Food Chemistry 270 (2019) 229–235

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Enhanced storability of blueberries by acidic electrolyzed oxidizing water application may be mediated by regulating ROS metabolism

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ARTICLE INFO

Keywords:
Blueberry (*Vaccinium* spp.)
Storability
Reactive oxygen species (ROS)
ROS metabolism
ROS scavenging enzymes
ROS scavenging capacity
Antioxidant activity
Acidic electrolyzed oxidizing water (AEW)

ABSTRACT

This study aimed to investigate the effects of acidic electrolyzed oxidizing water (AEW) treatment on storability and metabolism of reactive oxygen species (ROS) in blueberries cv. 'Brightwell' during storage at 4 °C. Results showed that, compared with the control blueberries, AEW treated-blueberries exhibited lower incidence of fruit decay, higher rate of commercially acceptable fruit, higher fruit firmness and skin hardness, and higher anthocyanin and total phenolics contents, along with higher activities of SOD, CAT and APX, higher antioxidant activity, but lower generation rate of superoxide anion and cell membrane permeability. These results demonstrated that AEW treatment for enhancing storability of harvested blueberries during storage may be mediated by regulating ROS metabolism, manifested as AEW increasing ROS scavenging capacity and reducing ROS accumulation, and thereby maintained the structural integrity of cellular membrane, which indicated that AEW treatment was a facile postharvest method for extending the shelf life of harvested blueberries.

1. Introduction

The fruits of blueberry (*Vaccinium* spp.) are popular fruits all over the world owning to their unique flavor and nutritive components including anthocyanin, flavonol, vitamin, phenolic acid, and mineral (Li et al., 2017; Morita, Naito, Yoshikawa, & Niki, 2017; Xu, Wang, Xu, Liu, & Li, 2016; Zheng & Wang, 2003). However, blueberries are extremely perishable and susceptible to microbial decay during postharvest storage. Fruit decay is the primary reason that affects the quality, commercial value, and shelf life of blueberries (Chu, Gao, Chen, Fang, & Zheng, 2018; Xu et al., 2016).

Recent studies showed that fruit decay was associated with the disturbed reactive oxygen species (ROS) production-scavenging system, including increases in generation rate of superoxide anion and content of malondialdehyde (MDA), decline in ROS-scavenging enzyme activities including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), and the reduction of non-enzymatic antioxidant content like anthocyanin and total phenolics (Chen et al., 2015; Xu et al., 2016). The consequential excessive ROS damaged the cellular membrane structure (Chu et al., 2018; Jin et al., 2012; Lin et al., 2014; Sun et al., 2018; Wang, Gao, Tao, Wu, & Zhibo, 2017), reduced disease resistance ability and accelerated decay development of harvested fruits (Chen et al., 2014; Lin et al., 2017; Sun et al., 2018). Other previous studies showed that the applications of pure oxygen atmosphere (Duan et al., 2011), essential oils (Jin et al., 2012; Wang, Wang, Yin, Parry, & Yu, 2007), or propyl gallate (Lin et al., 2015) for harvested fruits could lead to retain higher antioxidant activity and ROS scavenging capacity, and alleviate the physiological disorder, enhance the disease resistance against pathogens infection, and inhibit the development of fruit decay.

Electrolyzed oxidizing (EO) water is an environmentally friendly and safe antimicrobial sanitizer. It is considered an emerging technique in recent years. EO water has been used for inactivation of bacteria on fruit surface during postharvest handling (Chen, Hung, Chen, & Lin, 2017; Jadeja, Hung, & Bosilevac, 2013; Qi, Huang, & Hung, 2018). EO water exhibited great antimicrobial effects against *Fusarium* sp. *in vitro* (Khayankarn, Uthaibutra, Setha, & Whangchai, 2013) and obviously lowered the decay incidence and prolonged the shelf life of pineapples up to twenty days (Khayankarn et al., 2013). Another study also reported that EO water inhibited the growth of *Botrytis cinerea* and *Monilinia fructicola* and mitigated fungal infection on the surfaces of peaches and grapes (Guentzel, Lam, Callan, Emmons, & Dunham, 2010).

EO water can be conveniently produced by electrolysis of tap water and diluted sodium chloride solution. Acidic electrolyzed oxidizing water (AEW) generated at the anode side has properties of high available chlorine concentration (ACC) ($> 5\ mg\ L^{-1}$), low pH (< 2.8) and

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https://doi.org/10.1016/j.foodchem.2018.07.095
Received 31 March 2018; Received in revised form 14 July 2018; Accepted 16 July 2018
Available online 17 July 2018

strong oxidation–reduction potential (ORP) (> 1050 mV) (Chen et al., 2017; Huang, Hung, Hsu, Huang, & Hwang, 2008; Qi et al., 2018). It has been shown that *Botryosphaeria berengeriana*-induced postharvest fungal decay of pears was significantly inhibited by AEW treatment (Al-Haq, Seo, Oshita, & Kawagoe, 2002). Moreover, the antimicrobial effects of AEW on lettuce, spinach, potato, celery, and cilantro have also been reported (Luo, Kim, Wang, & Oh, 2016; Park, Alexander, Costa, & Kang, 2008; Zhang, Cao, Hung, & Li, 2016). Nonetheless, rare effort has been put on the impacts of AEW treatment on storability and ROS metabolism in harvested blueberries. In this work, the changes induced by AEW treatment in the incidence of fruit decay, the rate of commercially acceptable fruit, fruit firmness, skin hardness, anthocyanin and total phenolics contents, generation rate of superoxide anion, cell membrane permeability, activities of ROS scavenging enzymes, and antioxidant activity in harvested blueberries during storage were investigated. The aims of this investigation were to clarify the role of ROS metabolism in AEW-inducing the change of storability in harvested blueberries, as well as to evaluate the effectiveness of AEW treatment as a facile, safe, eco-friendly and promising postharvest method for increasing storability and extending shelf life of harvested blueberries.

2. Materials and methods

2.1. Preparation of blueberries

Rabbiteye blueberries (*Vaccinium ashei* Reade) cv. 'Brightwell' at commercially maturity (skin color 100% blue, fruit firmness 18.55 ± 1.12 N) were harvested from an experimental station in Alapaha, Georgia, USA, and immediately transported to the University of Georgia, Griffin, Georgia, USA. Fruits selected for experiment were in uniform maturity and size. Any rotten or damaged fruits were not selected.

2.2. Preparation of AEW

EO water was prepared with an EO water generator (P30HST44T, EAU Technologies, Inc., GA, USA). The selection of suitable AEW conditions (pH of 2.8, ORP of 1125 mV and ACC of 48 mg L^{-1}) were performed following our recent publication (Chen et al., 2017). The pH, ORP and ACC of AEW were determined based on previous work (Chen et al., 2017).

2.3. Procedures for treating blueberries

A total of 300 fruits were used in evaluating the initial fruit properties on harvest day (day 0). Another 3000 blueberries were randomly divided into two groups with each group of 1500 fruits for the following treatments: one group was the control group (with 1500 fruits immersed in 15 L of distilled water for 5 min), and the other group was the AEW group (with 1500 fruits immersed in 15 L of AEW for 5 min at pH 2.8, 48 mg L^{-1} of ACC, ORP of 1125 mV) at 25 °C. After treatment, blueberries of each group were air-dried in a hood for 20 min, packaged in vented clamshell containers (100 fruits per box, 15 boxes for each treatment), and stored at 4 °C and 90% relative humidity for up to 15 days. On days 3, 6, 9, 12, and 15, three boxes of blueberries from each treatment group were randomly selected for the assessment of decay incidence and commercially acceptable fruit rate. Ten blueberries from each box were then selected for measuring fruit firmness, and another ten blueberries from each box were used to determine skin hardness, and ten more blueberries from each box were used for determining cellular membrane permeability. The remaining 70 fruits from each box were then frozen at −70 °C for further analyses. All analyses were executed thrice on each box besides the studies of fruit decay incidence, commercially acceptable fruit rate, firmness, and skin hardness.

2.4. Assessments of fruit decay incidence and commercially acceptable fruit rate

One hundred blueberries from a box were used for assessing decay incidence and commercially acceptable fruit rate. The decay incidence of blueberries was measured with the method introduced by Xu et al. (2016). Decay incidence (%) = (Number of decayed fruits/Total number of fruits) × 100. Decayed fruits with visible fungal or bacterial lesions were regarded as rotten.

$$\text{Commercially acceptable fruit rate}(\%) = (\%)100(\%) - \text{decay incidence}(\%)$$

2.5. Texture measurement

The method of Chen et al. (2017) was used for measuring fruit firmness of blueberries. Compression tests of ten individual blueberries from a box were conducted using a texture analyzer (Model 5542, Instron Corporation, MA, USA) with a 35-mm diameter cylindrical probe at the speed of 1 mm/s until blueberries started releasing juice. The recorded maximum force (N) of compression was used to represent the blueberry firmness.

The method of Qi et al. (2018) was used for determining skin hardness of blueberry. Puncture tests of ten individual blueberries from a box were conducted using a texture analyzer (Model 5542, Instron Corporation, MA, USA). Puncture test was conducted according to the procedure described in Qi et al. (2018) and the maximum force (N) required to penetrate the skin was recorded as blueberry skin hardness.

2.6. Assay of anthocyanin and total phenolics contents

The procedures for measuring anthocyanin and total phenolics contents in blueberry peel were performed following the methods of Jiang, Lin, Shi et al. (2018) and Kim, Kim, Kim, & Park (2013) with some modifications. Blueberry peel tissues (2 g) from 10 frozen fruits were homogenized with 5 mL of a mixture of ethanol, distilled water, and HCl (70:30:1, *v/v/v*). Resulting homogenate was then centrifuged (Allegra 64R centrifuge, Beckman Coulter, CA, USA) at 14 000 × *g* and 4 °C for 20 min. Above extraction was repeated to collect the combined supernatant which was then replenished to 20 mL with extraction solution. The final extract was collected to determine anthocyanin and total phenolics contents

Anthocyanin content was measured using the modified methods of Duan et al. (2011) and Jin et al. (2012). Absorbance was recorded at 510 nm (buffer pH 1.0) and 700 nm (buffer pH 4.5) with a Beckman Spectrophotometer (Beckman DU 520, Beckman Co., USA). The content of anthocyanin was calculated following Jiang, Lin, Shi et al. (2018), which was based on cyanidin-3-glucoside (C3G) equivalents, and represented with mg C3G 100 g^{-1} of fresh blueberry peel weight.

The modified methods of Duan et al. (2011), Jiang, Lin, Shi et al. (2018) and Kim et al. (2013) were employed for measuring the content of total phenolics. The extract (1 mL) and the same amount of 1 M Folin-Ciocalteu reagent were mixed, followed by adding 3 mL of 7.5% sodium carbonate solution and 8 mL of distilled water. After 2 h incubation at room temperature, the absorbance was measured at 725 nm. Total phenolics content was calculated following Jiang, Lin, Shi et al. (2018), which was based on the standard of gallic acid (GA), and expressed as mg GA 100 g^{-1} of fresh blueberry peel weight.

2.7. Determination of cell membrane permeability

The methods described by Lin et al. (2016) and Zhou et al. (2014) were employed to measure cell membrane permeability with minor modifications. Two grams peel tissues of 10 blueberries was rinsed thrice with 30 mL of distilled water and then immersed in 30 mL of distilled water. Its electrolyte leakage (C_1) was recorded at 25 °C after 3 h with a conductivity meter (AR50, Fisher Scientific, PA, USA). The