Food Chemistry 239 (2018) 68-74

Contents lists available at ScienceDirect

Food Chemistry

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

Effects of cuticular wax on the postharvest quality of blueberry fruit



^a College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

^b Food Science Institute, Zhejiang Academy of Agricultural Science, Key Laboratory of Post-Harvest Handling of Fruits, Ministry of Agriculture, Key Laboratory of Fruits and Vegetables Postharvest and Processing Technology Research of Zhejiang Province, Hangzhou 310021, China

ARTICLE INFO

Article history: Received 4 April 2017 Received in revised form 2 June 2017 Accepted 5 June 2017 Available online 7 June 2017

Keywords: Blueberry fruit Wax removal Postharvest quality Reactive oxygen species Membrane structure

ABSTRACT

The blueberry fruit has a light-blue appearance because its blue-black skin is covered with a waxy bloom. This layer is easily damaged or removed during fruit harvesting and postharvest handling. We investigated the effects of wax removal on the postharvest quality of blueberry fruit and their possible mechanisms. The removal of natural wax on the fruit was found to accelerate the postharvest water loss and decay, reduce the sensory and nutritional qualities, and shorten the shelf-life. Wax removal decreased the activities of antioxidant enzymes and contents of antioxidants, and accelerated accumulation of ROS and lipid peroxidation, especially at the later period of storage. Moreover, the organellar membrane structure was disrupted in fruit with wax removed. These results indicate that cuticular wax plays an important role in maintaining the postharvest quality and delaying fruit senescence. The results should improve our understanding for better preservation of postharvest quality of blueberry fruit.

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1. Introduction

The outermost layer of plants is covered with cuticular wax, which is visible as a white or bluish coating on the surface of some fruits, such as blueberry, plum, and grape (Saftner, Polashock, Ehlenfeldt, & Vinyard, 2008; Wisuthiphaet, Damerow, & Blanke, 2014). Cuticular wax is the first protective barrier against biotic and abiotic stresses; it plays a vital role in limiting the nonstomatal water loss and in preventing the spore germination of pathogenic microbes (Bernard & Joubès, 2013; Samuels, Kunst, & Jetter, 2008). In recent years, several studies have demonstrated that cuticular wax is closely related to the postharvest quality of fruit (Lara, Belge, & Goulao, 2014; Martin & Rose, 2014). A sharp increase in postharvest water loss was observed upon the removal of wax from the surface of fruits, such as citrus and European plum (Mukhtar, Damerow, & Blanke, 2014; Wang et al., 2014). In addition, cuticular wax could inhibit spore germination and mycelial growth of Alternaria alternata in Asian pear fruit (Yin et al., 2011).

Blueberry (*Vaccinium* spp.) is widely popular among consumers because of its high nutritive value and pleasing flavor. The lightblue appearance of blueberry is because of its blue-black skin, which is covered with a waxy bloom. However, this conspicuous wax layer is vulnerable to damage, and it might be removed during

E-mail address: spsghy@163.com (H. Gao).

the harvesting, packaging, and transport of fruit, resulting in unattractive appearance and giving the impression of poor fruit quality (Mukhtar et al., 2014). It is unclear whether the removal of wax affects the blueberry quality during storage.

In general, various abiotic stresses, such as wound, cold, drought, and salt can disturb the cellular homeostasis and enhance the production of reactive oxygen species (ROS) in plants (Gill & Tuteja, 2010; Mittler, 2002). ROS are highly reactive and toxic, and can cause damage to lipids, proteins, carbohydrates, and DNA, eventually leading to oxidative stress (Gill & Tuteja, 2010; Mittler, 2002). The mitochondria, being the primary sites of endogenous ROS generation, are susceptible to oxidative damage (Sweetlove et al., 2002). The accumulation of ROS in cells could cause oxidative damage of the mitochondrial proteins, finally resulting in the mitochondrial dysfunction or cell death (Tian, Qin, & Li, 2013). It has been demonstrated that ROS initiate fruit senescence, and thereby, directly affect the postharvest quality of fruit (Qin, Wang, Liu, Li, & Tian, 2009; Tian et al., 2013; Xia, Chen, Qin, Li, & Tian, 2016). To some extent, the damage or removal of blueberry wax by mechanical abrasion during harvesting and postharvest handling is ineluctable. However, until date, there are no studies on whether the removal of wax laver is related to ROS metabolism and fruit senescence, and affects the postharvest quality.

In this context, the present study was conducted to assess the roles of cuticular wax on the postharvest quality of blueberry fruit during cold storage, by removing the wax. We also investigated the





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^{*} Corresponding author at: College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China.

possible mechanism for the observed roles of cuticular wax with respect to the ROS metabolism.

2. Materials and methods

2.1. Blueberry samples and treatment

Mature blueberry fruit (*Vaccinium ashei* 'Britewell') were harvested from a local blueberry orchard in Zhejiang Province of China on July 23, 2015. In this study, mature fruit were considered as those that remained for a maximum of 3 days on the vine after attaining the maximum blue color. The fruit were carefully handpicked with polyvinyl chloride gloves and were directly placed in plastic baskets. They were then transported within 2 h to the laboratory at about 10 °C in a refrigerated car. The fruit with no physical damage and uniform size and color were selected for the subsequent experiments.

To mimic the damage to the wax layer on the fruit surface during commercial handling, this layer was removed by slightly wiping with Blu-tack adhesive, instead of immersing the fruit in an organic solvent. The visual appearance and scanning electron microscope (SEM) images taken before and after the wax removal are shown in Fig. 1A. The natural wax layer of blueberry fruit contained a large amount of tubular wax crystals; after the removal of epicuticular wax, the surface became smoother. Both the untreated fruit (control group) and those with the wax removed were subpackaged into 60 plastic clamshells (125 g), containing 50 fruit each, and then wrapped in 0.05-mm thick unsealed polyethylene bags. All the fruit were stored at 4 °C in about 90% relative humidity for 36 d. Each treatment had three replicates.

2.2. Measurement of the quality parameters

The color of the blueberry surface was determined by a chroma meter CR-400 (Konica Minolta Sensing Inc., Japan). The CIELAB

software was employed to measure the L^* , a^* , and b^* values. The L^* value represents the lightness from black ($L^* = 0$) to white ($L^* = 100$), the a^* value indicates the color from green (-) to red (+), and the b^* value indicates the color from blue (-) to yellow (+). Twenty blueberry fruit were randomly selected from each treatment to measure the surface color.

The firmness of blueberry fruit was determined using a TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., U.K.) with a 5 mm diameter stainless steel probe. Twenty blueberry fruit were randomly selected from each treatment to measure the firmness. Each fruit was equatorially compressed by 5 mm at a test speed of 1.0 mm s^{-1} and the maximum force (N) during the test was recorded.

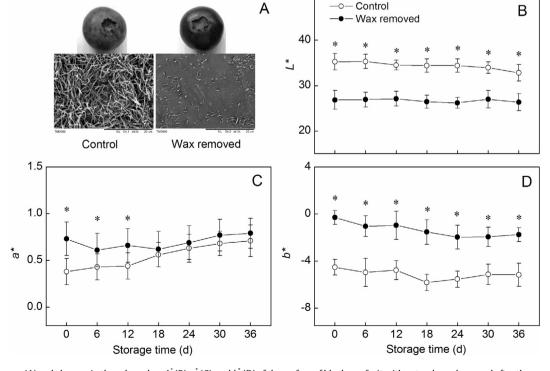
For the assessment of total soluble solids (TSS) and titratable acidity (TA), three replicate samples (8 fruit each) were wrapped in gauze and squeezed until no more juice was released. TSS was determined with a digital hand-held refractometer (Atago PAL-1, Japan). TA was determined by titration with 0.05 M NaOH to pH 8.2, and the result was expressed as a percentage of citric acid.

The weight loss was determined by weighing the blueberry fruit before and after the storage period and was expressed as the percentage of weight loss compared to the initial weight.

The decay incidence was evaluated by the number of decayed fruit relative to the total number of fruit. The decayed fruit were considered as rotten and had visible fungal growth or bacterial lesions on their surface.

2.3. Measurement of ascorbic acid, total phenolic and anthocyanin content

The ascorbic acid content was determined using a colorimetric method with 4, 7-diphenyl- l, 10-phenanthroline according to the method described by Arakawa, Tsutsumi, Sanceda, Kurata, and Inagaki (1981), by measuring the absorbance at 534 nm based on



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Fig. 1. The appearance (A) and changes in the color values $L^{*}(B)$, $a^{*}(C)$, and $b^{*}(D)$ of the surface of blueberry fruit with natural wax layer and after the removal of wax, during cold storage. Error bars represent the standard deviation of the means. The asterisk (*) shows significant difference at P < 0.05 for each day.

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