1   **Dual Effectiveness of Ascorbic Acid and Ethanol Combined Treatment to Inhibit**
2   **Browning and Inactivate Pathogens on Fresh-cut Apples**

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ABSTRACT

Fresh-cut apples have recently been associated with several recalls due to food-borne pathogen contamination. Simultaneous control of enzymatic browning and pathogen growth is challenging, because of the incompatibility between most chemicals applied for these two purposes. This study evaluated the efficacy of ethanol in combination with ascorbic acid to inhibit browning and inactivate pathogens on apple slices. Apple slices were dipped in solutions containing 10%-40% (v/v) ethanol (E) and 1% (m/v) ascorbic acid (AA), dewatered, packaged, and stored at 1.5 °C. In parallel experiments, apple slices were dip-inoculated with *Escherichia coli* (*E. coli*) O157:H7 and *Listeria monocytogenes* (*L. monocytogenes*) prior to treatment. Treatments with 20-40% E significantly inhibited the growth of aerobic bacteria, and yeast and mold during storage. Treatment with 20% E plus 1% AA maintained the lightest flesh color and highest tissue firmness. Meanwhile, treatment with 30% E plus 1% AA led to highest microbial inactivation, together with more effective browning inhibition and firmness maintenance. Furthermore, 30% E plus 3% AA reduced pathogen populations immediately after treatment and further during cold storage, demonstrating that this combined treatment can enhance safety, as well as quality of fresh-cut apples.

Key words: Ethanol, Ascorbic acid, Anti-browning, Fresh-cut apple, Pathogen

1. Introduction

Changing lifestyles and eating habits as well as preference for products with fewer additives have resulted in an increased demand for fresh-cut produce (Hussain, Omeera, Suradkar, & Dar, 2014). Guaranteeing food quality and safety of fresh produce is a challenge to food processors because the range of available processing technologies is restricted by the necessity to maintain the freshness of the product. Two of the major challenges for food processing companies are the control of enzymatic browning and avoiding pathogen growth of fresh-cut produce (Lu, Yu, Gao, Lu, & Zhang, 2004; Luo, 2007; Luo, Lu, Zhou, & Feng, 2011; Wang, Nie, & Cantwell, 2014; Matan, Puangjinda, Phothisuwan, & Nisoa, 2015).

Browning discoloration is the main factor limiting the sensorial shelf-life of fresh-cut apples (Supapvanich, Pimsaga, & Srisujan, 2011). Many approaches to inhibit browning of fresh-cut fruit have been reported including a wide variety of chemical compounds, edible films (Sharma & Rao, 2015; Mahmoud & Ommol, 2016), modified atmosphere packaging (MAP) (Cortellino, Gobbi, Bianchi, & Rizzolo, 2015), heat shock treatment (Aguayo, Requejo-Jackman, Stanley, & Woolf, 2015), and temperature control (Oms-Oliu et al., 2010; Rojas-Graü, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009). Although chemical treatments have been among the most successful methods, they have often raised health concerns regarding residues, sulfites have traditionally been used to prevent browning; however, their use on fresh-cut apples has been banned by the FDA due to concerns over allergic reactions in some individuals (Sidney & Mark, 2014). The most widely used

alternative to sulfite is an AA wash solution, an antioxidant that is generally recognized as safe (GRAS) (U.S. Food and Drug Administration, 1986, 2011).

Studies from Bhagwat, Saftner, and Abbott (2004) indicated that anti-browning solutions should not be used on multiple batches of sliced apples because, lacking anti-bacterial activity, they may become sources of contamination due to microbial build-up in the anti-browning solution (Pérez-Rodríguez, et al., 2014; Holvoet, et al., 2014). Even when the wash solution reduces the native microbes, pathogens may flourish as competition for space and nutrients is reduced (Alegre et al., 2013). However, the expense of using fresh wash solutions for each batch of apples may be prohibitive. Sanitizers are essential components of produce wash solutions to prevent microbial cross-contamination among produce pieces and enable the reuse of wash solutions. However, all of the sanitizers generally used by the fresh-cut produce industry, including chlorine (Gómez-López, Lannoo, Gil, & Allende, 2014), ozone (Alwi & Ali, 2014) chlorine dioxide (Chen, Zhu, Zhang, Niu, & Du, 2010), peroxyacetic acid (Gil, Selma, López-Gálvez, & Allende, 2009), and acidic electrolyzed water (Hao, Li, Wan, & Liu, 2015) are oxidizing agents. The anti-browning properties of AA are due to it being a reducing agent. As such, AA neutralizes and renders ineffective the sanitizers widely used for pathogen control. Therefore, a treatment that can both control enzymatic browning and inactivate pathogens is greatly needed to overcome this technical challenge and maintain the quality and safety of fresh-cut apples (Luo, Lu, Zhou, & Feng, 2011).

Few studies have evaluated techniques for the simultaneous inactivation of foodborne pathogens and control of browning in fresh-cut apples. Fan, Sokorai, Sommers, Niemera, and Mattheis (2005) used ionizing radiation to treat sliced apples inoculated with an *L. monocytogenes*-contaminated calcium ascorbate dip. Wang et al. (2007) controlled browning and microbial growth on fresh-cut apples by sequential treatment of sodium hypochlorite and calcium ascorbate. Xiao, Luo, Luo, and Wang (2011) used sodium chlorite dip treatment and chitosan coatings to maintain quality and inactivate *E. coli* O157:H7 on fresh-cut pears. The combination treatment of gamma irradiation and AA was employed to maintain quality and safety of fresh-cut eggplant (Hussain, Omeera, Suradkar, & Dar, 2014). New apple pectin-based edible coatings incorporating a combination of antioxidants and antimicrobial agents were developed to control enzymatic browning and growth of food-borne human pathogens on fresh-cut ‘Rojo Brillante’ persimmon (Sanchís et al., 2016). While research into development of sanitizer and anti-browning agent combinations has been the subject of some study in recent years, more cost-effective, non-radiological treatment options that cause minimal tissue injury are currently needed by the fresh-cut industry.

Ethanol is a generally recognized as safe (GRAS) substance and exists naturally in plants, with low toxicity for fruits and vegetables. It has been used to retard tissue senescence (Perata & Alpi, 1991), maintain the quality of intact apples, grape, cherry, peaches, mango, fresh-cut eggplant (Chervin, Westercamp, & Monteils, 2005; Hu, Jiang, Tian, Liu, & Wang, 2010), asparagus spears (Herppich, Huyskens-Keil, & Hassenberg, 2014) and fresh-cut sunchoke tubers (Wang, Nie, & Cantwell, 2014).

Ethanol is a strong bactericide and is widely applied as disinfectant. *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens* are killed by 10 s of exposure to 40-100% ethanol (Morton, 1950). Ethanol concentrations greater than 25% can kill vegetative cells in mixed-culture samples (Koransky, Allen, & Dowell, 1978).

The objective of this work was to evaluate the combined effect of AA and ethanol on maintaining quality of fresh-cut apples and inhibiting the growth of the human pathogens, *E. coli* O157:H7 and *L. monocytogenes*, during apple storage.

2. Materials and methods

2.1. Materials

Granny Smith apples (*Malus domestica* Borkh) of approximately uniform size and color were obtained from a wholesale produce market in Jessup, MD, USA. The fruits were stored at 0-1 °C and used within 2 weeks. Thirty-eight apples free of defects were washed in 200 mg L⁻¹ free chlorine solution for 2 min and then rinsed with running tap water. Apples were cut and cored into 12 equal slices with a sterilized stainless steel knife. For each treatment, 60 apple slices were immersed in each treatment solution for 2 min, and then de-watered with a hand held salad spinner (Progressive International® Corp. Kent, Washington, USA). Six apple slices were packaged in sealed bags (16 cm×16 cm, polyethylene film, oxygen transmission rate of 16.6 pmol s⁻¹m⁻² Pa⁻¹). The packaged samples were stored at 1.5 °C, and quality evaluations were performed on days 1, 4, 7, 10, and 14.

2.2. Treatments and quality evaluation

2.2.1. Treatments

Five different wash solutions were prepared as described below: water wash (control) (WW), 10 g L⁻¹ (w/v) AA (1% AA), combination of 10 g L⁻¹ (w/v) AA and 0.2 L L⁻¹ (v/v) ethanol (1% AA+ 20% E), combination of 10 g L⁻¹ (w/v) AA and 0.3 L L⁻¹ (v/v) ethanol (1% AA + 30% E), combination of 10 g L⁻¹ (w/v) AA and 0.4 L L⁻¹ (v/v) ethanol (1% AA + 40% E). Ethanol was obtained from Sigma-Aldrich (St. Louis, MO, USA), AA from EM SCIENCE (Darmstadt, Germany) and sodium hypochlorite (Clorox Co., Oakland, CA, USA) from the local supermarket.

2.2.2. Gas composition

Package headspace gas composition was determined immediately after removing the samples from cold storage on the day of the evaluation. The O₂ and CO₂ levels were determined with a head space gas analyzer (PBI Dansensor, Checkmate 9900, Ringsted, Denmark).

2.2.3. Color

The color of apple slices was measured on both cut surfaces of each slice using a tri-stimulus CR-400 Minolta Chromo Meter (Minolta Co. Ltd, Japan), calibrated with a standard white plate ($Y = 94.00$, $x = 0.3158$, $y = 0.3322$). In consideration of color variations among the cut surfaces within each apple and among different apples, a total of 8 readings for each treatment (2 readings per slice, 1 slice per bag and 4 replicate bags) were taken to ensure that the data obtained truly represented the color of the samples. The L^* , a^* , and b^* values for each apple wedges were recorded on each sampling day.

2.2.4. Texture

Firmness of the apple slices was determined using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, N.Y., USA) with a TA cylinder probe (4 mm ID). The middle position of each slice was used to measure the firmness. The slices were fixed on a “V” model platform to avoid the slant slice slipping when the probe pressed it. The firmness peak force of the measurement was determined by the peak force required to penetrate the cut surface of apple slices by 10 mm with a 4 mm diameter probe. Eight measurements were conducted for each treatment.

2.3. Optimization of treatment parameters – treatment effects on natural apple wedge microbiota

Six different wash solutions were prepared as described below: WW (pH 5.5), 1% AA (pH 2.76), 30% E (pH 6.38), 1% AA + 30% E (pH 3.00), 200 mg L⁻¹ sodium hypochlorite solution (200 mg L⁻¹ chlorine, pH 6.5), 50 mg L⁻¹ sodium hypochlorite plus 10 g L⁻¹ (w/v) AA solution (50 mg L⁻¹ chlorine + 1% AA, pH 2.6). The apple slices were prepared as previously described and randomly sorted into groups. Each group (approx. 64 slices) was immersed for two minutes into one of the six treatment solutions, then dewatered (approx. 2 minutes) and packaged separately, then the bags were resealed after each sampling except the bags for gas composition determination.

In four replicates, two apple slices (about 15 g per slice) from each package were placed in a stomacher bag and macerated in 100 mL sterile phosphate buffered saline (PBS, 10X solution, Fisher Scientific, Pittsburgh, PA, USA) using a laboratory blender (Seward Stomacher 400 Biomaster, London, UK); the solution was serially diluted with PBS, and plated on an appropriate medium using a spiral plater (WASP2,

Don Whitley Scientific Ltd., Shipley, United Kingdom). The total aerobic plate count (APC) was determined by plating the samples on trypticase soy agar (TSA, Difco, Detroit, MI, USA) and incubating at 28°C for 48h. Yeast and mold counts were determined by plating on potato dextrose agar (PDA, Difco, Detroit, MI, USA) supplemented with 200 mg L⁻¹ chloramphenicol (Sigma–Aldrich, St. Louis, MO) and incubating at 22°C for 72 h.

2.4. Inactivation efficiency of *E. coli* O157:H7 and *L. monocytogenes*

A six strain cocktail of *E. coli* O157:H7 (RM4406, RM4407, RM1918) and *L. monocytogenes* (Scott A 45A54, Scott A 45A6, Scott A NFPA) was prepared using cultures obtained from the Environmental Microbial and Food Safety Laboratory (USDA-ARS, Beltsville, MD, USA). All isolates were streaked from frozen stocks onto either MacConkey Agar (MACN; Difco Laboratories, Detroit, MI) supplemented with 50 mg L⁻¹ nalidixic acid (Sigma-Aldrich, St. Louis, MO) for *E. coli* or Modified Oxford Medium (MOX; Difco Laboratories, Detroit, MI) for *L. monocytogenes*. Each strain was individually enriched in separate 50 mL conical centrifuge tubes containing 30 mL trypticase soy broth (TSB), and incubated at 37 °C with shaking (125 rpm) for 18h. Each culture was pelleted, washed twice in PBS and resuspended in 30 mL PBS. Each genus (3 × 30 mL PBS) was combined into a separate 90 mL cocktail solution, diluted in buffered peptone water (BPW, Difco) and plated on either MACN or MOX to determine the bacterial concentration. The cocktails were maintained in a wet-ice bucket and placed at 4 °C overnight to maintain population density for subsequent inoculation the following day.

On the day of inoculation, approximately 1 mL from each of the *E. coli* and *L. monocytogenes* inoculation cocktails were inoculated into a 4 L beaker containing 2 L PBS to prepare an inoculation bath containing 6 Log CFU/ mL *E. coli* O157:H7 and 6.5 Log CFU/ mL *L. monocytogenes*.

Twenty-four Granny Smith apples were rinsed with deionized water. Each apple was cut and cored into 12 slices with a fruit slicer and a sterilized knife. The apple slices (288 slices) were randomized and stored on ice prior to inoculation. Apple slices were immersed in the inoculation bath for 2 min in a BSL-2 laminar flow hood, and then surface-dried in a laminar flow hood for approximately two minutes. Five wash treatments were prepared with deionized water using the methodology above and included: 1) WW, 2) 30% E, 3) 30% E + 3% AA, 4) 50 mg L⁻¹ chlorine, 5) 50 mg L⁻¹ chlorine + 3% AA solution. Additionally, 6) an unwashed control was prepared. For each treatment, 3 sets of 8 inoculated apple slices were submerged in the wash solutions and manually agitated for 2 min. The samples were drained, then randomized and packaged in zipper bags as above (8 slices per bag) and stored under 5 °C isothermal conditions (Revco 4504, Asheville, NC). Microbiological analysis was performed on day 0, 3, 7, and 14.

On each sampling day, three bags from each treatment were randomly retrieved from the storage, and two slices from each bag were randomly selected for analysis. Remaining slices were immediately returned to 5 °C storage. Apple slices were placed in sterile filter stomacher bags and manually massaged for 1 min in 100 mL PBS, followed by 30 sec sonication at 40KHz (Branson model 5510, 40KHz, Thomas

Scientific, Swedesboro, NJ, USA). The sample solution was plated using a spiral plater (WASP2, Microbiology Int., Frederick, MD) onto MACN and MOX, incubated for 24 h at 37 °C and enumerated with an automated plate counter (ProtoCOL2, Microbiology Int., Frederick, MD).

2.5. Statistical analysis

The experiment followed a completely randomized design with three replications. Data were processed through OriginPro 8.0 (Origin Lab Corporation, Northampton, MA, USA) and presented as the means and standard deviations. Microbiological data were log transformed prior to analysis. All data were tested by two factor analysis of variance (ANOVA) using the SPSS statistical package version 21 (SPSS Inc., Chicago, IL). Two-way analysis of variance and least significant difference (LSD) were used to differentiate mean values ($P < 0.05$).

3. Results and discussion

3.1. Effects of wash treatments on browning control in apple slices

Values of L^* have been widely used to indicate the lightness/darkness of the cut surface of apple slice with decreases in L^* indicating the browning of apple surfaces (Castaner, Gil, Ruiz, & Artes, 1999). On day 0, there was a significant ($P < 0.05$) decline in L^* value for the water only control within 15 minutes after dipping as well as throughout cold storage of this treatment (Fig. 1), which agrees with the substantial browning observed on these samples. Treatment containing 1% AA, and its combination with E (from 20% to 40%) maintained stable L^* values throughout the entire 14-day storage period. The values of a^* indicate the redness of cut surface of

apple slices with higher a^* values indicating redder (brownier) color. During the storage time of 14 days, the a^* values of the apple slices in the combined AA and E treatment groups were lower than for AA only treatment. This result suggests that E, in combination with AA could be used as an effective (non-antagonistic) additive for controlling enzymatic browning in fresh-cut apples. The values of b^* indicate changes in blue and yellow on the tristimulus color coordinator with an increase in b^* indicating yellowing which often occurs as part of the browning process. As shown in Figure 1C, there was a significant increase in b^* value on the water washed samples, while treatments containing 1% AA alone, and in combination with E maintained stable b^* values during the entire storage. The visual properties of the apple slices in different treatment groups and storage times are shown in table 1 and table 2, which confirm the effect of the combination of AA and E on maintaining quality of fresh-cut apple slices.

3.2. Effect of treatments on the firmness of apple slices

Table 3 shows the effect of ethanol concentration on apple tissue firmness. The firmness of the apple slices subjected to water wash decreased over time during storage; however, samples that received the combination treatment of 1% AA and 20% E maintained firmness during the entire storage. Increasing ethanol concentration, beyond 20% decreased firmness, but still maintained greater firmness than water wash or 1% AA alone. The effect of E on firmness may be complicated by 2 counteracting processes. On one hand, the E could penetrate into tissues and cause tissue injury, and consequent loss of firmness as observed in treatments containing 30% and 40% E

(Table 3). On the other hand, at lower concentrations, i.e. 20%, E may retard tissue senescence (Perata & Alpi, 1991), and maintain cell membrane integrity (Hu et al., 2010; Saltveit, 1989) by inhibiting enzymes involved in ethylene production (Asoda, Terai, Kato, & Suzuki, 2009; Bai et al., 2004; Liu et al., 2012).

3.3. Changes in package atmosphere composition

Package headspace O₂ partial pressures for all treatments declined steeply initially and then more gradually over the remainder of the storage duration, while CO₂ partial pressures increased steeply initially and subsequently more gradually (Fig. 2). The water wash control maintained the highest O₂ levels in the packages throughout the 2 week storage period. After the initial decline, packages containing samples treated with 1% AA plus 20-30% E maintained the greatest stability in O₂ levels from day 4 through the end of storage. After 14 days of storage, no significant differences in O₂ levels remained among the treatments. The combination treatments 1% AA plus 20-30% E maintained lower CO₂ levels than WW throughout the experiment and lower than 1% AA treatment from day 7 through the end of storage. This result is consistent with that of Bai et al. (2004) who reported that E vapor treatments (5 mL kg⁻¹ fruit) for 24 hours at 25 °C before processing into slices, reduced respiration rates in fresh-cut apple. In general there is an inverse relationship between respiration rate and shelf life since commodities with higher respiration rates tend to senesce faster (Saltveit, 2004). Among all treatments, packages containing samples treated with 1% AA had the greatest overall decline in O₂ and increase in CO₂. Packages of samples treated with the combination of 1% AA plus 40% E also had more pronounced changes in O₂ and

CO₂ than the combinations containing 20% and 30% E. The dramatic changes in firmness and package headspace pressure also suggest that the use of 40% E and 1% AA may have caused tissue injury.

3.4. Microbiological analysis

The total aerobic bacterial counts (APC) on apple wedges were monitored on days 0, 1, 4, 7, 10, 14 and 21. As shown in Fig. 3, APC was below the detection limit of 100 CFU/g on day 0, reflecting the aseptic processing conditions and the effectiveness of 200 mg L⁻¹ chlorine for prevention of bacterial transference from surface to internal tissues. On day 14, the water wash control samples and those treated with 1% AA had sharp increases in APC, reaching 3 log and 5 log CFU/g, respectively. The addition of 20% E to the 1% AA treatment slightly reduced the increase in APC during storage, while higher E concentrations (e.g. 30% and 40% E) suppressed the growth of aerobic bacteria, with APC remaining below detection level on day 14.

Given the significantly lower APC noted at the end of storage on apple slices treated with 30% or higher E concentrations, an additional study (21 days duration) was conducted to compare the antimicrobial effect of 1% AA and 30% E alone, and in combination on apple slices. The commonly used sanitizer chlorine and its combination with 1% AA are also included in the study. As shown in Fig. 4, no bacterial growth was observed within the first 8 days after processing, but a sharp increase in APC was noticed on samples treated with 1% AA, starting on day 10. Samples treated with chlorine and its combination with 1% AA also had significant increases in APC toward the end of storage life. However, APC remained low on

apple slices treated with 30% E and its combination with 1% AA throughout the entire 21-day storage. Similar trends were observed for yeast and mold.

The results of this study indicate that the combination treatment of 30% E and 1% AA is more effective than chlorine to maintain quality, and as effective as chlorine to suppress microbial growth on fresh-cut apple slices.

Apple slices were co-inoculated with approximately 6 Log CFU/ mL of both *E. coli* O157:H7 and *L. monocytogenes* prior to wash treatment to test the efficacy of wash solutions for pathogen inactivation. Initial recovery of both pathogens on day 0, for unwashed controls (Fig. 5), approximated 6 Log CFU per apple slice, which suggested that our recovery and detection methods were sound. Apple slices treated with 30% E, and with the combination of 30% E + 3% AA significantly reduced *E. coli* O157:H7 populations on day 0. Stronger pathogen reduction was obtained by the combination treatment of 30% E + 3% AA than with 30% E alone. The AA treatment alone achieved no pathogen reduction. The greater pathogen reduction attained when combining AA and E may be attributable to the lowered pH. Previously reported E dips at 30% and 50% concentration resulted in 1 and 2 Log CFUs per apple slice reductions, respectively (Pino, Lichter, Danshin, & Sela, 2006). On the other hand, during storage, the *E. coli* O157:H7 populations significantly declined over time on all wash and non-wash treatments. On day 14, the combination treatment of 3% AA + 30% E had the lowest *E. coli* O157:H7 populations, followed by WW and chlorine.

All wash treatments resulted in a significant reduction of *L. monocytogenes* populations from apple slices on day 0 (Figure 5). The strongest *L. monocytogenes*

reduction was seen with 30% E, followed by the combination treatment of 30%E + 3%AA. During storage, *L. monocytogenes* populations on apples from all treatments showed a declining trend over time except water wash and 3% AA treatment.

E. coli populations naturally decline when exposed to refrigerated conditions below 5°C, whereas psychrotrophic *L. monocytogenes* populations have been shown to be stable or even increase during cold storage. All recovered populations of *E. coli* for all treatments were significantly lower on day 14 than on day 0. Conversely, the populations of *L. monocytogenes* were stable throughout the 14 days storage on apple slices treated with WW, 3%AA, and 50 mg L⁻¹ chlorine + 3% AA. However, on apple slices treated with 30% E *L. monocytogenes* populations declined after 3 days storage and on those treated with 50 mg L⁻¹ chlorine and 30%E + 3%AA, the psychrotroph declined throughout storage. These 3 treatment groups had significantly lower *L. monocytogenes* populations than others on day 14 (Fig. 5).

Ethanol dip or vapor had previously been used to sanitize table grapes and fresh-cut eggplant as an alternative to SO₂ (Karabulut, Gabler, Mansour, & Smilanick, 2004; Lichter et al., 2002; Hu et al., 2010). Ethanol kills organisms by denaturing proteins and dissolving lipids. E is effective against most bacteria, but ineffective against spores (McDonnell & Denver, 1999). Additionally, E causes water stress by lowering water activity and thereby interferes with hydrogen bonds within and between hydrated cell compounds, ultimately disrupting enzyme and membrane structure and function (Hallsworth, 1998). The results in this study show that E concentrations of 20% to 40% can either completely or partially inhibit the growth of

the APC on apple slices during storage and there is a dose-response relationship between the suppression of APC and E concentration. Treatment with 16.5% (v/v) E (Giotis, Julotok, Wilkinson, Blair, & McDowell, 2008) and 2.5%-20% (v/v) E (Hassani, et al., 2009) were employed to evaluate their efficacies to inhibit growth of *L. monocytogenes* and *S. typhimurium*, respectively. Giotis et al. (2008) found that prior exposure to alkaline stress caused *L. monocytogenes* to be more resistant to E treatment; while Hassani et al. (2009) found that stress induced by using 10-12.5% E treatment caused the formation of a novel phage shock protein G in *S. typhimurium*, which helps bacterial cells during E stress. Expression of the phage shock protein G did not occur at levels of E of 20%. The above two reports warn of the potential to cause resistant strains to spread in environments by use of mild hurdle treatments. In this work the results showed that the 30% E and 1% AA can reduce the APC to a non-detectable level.

AA is a GRAS commercial anti-browning additive commonly used for fresh-cuts. Myrvik and Valk (1954) reported that the AA exerted an antibacterial action due to the oxidation of its unstable enediol to a diketone group. Rawal (1978) reported synergic bactericidal action of AA on *Pseudomonas aeruginosa*, as it altered the cell surface to render it increasingly permeable to antibiotics. However, Van Asbeck et al. (1983) reported that AA alone had no bactericidal properties. Giannuzzi and Zaritzky (1993) reported that 1% AA, individually or combined with citric acid showed antimicrobial action in a model system of refrigerated pre-peeled potatoes at 4 °C, and the apparent synergic effect of these acids when they were applied together

was demonstrated to be actually an additive effect when concentrations of undissociated acid in the mixtures were considered in addition to dissociated acid concentration. The results in this study indicate that the combination of AA and E can control the bacterial growth during storage more effectively than other treatments. Alegre, Abadias, Anguera, Oliveira, and Viñas, (2010) found that 2 % AA had no bactericidal action and even stimulated the growth of the native microbes. Chlorine is an oxidizer and sanitizer, but its reaction with 30 mg/mL AA results in its reduction and consequent loss of its bactericidal action. On the other hand, E coupled with AA seems to enhance the antimicrobial action beyond what either substance can contribute alone.

4. Conclusion

The combination of E (30%) and AA (1-3%) can inhibit browning and softening of Granny Smith apple slices and control the growth of APC as well as reduce *E. coli* O157:H7 and *L. monocytogenes* populations on the apple slices. Additionally, the combination treatment of 30% E and 1% AA was able to reduce respiration rate in apple slices, as evidenced by lower CO₂ production rate in packages compared to that observed in packages of water washed controls. E (30%) supplemented with AA (3%) can achieve browning inhibition as well as pathogen inactivation. This treatment is a promising and greatly needed solution to meet the increasing demand for quality and safety of fresh-cuts.

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557

Figure Legend

Figure 1. Effect of chemical treatments on the color parameters, A.) L*, B.) a* and C.) b* values of the cut surfaces of Granny Smith apple slices.

Figure 2. Changes in headspace gas composition, including A.) O₂ and B.) CO₂ partial pressures in apple slice packages in different treatments during 14 d storage.

Figure 3. Effect of treatments on A.) the total aerobic count and B.) the yeast and mold count on fresh-cut Granny Smith apple wedges during 1.5 °C storage.

Figure 4. Effect of treatments A.) on the total aerobic count and B.) the yeast and mold of fresh-cut Granny Smith apple wedges during 1.5 °C storage. Below detection level (< 100 CFU/g fresh tissue).

Figure 5. The effect of wash treatment and storage duration on the survival and growth of *E. coli* O157:H7 and *L. Monocytogenes* on apple slices stored at 5 °C. Different upper case letters indicate significant differences at P<0.05 among treatments within a day. Different lower case letters indicate significant differences at P<0.05 among days within a treatment.

Table 1. Photographs of the apple slices for different treatments (WW, 1% AA, 1%AA+20%E, 1%AA+30%E, 1%AA+40%E) after different storage durations (0-14 days).

579 Table 2. Photographs of the apple slices in different treatments (30%E, Ch,
580 Ch+1%AA) after different storage durations (0-21 days).

581

582 Table 3. Effects of treatments on the firmness of Granny Smith apple slices. Different
583 upper case letters indicate significant differences at $P < 0.05$ among treatments within a
584 day. Different lower case letters indicate significant differences at $P < 0.05$ among days
585 within a treatment.

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587 Figure 1

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590 Figure 2

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593   Figure 3

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Table 1 Photographs of the apple slices stored at 5 °C

Treatment Time	WW	1%AA	1% AA+20%E	1% AA+30%E	1% AA+40%E
Day 0					
Day 1					
Day 4					
Day 7					
Day 10					
Day 14					

Table 2 Photographs of the apple slices stored at 5 °C

Treatment Time	30%E	Chl	Chl+1%AA	1% AA+30%E
Day 0				
Day 1				
Day 4				
Day 7				
Day 10				
Day 14				
Day 21				

Table 3 Changes in firmness of Granny Smith apple slices, stored at 5 °C

Treatments	Firmness (N) for different storage durations				
	Day 1	Day 3	Day 5	Day 7	Day 14
Water wash (WW)	8.74±0.16Ca	8.99±0.80Ca	9.14±0.99Ba	8.35±1.37Ba	6.86±0.92Db
1% AA	8.96±0.66Ca	9.17±1.12BCa	9.06±1.77Ba	8.74±1.74Ba	8.05±0.62CDa
1% AA+20% E	12.48±0.69Aa	12.70±0.63Aa	13.40±0.69Aa	13.10±1.65Aa	12.32±1.02Aa
1% AA+30% E	10.28±1.35Bb	10.48±1.10Bab	10.24±0.87Bb	12.05±1.29Aa	9.39±0.87BCb
1% AA+40% E	10.48±0.52Bab	9.67±1.06BCb	11.38±1.49ABa	9.55±0.59Bb	10.27±1.33Bab

Different capital letters and lowercase letters indicate significant differences at $P < 0.05$ between different treatments and storage durations, respectively.