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Effects of acidic electrolyzed oxidizing water on retarding cell wall degradation and delaying softening of blueberries during postharvest storage

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ABSTRACT

This study was to evaluate the effect of acidic electrolyzed oxidizing water (AEW) on extending the shelf-life of blueberries during postharvest storage. On day 0, two cultivars of blueberries (Brightwell and Camellia) were washed by AEW with available chlorine concentration (ACC) of 48 mg/L at pH 2.8 for 5 min, and then stored at 4 °C until analyzing. Quality attributes of blueberries including firmness, cell wall composition and cell wall degrading enzymes were analyzed on days 0, 3, 6, 9, 12 and 15. Results showed that Brightwell softened faster than Camellia. AEW treatment slowed down the softening of both cultivars, while it had higher efficacy on delaying the softening of Camellia blueberries than the Brightwell blueberries. In addition, AEW treatment deactivated different cell wall degrading enzymes (CWDEs, polygalacturonase (PG), cellulase and β-galactosidase) and prevented the degradation of cell wall components including Na_2CO_3-soluble pectin (NSP), hemicellulose and cellulose. These results suggested that AEW treatment can be used as a postharvest technique to delay the softening process of blueberries during storage through lowering CWDEs activities and preventing degradation of cell wall components.

1. Introduction

Blueberry (*Vaccinium* spp.) fruits containing high levels of anthocyanins, vitamins, flavonols and dietary fiber have been shown to have health benefits such as antioxidant, heart protection and chronic diseases prevention (Chen, Cao et al., 2015; Wang, Camp, & Ehlenfeldt, 2012). In recent decades, production and consumption of blueberries have increased multifold. However, rapid softening of blueberries during storage greatly limits their storage, transportation and marketing potential (Chen, Cao et al., 2015; Deng, Shi, Li, & Liu, 2014).

Fruit softening is closely related to the disassembly of pectin substances, hemicellulose and cellulose which are main components of the cell wall and middle lamella (Deng, Wu, & Li, 2005; Lin, Zhao, & Xi, 2007; Duan et al., 2011; Zhao, Lin, Wang, Lin, & Chen, 2014; Wang et al., 2015; Chen et al., 2017; Xie et al., 2017). Activities of cell wall degrading enzymes (CWDEs) including polygalacturonase (PG), cellulase and β-galactosidase are main factors causing degradation of cell wall components and fruit softening (Bu, Yu, Aisikaer, & Ying, 2013; Deng et al., 2005; Wei et al., 2010). Such changes in cell wall structure, composition and activities of CWDEs have previously been reported in grapes (Deng et al., 2005), longans (Duan et al., 2011; Lin et al., 2007; Zhao et al., 2014), persimmons (Luo, 2007), Huanghua pears (Chen et al., 2017) and cherries (Wang et al., 2015). Deng et al. (2005) found that the firmness of Kyoho grapes decreased rapidly during storage with a progressive increase in water-soluble pectin (WSP) content and decrease in contents of cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA)-soluble pectin (CSP), Na_2CO_3-soluble pectin (NSP), cellulose and hemicellulose.

Electrolyzed oxidizing (EO) water is an environment-friendly and emerging antimicrobial sanitizer. EO water has been used to mitigate the fungal infections on fruits surface during postharvest storage and handling (Guentzel, Lam, Callan, Emmons, & Dunham, 2010; Jadeja, Hung, & Bosilevac, 2013). EO water is synthesized by

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electrolyzing NaCl solution (<0.1 g/100 mL) in an electrolysis chamber with an anode and a cathode divided by a diaphragm membrane. Electrolysis of diluted NaCl solution results in the formation of hydrochloric acid, hypochlorous acid and chlorine gas on the anode side. Solution produced from this process is known as the acidic electrolyzed oxidizing water (AEW). Generally, AEW has a low pH (<2.8), high redox potential or oxidation-reduction potential (ORP) (ORP > 1050 mV) and high available chlorine concentration (ACC, greater than 5 mg/L). The efficacy of AEW on reducing foodborne pathogens on fresh vegetables and fruits has been reported in previous studies (Fang, Connon, & Hung, 2016; Jadeja et al., 2013; Zhang, Cao, Hung, & Li, 2016; Zhang, Li, Jadeja, Fang, & Hung, 2016). Santo, Graça, Nunes, and Quintas (2016) reported that AEW treatment (pH of 2.82, ORP of 1121 mV and ACC of 103 mg/L) for 5 min significantly reduced the *Cronobacter sakazakii* population of fresh-cut apple, pear and melon. Park, Alexander, Costa, and Kang (2008) found that AEW treatment (pH of 2.06 and ACC of 37.5 mg/L) completely eliminated *Listeria monocytogenes*, *Salmonella* Typhimurium and *Escherichia coli* O157:H7 from lettuce and spinach leaves. However, to our knowledge, the effect of AEW as a postharvest treatment on fruit quality particularly regarding softening of blueberries has not been reported.

This study was conducted to investigate the change of firmness of AEW-treated Brightwell and Camellia blueberries during postharvest storage. In order to gain insights into softening delaying process of blueberries during storage and between the two cultivars, changes in cell wall composition and activity of CWDEs in response to AEW treatment were also determined.

2. Materials and methods

2.1. Chemicals

D-galacturonic acid, polygalacturonic acid, *p*-nitrophenyl-β-D-galactopyranoside and carboxymethyl cellulose were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Anthrone, ethanol, chloroform, methanol, acetone, polyvinyl pyrrolidone, mercaptoethanol, 3,5-dinitrosalicylic acid, sodium carbonate, sodium hydroxide and sodium borohydride were obtained from Fisher Scientific Co. (Suwanee, Georgia, USA). The above reagents in this experiment were analytical grade.

2.2. Preparation of blueberries

Rabbiteye blueberries (*Vaccinium ashei* Reade cv. Brightwell) and Highbush blueberries (*Vaccinium corymbosum* L. cv. Camellia) at full maturity, as determined by complete blue skin color, were hand-harvested from research farm in coastal plain experiment station (Alapaha, Georgia, USA). Harvested fruits were packed in commercial vented clamshell containers, and then placed in a 375 L incubator with ice in the bottom. It took 4 h for fruit collection and 3 h of transportation to the laboratory at the University of Georgia, Griffin. Fruits in uniform size and color were selected for experiment. Any rotten and damaged fruits were discarded.

2.3. Preparation of AEW

In order to produce the AEW, 0.03 g/100 mL NaCl solution was electrolyzed using an EO water generator (P30HST44T, EAU Technologies, Inc., GA, USA). The pH and ORP of AEW was determined using a dual channel ACCUMET meter (AR50, Fisher Scientific, PA, USA) with pH and ORP electrodes. DPD-FEAS method (Hach Co., Loveland, CO, USA) was used to measure the initial ACC of AEW. AEW with ACC of 48 mg/L was obtained and pH of AEW was adjusted to 2.8 using 0.1 mol/L HCl.

2.4. Procedures for treating blueberries

A total of 5100 blueberries were selected from each cultivar. Immediately after harvest (day 0), 300 blueberries were used for evaluating the initial fruit quality and the remaining 4800 fruits were separated into two groups randomly each of 2400 fruits for the following treatments in triplicate: (1) control group (2400 fruits immersed in 20 L of distilled water for 5 min at 25 °C) and (2) AEW group (2400 fruits immersed in 20 L of AEW for 5 min at pH 2.8, 48 mg/L of ACC, ORP of 1125 mV, and 25 °C). After washing, blueberries in each treatment were air-dried in a hood for 30 min, packaged in vented clamshell containers (100 fruits per box, 24 boxes of each treatment, 8 boxes of each replicate), and stored at 4 °C and 90% relative humidity for up to 15 days. On days 3, 6, 9, 12 and 15, three boxes per treatment group were selected randomly for quality analysis. For each box, ten blueberries were used for measuring firmness and the remaining 90 fruits were then frozen at −70 °C for further analysis. All analyses were triplicated for each replicate except fruit firmness.

In addition, fruits of remaining three boxes (total 300 fruits) from each replicate and treatment were used for evaluating weight loss. At 3-day intervals during storage, weight loss was determined in each box by percentage of weight loss with respect to the harvest day.

2.5. Measurement of fruit firmness and weight loss

Fruit firmness was measured by compression the individual blueberry using a texture analyzer (5542, Instron Co., MA, USA) with a 35 mm diameter cylindrical probe at the speed of 1 mm/s until the blueberry began releasing juice. The maximum force (N) of compression was recorded as blueberry firmness.

The weight loss of blueberries during storage was recorded according to the method of Chen, Lin et al., (2015). The percentage of weight loss was calculated compared to the initial weight.

2.6. Preparation of cell wall material (CWM)

Extraction of CWM was carried out as described by Lin et al. (2007), Deng et al. (2014) and Chen et al. (2017). Frozen blueberries (30 g) were added to 250 mL of 95 mL/100 mL ethanol and homogenized. Resulting slurry was boiled with continuous stirring for 30 min, cooled and then filtrated with filter papers (No.4, Whatman™, Boca Raton, FL, USA). The residues were subsequently washed three times with 85 mL/100 mL ethanol, chloroform: methanol (1:1, mL:mL), and acetone, respectively, until the insoluble material being colorless, then dried overnight at 37 °C to get the final weight of CWM.

2.7. Fractionation and analysis of cell wall components

Cell wall components fractionation and analysis were followed by the procedures described in Deng et al. (2014) and Chen et al. (2017). Water-soluble pectin (WSP) was obtained by dispersing CWM (500 mg) in water for 4 h on an incubator shaker (CLASSIC C24, New Brunswick Scientific Co., Inc., NJ, USA), then collecting supernatant by centrifugation (Allegra 64R centrifuge, Beckman Coulter, CA, USA) at 11,000×*g* for 10 min at 4 °C. The sediment was immersed in 50 mmol/L Na_2CO_3 with 0.01 mol/L $NaBH_4$ for 4 h with shaking. The supernatant was collected by centrifugation at 11,000×*g* for 10 min as Na_2CO_3-soluble pectin (NSP). Residues were further soaked in 4 mol/L NaOH solution with 100 mmol/L $NaBH_4$ with shaking for 4 h. Following centrifugation condition described above, the supernatant was designated as hemicellulose. Double-distilled deionized water was then added to the remaining