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Dual effectiveness of ascorbic acid and ethanol combined treatment to inhibit 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 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.

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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 on fresh-cut produce (Lu, Yu, Gao, Lu, & Zhang, 2004; Luo, 2007; Luo, Lu, Zhou, & Feng, 2011; Matan, Puangjinda, Phothisuwan, & Nisoa, 2015; Wang, Nie, & Cantwell, 2014).

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 (Mahmoud & Ommol, 2016; Sharma & Rao, 2015), 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).

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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 et al., 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, Feng, and Luo (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 et al., 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 generally recognized as safe (GRAS), exists naturally in plants, and has 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 et al., 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 similarsize 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 cored and cut into 12 equal slices with a fruit slicer and a sterilized stainless steel knife. For each treatment, 60 apple slices were immersed in each treatment solution for 2 min, and then dewatered 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^{-1} m^{-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 O_2 and CO_2 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.