bags. At the end of storage (21 d), samples treated with POAA-CaA were significantly less firm ($P < 0.05$) in all package types than other treatments except for the POAA-washed apple wedges in the 225 OTR bags. Similar to the control, the AEW-CaA treatment retained firmness better than other treatments at the 21st day, regardless of the packaging used, with the exception of samples washed with AEW and packed in 225 OTR bags. This may suggest that the CaA treatment, when combined with AEW, functioned to retard firmness reduction of fresh-cut apples. Fan and others (2005a) also noticed that CaA treatment reduced firmness loss of ionic irradiated fresh-cut apples during storage. It was postulated that the Ca might have helped to inhibit stress-induced senescence in apples by maintaining membrane integrity (Fan and others 2005b).
Browning and microbial growth control for fresh-cut apples

**Microbiological analysis**

There were less than 2-log total aerobic bacteria and no yeast and mold detected on unwashed fresh-cut apples, which were relatively clean when obtained from a local market and processed under sterile conditions. On day 0, no significant differences ($P > 0.05$) in TAPC were found between the control (sterilized water) and the sanitizing treatments in each package type (Figure 4). During storage, a gradual increase in TAPC was observed for all the treatments and packaging bags. Although variability in the response of microflora to the sanitizing treatments was observed during storage, similar to that reported by Sapers and others (2001), the AEW wash exhibited a better inhibition to aerobic bacterial growth than other treatments. The elevated CO$_2$ partial pressure in 158 OTR and 225 OTR bags did not limit the growth of microflora. Instead, a retarded growth of aerobic bacteria, compared to other packaging materials, was observed in Ziploc bags that had lower CO$_2$ partial pressure.

The yeast and mold counts on fresh-cut apples at the 21st day of storage are shown in Figure 5. There was no yeast or mold growth on fresh-cut apples treated with AEW, POAA, AEW-CaA, or POAA-CaA until the 11th day. On the 21st day a significant growth in yeast and mold was observed for all 3 packaging bags. Statistically, the growths in 158 OTR, 225 OTR, and Ziploc bags were all significantly ($P < 0.05$) different if data from all the treatments in 1 type of packaging are lumped together for the comparison. A significant ($P < 0.05$) interaction was also found between packaging types and treatments in their effect on yeast and mold population. Yeast and mold populations on apple wedges treated with AEW and AEW-CaA were significantly ($P < 0.05$) lower than control, POAA, and POAA-CaA in all 3 packages. Ziploc bags and 225 OTR film allowed better growth of yeast and mold in samples treated with POAA and POAA-CaA. In all package types, POAA-CaA treatment resulted in the highest yeast and mold populations as it did for total aerobic bacteria population at the end of storage. This may indicate that POAA-CaA treatment facilitated favorable conditions for microbial growth.

**Sensory evaluation**

Since the total aerobic bacteria started to grow rapidly after 8 storage days, sensory attributes were compared and reported only...
Browning and microbial growth control for fresh-cut apples...
Acknowledgments

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References


