Dual effectiveness of sodium chlorite for enzymatic browning inhibition and microbial inactivation on fresh-cut apples

Yaguang Luo a, *, Shengmin Lu a, b, Bin Zhou a, c, Hao Feng c

a Environmental Microbial and Food Safety Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, USA
b Institute of Food Processing, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
c Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

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A B S T R A C T

The dual effectiveness of sodium chlorite for browning inhibition and microbial inactivation on fresh-cut apples was investigated and compared to other anti-browning and antimicrobial agents. Results indicate that sodium chlorite significantly (P < 0.001) inhibited the browning reaction of fresh-cut Red Delicious apples stored at 5 °C for 14 days. This treatment also significantly reduced polyphenol oxidase activities. Treatments with acidified sodium chlorite, calcium chloride, or calcium ascorbate exhibited strong inhibition on apple browning during the early storage, these treatment effects diminished after 7 days in storage. Combining calcium chloride with sodium chlorite further significantly (P < 0.001) improved the firmness of apple slices, and browning inhibition during storage. Additionally, treatments with acidified sodium chlorite, sodium chlorite, or the combination of sodium chlorite and calcium chloride significantly (P < 0.001) reduced Escherichia coli populations on fresh-cut apples by 3.0, 3.6, and 3.9 log cfu g−1 over the water control. The dual effectiveness of sodium chlorite to inhibit enzymatic browning and inactivate E. coli may allow this compound to achieve a prominent role in improving the quality and safety of products in the fresh-cut apple and other food industries.

1. Introduction

Fresh-cut apples have emerged as popular products for consumers who demand healthy alternatives to conventional snack foods and have been approved for school lunch programs in the US (Gorny, 2004; UFPA, 2009). The production and consumption of fresh-cut apples and other fresh-cut produce items is projected to continue growing as more consumers demand fresh, convenient and nutritious foods. The produce industry still faces two important challenges to the quality and safety of fresh-cut products, i.e. control of enzymatic browning and control of microorganisms. More effective methods must be developed to simultaneously control both the enzymatic browning of apples and other fresh-cut produce items and the growth of spoilage microorganisms and pathogens if contaminated.

Enzymatic browning is the primary physiological disorder that causes the decline of sensory quality and shelf life of fresh-cut apples (He, Luo, & Chen, 2008; Lu, Luo, Feng, & Turner, 2007). Browning reaction is typically catalyzed by polyphenol oxidase (PPO), followed by non-enzymatic formation of melanins. Researchers have given much attention and explored many approaches to solve the browning problem of fresh-cut apples (Martinez & Whitaker, 1995). However, despite intensive research activities, concerns over off-flavors and odors, food safety, economic feasibility, and effectiveness of inhibition have resulted in very few browning inhibitors demonstrating potential to be useful in the food industry (McEvily, Iyengar, & Otwell, 1992; Soliva-Fortuny & Martin-Belloso, 2003).

Treatment with calcium ascorbate is the major technology that has been widely employed by the fresh-cut apple industry to inhibit the enzymatic browning reaction and to maintain the quality and shelf life of fresh-cut apples (Gorny, 2004). However, the reductive nature of calcium ascorbate (CaAs) makes proper sanitizing treatment for pathogen control a technical challenge due to the incompatibility of CaAs with the widely used oxidative sanitizing agents, i.e. chlorine, ozone and chlorine dioxide (He & Luo, 2007; Luo, 2007). The frequent reuse of browning control solutions for many batches of apples creates a high risk for food-borne illness outbreaks without proper sanitizing treatment to control the potential cross-contamination of apples by food-borne human pathogens. In fact, the contamination of sliced apples with human pathogenic bacterium, Listeria monocytogenes, has resulted in costly...
Recalls in the US (FDA, 2006, 2009). There is an urgent need for either a sanitizer that is compatible with the current widely used anti-browning solution, or preferably, a solution that can provide dual control of browning reaction and microbial growth to maintain the safety and quality of fresh-cut apples.

Acidified sodium chlorite (ASC) is a sanitizing agent recently approved by the FDA for dip or spray application for food items, including fresh and fresh-cut fruits and vegetables. It has been shown to be effective for the control of pathogens (Escherichia coli O157:11, L. monocytogenes, Salmonella Poona etc.) on fresh-cut carrots and ciliantro (Allende, McEvoy, Tao, & Luo, 2009; Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004; Ruiz-Cruz, Luo, Gonzalez, & Tao, 2006). Due to its low pH (~2.5), it is hypothesized that ASC may be able to control browning reaction of fresh-cut apples, and therefore serve as the aforementioned dual control agent much needed by the apple industry. Extensive studies in our laboratory using Golden Delicious and Red Delicious apples have demonstrated that ASC is a potential browning inhibitor.

In elucidating the mechanism of browning control by ASC, an additional experiment was conducted to compare the browning inhibition by ASC with that of its major components: sodium chlorite (SC) and citric acid (CA). Results indicated that SC had stronger browning inhibition than ASC. Further studies on the mechanism of browning inhibition indicated that SC is a mixed type of inhibitor for polyphenol oxidase activity (He & Luo, 2007; Lu, Luo, & Feng, 2006; Lu et al., 2007). Additionally, during these early studies, we also noticed that both SC, and especially ASC caused tissue softening of apples. Therefore, incorporation of calcium chloride (CC) into the formula to solve the tissue softening issues was investigated. Preliminary studies determined that 20 g L\(^{-1}\) CC was effective in improving apple texture without incurring the bitter taste discerned at higher concentrations. The authors tested many dosages of SC on fresh-cut apple and lettuce for its effects on both microbial and browning control and determined that a dosage of 300 mg L\(^{-1}\) was optimal for maintaining quality of apple slices while inhibiting microbial growth. The object of this research was to determine the dual effectiveness of SC, compared to ASC, CA, CaAs and calcium chloride, for browning inhibition and inactivation of human pathogen surrogate, Escherichia coli HB101.

2. Materials and methods

2.1. Materials

Seven different wash solutions were prepared as described below: water control (CK), 300 mg L\(^{-1}\) sodium chlorite (SC), 300 mg L\(^{-1}\) acidified sodium chlorite (ASC), 300 mg L\(^{-1}\) citric acid (CA), 20 g L\(^{-1}\) calcium chloride (CC), combination of SC 300 mg L\(^{-1}\) and CC 20 g L\(^{-1}\) (SC & CC), and 20 g L\(^{-1}\) calcium ascorbate (CaAs), and all chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA), with the exception that ASC was obtained from EcoLab (St Paul, MN, USA).

Red Delicious apples (Malus domestica Borkh) were obtained from a wholesale produce market in Jessup, MD, USA. The fruits were stored at 0–1 °C and used within 2 weeks. Apples with uniform size and color were rinsed in tap water and cut into 8 equal wedges using a sharp stainless steel knife. Five wedges, one from each of five apples were pooled together and dipped within 1 min of cutting in one of the aforementioned seven treatment solutions for 1 min, drained on clean paper towel, sealed in 16 cm × 16 cm polypropylene film with oxygen transmission rate of 16.8 pmol s\(^{-1}\) m\(^{-2}\) Pa\(^{-1}\) and stored at 5 °C for 2 weeks. Time zero color measurements were made on control samples and those samples most vulnerable to browning first. All samples were measured within 1 h of dipping.

2.2. Methods

2.2.1. Color and texture determination

Color of apple wedges was measured on both sides of each wedge using a tri-stimulus CR-400 Minolta Chromo Meter (Minolta Co. Ltd, Japan) on days 0, 1, 3, 7 and 14 of storage. The Chroma meter was calibrated with a standard white plate (Y = 94.00, x = 0.3158, y = 0.3322). The values reported are the means of 10 lightness (L\(^{*}\)) values.

Firmness of the apple wedges was evaluated using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, N.Y., USA) following a modified procedure described by Rojas-Grau, Grasa-Guillem, and Martin-Belloso (2007). Firmness was expressed by the peak force required to penetrate the apple wedges by 10 mm with a 5 mm diameter probe.

2.2.2. PPO activity determination

Apple PPO was extracted from an apple acetone powder according to Yoruk, Hogsette, Rolle, and Marshall (2003). Enzyme activity was determined spectrophotometrically using chlorogenic acid as the substrate. The 3 mL standard reaction mixture, prepared in a cuvette, contained 2.35 mL of 0.05 mol L\(^{-1}\) acetate buffer, pH 5.2, 0.3 mL inhibition stock solution (or acetate buffer for the control), 0.3 mL of 20 mmol L\(^{-1}\) chlorogenic acid solution prepared in the same acetate buffer, and 0.05 mL PPO extract. The reference cuvette contained only the chlorogenic acid solution and acetate buffer. The changes in absorbance at 420 nm and 25 °C for 2 min were measured using a Shimadzu PharmaSpec UV-1700 UV–vis spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). The enzyme activity was determined based on the initial reaction rates. The inhibition of PPO activity was expressed as percent inhibition which was calculated by the formula:

\[
\text{inhibition(%) = \left( \frac{PPO_{\text{control}} - PPO_{\text{treatment}}}{PPO_{\text{control}}} \right) \times 100}.
\]

2.2.3. Escherichia coli inactivation

A streptomycin resistant E. coli strain HB101 was used in this study. E. coli was grown overnight in Luria Bertani broth (Difco Laboratories, Detroit, MI) supplemented with Streptomycin at 37 °C with constant agitation at 175 rpm. Cultures were washed by centrifugation (4000 × g, 15 min, 4 °C) with 1 g L\(^{-1}\) peptone saline water, and added proportionally to distilled water to obtain an initial inoculum solution. The concentration of E. coli cells in the inoculation solution was diluted in order to reach a desired E. coli inoculation of 10\(^{2}\)–10\(^{8}\) CFU g\(^{-1}\) on fresh-cut apples.

Each Red Delicious apple was cut into eight wedges, which were then cut into 4 pieces of approximately 5 g each. The pieces from all apples were pooled together and 4 pieces (20 g) were randomly assigned to each treatment. Samples were immersed in the E. coli inoculum solution under constant agitation for 10 min, followed by drainage for 1 min and air drying for 30 min. Then, the inoculated apples were dipped into the aforementioned seven treatment solutions at a sample/wash water ratio of 1:20 (w/t), and kept submerged for 1 min, followed by drainage for 30 s and air drying. The samples were homogenized in 180 mL of sterile phosphate buffered saline in a stomacher 400 Biomaster (Seward Limited, London, UK). Populations of E. coli were enumerated by plating serially diluted samples using a spiral plating device (Wasp ll Spiral Plater, DW Scientific, West Yorkshire, UK) onto Luria Bertani (LB) agar (Difco) supplemented with 100 µg mL\(^{-1}\) streptomycin. The LB
agar plates were incubated at 37 °C for 24 h. Colonies were enumerated with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK).

2.3. Statistical analysis

Experiments were conducted with three replications per treatment. E. coli counts (cfu g⁻¹) were log transformed before statistical analysis. Data were analyzed according to a general linear model using PROC MIXED (SAS Institute Inc., Cary, NC). Enzyme inhibition data were expressed as % inhibition and were sin transformed prior to statistical analysis. Assumptions of normality and variance homogeneity of the linear model were checked for all data. A variance grouping technique was used to address variance heterogeneity for means comparisons. For all data, when effects were statistically significant, means were compared using Sidak adjusted p-values to maintain experiment-wise error ≤0.05.

3. Results and discussion

3.1. Color changes of apple slices

Red Delicious apple slices subjected to each of the seven wash treatments, i.e. water control (CK), sodium chlorite (SC), acidified sodium chlorite (ASC), calcium chloride (CC), the combination of SC and CC (SC & CC), citric acid (CA), and calcium ascorbate (CaAs), were monitored for color changes (L* value) during storage. As shown in Fig. 1, wash treatment significantly (P<0.0001) affected the L* values of apple slices on day 0 and throughout the storage. On day 0, although all samples still appeared fresh visually, samples receiving the control and CA treatment had significantly lower L* values than those receiving the other treatments. Red Delicious apples are known to have severe enzymatic browning problems, and browning often occurs within minutes after cutting (Luo & Barbosa-Cánovas, 1996, 1997). Since the decline in L* value is closely related to the browning reaction of fresh-cut apples (Lu et al., 2007; Sapers & Douglas, 1987), the significantly lower L* values on samples received CK and CA treatments suggest strong browning reactions on those samples during the delay between dipping and color measurement on day 0. During storage, the L* values of all samples decreased progressively, with the sharpest decline occurring within the first 3 days, followed by additional decline during the remainder of the storage period. Although CaAs had significantly larger L* values on days 0, 1, and 3 than the control and CA treatments, the differences in L* diminished after 7 days in storage. Similarly, although CC and ASC treatment maintained larger L* values during the early storage, the differences in L* diminished towards the end of storage. However, treatment containing SC and SC&CC maintained significantly larger L* values than all other treatments throughout the entire 14 day storage, suggesting that SC and SC&CC effectively delayed browning reaction of apple slices during storage.

Calcium ascorbate has been successfully employed to control the browning of apple slices, attributable to the components of ascorbic acid and calcium ion in the solution. However, its inhibitory effect depends on the concentration used. Although 50 g L⁻¹ CaAs (Wang, Feng, & Luo, 2007) has strong browning inhibition, its high price results in it more commonly being used at lower, less effective concentrations. Ascorbic acid and its derivatives are known for their ability to reduce quinones back to the original colorless diphenol and their effects disappear once they are oxidized (McEvily et al., 1992). In this study, 20 g L⁻¹ CaAs only partially inhibited browning during the first 3 days (Fig. 1). Calcium chloride is able to maintain fruit firmness, inhibit browning, and extend the shelf life; its effect also depends on the concentration used. Treatment with 20 g L⁻¹ CC maintained high L* value during the first seven days, but the L* value declined in later storage compared to water control. Citric acid has been widely used commercially as an anti-browning agent and is known to have inhibitory activity on polyphenol oxidase (Pizzocaro, Torreggini, & Gilardi, 1993) and anti-browning activity in minimally processed fruits and vegetables (Rocha, Brochado, & Morais, 1998). However, the browning inhibition activities of CA are concentration dependant. According to Jiang, Li, & Li (2004), at concentrations lower than 0.02 mol L⁻¹ (3.84 g L⁻¹), citric acid actually stimulated PPO activity of fresh-cut Chinese water chestnut. At a treatment concentration of as low as 0.3 g L⁻¹ used in this current study, the smaller L* value on apple slices treated with citric acid may suggest that low concentrations of CA treatment can stimulate browning reaction on apple slices. Given that ASC had significantly weaker browning inhibition than SC, and CA at the tested concentration did not exert any browning inhibition, the observed browning inhibition of ASC treatment is most likely attributed to SC rather than CA.

3.2. Firmness change of apple slices

Firmness of apple slices was affected by the calcium treatments, with samples treated with CC and SC&CC significantly (P = 0.001) firmer than those treated with CK, SC, and ASC (Fig. 2). Calcium cation is well known to increase firmness and delay membrane lipid catabolism in apple fruit (Picchioni, Watada, Conway, Whitaker, & Sams, 1998). Calcium chloride has been previously found to be effective in improving the firmness of fresh-cut apples treated with 4-hexylresorcinol (Luo & Barbosa-Cánovas, 1996) and isoascorbic acid (Buta, Moline, Spaulding, & Wang, 1999). In this study, SC, in combination with CC also maintained firmness of apple slices, which suggests a promising formula ideal for both controlling browning and improving firmness.

Fig. 1. Changes of L* values in apple slices subjected to the following treatments: 300 mg L⁻¹ sodium chloride (SC; △); 300 mg L⁻¹ acidified sodium chloride (ASC; ▽); 20 g L⁻¹ calcium chloride (CC; □); the combination of SC plus CC (SC&CC; ▪); 20 g L⁻¹ calcium ascorbate (CaAs; ●); 300 mg L⁻¹ citric acid (CA; ○); and water control (CK; ●). Data presented is the mean of ten sample measurements.
3.3. Inhibition of apple PPO activity

All treatments containing SC (SC, ASC, SC & CC), CC, or CaAs significantly reduced PPO activities compared to CK and CA treatments (Fig. 3). Although statistically insignificant, CC and CaAs treatments reduced PPO activity slightly more than SC and SC & CC, reaching nearly 100% reductions of PPO activities. Interestingly, ASC more than just inhibited PPO activity under the testing conditions, but probably caused bleaching of the substrate as a result of its potent oxidizing potential, thus resulting in the decrease of absorbance during reaction and an over 100% PPO inhibition. CA did not inhibit PPO activity, a result that was consistent with the gradual decrease in $L^*$ value for CA treated apple slices during storage (Fig. 1). Although both CC and CaAs resulted in nearly 100% PPO activity inhibition, the $L^*$ value of apple slices in these 2 treatment groups did not remain high during storage, as a result of gradual depletion of the CC and CaAs over time.

3.4. Inactivation of Escherichia coli

Apple slices that received water wash treatment had $E. coli$ populations averaging $5.46 \pm 0.12 \log$ CFU g$^{-1}$. While CA, CC and CaAs treatments exhibited no significant ($P > 0.05$) reduction of $E. coli$ populations compared to the water wash control, all treatments containing SC significantly ($P < 0.0001$) reduced $E. coli$ populations, with SC & CC, SC, and ASC reducing $E. coli$ population by 3.9, 3.6, and 3.0 log cfu g$^{-1}$, respectively (Fig. 4). As a strong oxidant, SC has been used in drinking water disinfection, bleaching of brined cherries, extending storage life of meat and fish products, and as the source for the formation of chlorine dioxide (Mullerat, Sheldon, & Klapes, 1995; Zhang, Lu, & Levin, 2003). Our earlier studies showed that both SC and ASC had strong antimicrobial effect against human pathogenic bacteria, $E. coli$ O157:H7, on cilantro leaves (Allende et al., 2009). This current study further confirmed that SC, either on its own or in combination with CC or CA, had a strong efficacy on microbial inactivation.

Fresh-cut processing of apples causes the loss of protective epidermal and sub-epidermal tissues, and results in the exposure of nutrient-rich internal tissue that support pathogen attachment and growth. In current commercial processing, CaAs is often used to maintain stability of color and inhibit browning. The large cost associated with the use of CaAs during the preparation of apple slices prohibits processors from discarding the treatment solutions after a single use. If contaminated, the treatment liquid could potentially become a source of pathogens and thus cause cross-contamination and pathogen spread for the subsequent batches as CaAs has no antimicrobial effect (Gorny, 2003). Therefore, the search for a chemical compound that is compatible with CaAs treatment, or better yet, a dual control agent for both browning inhibition and pathogen inactivation has received intensive
research attention. Several researchers have developed multi-chemical formulas that provide both browning inhibition and some degree of antimicrobial activity. Piloza and Sapers (2004) demonstrated the efficacy of an acidic browning inhibitor formula containing hexametaphosphate which is believed to sequester copper in polyphenol oxidase, thereby causing enzyme inhibition and preventing browning. This neutral polyphosphate seed was used in an acidic formula with CA, ascorbic acid and sodium chloride. The formula was expected to have antimicrobial activity against human pathogens, because of its low pH (2.9). Bhagwat, Saffter, and Abbott (2004) used a formula which contained reducing agents (isosorobic acid and ascorbate), a food preservative (propionate), and an inhibitor (N-acetylglucosamine) of PPO-mediated browning of fresh-cut produce. The formula was effective in reducing pathogenic bacteria growth for fresh-cut apples. Wang (2007) used a sequential treatment of acidified electrolyzed water followed by calcium ascorbate to achieve dual control of browning and bacterial growth on apple slices. All of those applications required combinations of multiple chemical compounds or preparation steps. The discovery of the dual effectiveness of browning inhibition and microbial inactivation of a single chemical compound, SC, thus makes this chemical compound uniquely suited for fresh-cut apple processing.

4. Conclusion

Sodium chlorite treatment significantly inhibited browning reaction and polyphenol oxidase activity of fresh-cut apples during the 14 day storage period. This treatment also significantly reduced E. coli populations by 3.6 log cfu g⁻¹. Combining calcium chloride with sodium chlorite did not detract from the solution’s anti-browning or antimicrobial properties and significantly improved the texture of apple slices. The effectiveness of sodium chlorite on browning inhibition and pathogen inactivation suggests that sodium chlorite has the potential to be applied to food products to achieve a much needed dual control for both browning and microbial contamination of food products.

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