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Effects of allyl isothiocyanate treatment on postharvest quality and the activities of antioxidant enzymes of mulberry fruit

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ABSTRACT

The effects of allyl isothiocyanate (AITC) treatment on fruit quality, anthocyanin and phenolic contents, and the activities of antioxidant enzymes of mulberries var. Dashi (*Morus alba* L.) were evaluated. Freshly harvested mulberry fruit were placed in plastic sealed containers and treated with AITC at 5 and 15 $\mu L\,L^{-1}$ for 15 d, respectively. Samples were randomly selected initially and at 3 d intervals during storage. The fruit treated with AITC were resistant to decay, and had high levels of total soluble solids as well as titratable acidity. The application of AITC in mulberry fruit was effective in decreasing malondialdehyde (MDA) accumulation and lipoxygenase (LOX) activity, inhibiting respiration, maintaining surface color and firmness, and suppressing total phenolic and anthocyanin contents. However, AITC treatment had no discernible effect on the activities of antioxidant enzymes. The results from this study indicated that AITC treatment improved the metabolism and postharvest quality of mulberry fruit and provided an effective method for prolonging the storage life of the fruit.

1. Introduction

Mulberry, a fast-growing deciduous plant, is distributed from temperate to subtropical regions of the Northern hemisphere and the tropical regions of the Southern hemisphere (Ercisli and Orhan, 2007). In China, mulberry (*Morus alba* L.) has been cultivated for its leaves as a food source for the domesticated silkworms (*Bombyx mori* L.). Mulberry is also a traditional Chinese edible fruit and used in folk medicines to protect liver from damage, strengthen the joints, facilitate discharge of urine, lower blood pressure, and treat weakness, fatigue, anemia, and premature graying of hair (Bae and Suh, 2007; Sheng et al., 2014). Other studies reveal that mulberry fruit contain high amounts of phenolics, flavonoids, polysaccharide and ascorbic acid and possess multiple biological activities such as hepatoprotective (Ou et al., 2013), antioxidative (Bae and Suh, 2007; Yang et al., 2010; Yang and Lee, 2012; Butkhup et al., 2013), antibacterial (Yang and Lee, 2012), hypolipidemic (Yang et al., 2010), immunomodulating (Lee et al., 2013), anti-inflammatory and anti-apoptotic (Liu and Lin, 2012) activities.

Mulberry fruit have a unique sweet taste, and a distinctive flavor. Most of them display an attractive bright red color before full ripeness and are purply-black when fully ripe (Butkhup et al., 2011). Due to the functional quality characteristics, good taste, and nutritional value, the production and consumption of mulberry fruit have increased rapidly in recent years (Hu et al., 2014). However, mulberry fruit have a brief harvest season of less than 1 month and are perishable after harvest due to the high water content of more than 70% (Yang et al., 2010; Chen et al., 2011). Because of their short harvesting season and sensitivity to storage, mulberry fruit are mainly consumed locally and processed to wine, fruit juice, jam and canned food. However, the sensory quality of mulberries can be seriously damaged/changed and anthocyanins and visual color could be degraded during processing (Chen et al., 2011; Kara and Erçelebi, 2013). Therefore, it is important to develop effective storage methods to improve the storage quality and shelf life of mulberry fruit.

Plant essential oils such as horseradish, lavender, oregano, basil, sage, mustard, thyme, rosemary and garlic extract have been known to possess antimicrobial activity against food-borne pathogens (Zou et al., 2013). Allyl isothiocyanate (AITC) is the major antimicrobial component in the essential oils from black

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(*Brassica nigra*) and brown mustard (*Brassica juncea*) and is approved as Generally Recognized As Safe (GRAS) by the Food and Drug Administration (FDA) in the United States (Zou et al., 2013; Otoni et al., 2014). AITC has demonstrated fungicidal activity against *Aspergillus flavus* in peanuts (Otoni et al., 2014), *Penicillium expansum* on pear (Mari et al., 2002), *Alternaria alternate* on netted melon (Troncoso-Rojas et al., 2009), *Botrytis cinerea* on strawberry (Ugolini et al., 2014), and bactericidal activity against pathogens on iceberg lettuce, apple, and tomato (Lin et al., 2000). Furthermore, AITC has been reported to reduce decay and extend the shelf life of cooked rice (Kim et al., 2002), *kimchi* (Ko et al., 2012), strawberries, blackberries, raspberries, and blueberry (Wang et al., 2010). Therefore, AITC might be a potential natural antimicrobial agent for food preservation.

A comprehensive review of the literature revealed no information available on the effects of AITC treatment on both postharvest quality and the antioxidant enzyme activities of mulberry fruit. The objective of the present work was to specifically evaluate the effects of AITC treatment on decay, color, firmness, total soluble solids, titratable acid, ascorbic acid, total phenolics, total anthocyanin, malondialdehyde (MDA) and lipoxygenase (LOX), superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT) and ascorbate peroxidase (As-POD) activity of mulberry fruit in plastic sealed box during storage.

2. Materials and methods

2.1. Fruit material and treatments

Mulberry (*Morus alba* L., cv. Dashi) fruit were hand-harvested at a commercially mature stage from a farm located in Meixi town, Anji county of Zhejiang Province, China on May 16, 2013, and then transported to the laboratory by a refrigerated car within 2 h. Uniform fruit being free from blemishes were selected by size and color.

Fifty fruit were placed into 1 L plastic sealed containers with snap-on lids. The volatile compound AITC (Sigma–Aldrich Shanghai Trading Co., Ltd., Shanghai, China) was spotted onto a piece of filter paper which was subsequently hung inside the plastic containers just before the lids were closed. The applied AITC concentrations were 5 and 15 $\mu L\,L^{-1}$, respectively, and distilled water was used as the control. The containers were stored at 5 °C. Three containers of each treatment were taken for analysis every 3 days within a total storage period of 15 d.

2.2. Measurement of CO_2 and O_2 content in fruit container

The contents of CO_2 and O_2 in the fruit containers were measured using a CYES-II carbon dioxide and oxygen analyzer (Xuelian Instrument Company, Shanghai, China) according to the instruction.

2.3. Fruit decay, firmness and surface color measurements

Fruit decay was visually evaluated. Fruit with visible mold growth was considered rotten. The severity of fruit decay was expressed as percentage of fruit showing decay symptoms.

Fruit firmness measurement was conducted by a TA-XT plus texture analyzer (Stable Micro Systems Ltd., U.K.) with a 5 mm diameter stainless probe. Firmness was measured on the equatorial region of each fruit. Ten fruit from each treatment were compressed 3 mm at a rate of 1.0 mm s^{-1} and the maximum force (Newtons) developed during the test was recorded.

Color attributes (L*, a* and b*) were measured with a Minolta Chroma meter (Konica Minolta, CR-400, Japan) using the CIE scale. The equipment was calibrated with a white plate before use. A total of 20 fruit measurements were examined from each treatment.

2.4. Measurements of total soluble solids and titratable acidity

Six fruit from each replicate were wrapped in cheese cloth and squeezed with a hand press, and the juice was analyzed for total soluble solids (TSS), titratable acidity (TA). TSS was determined with a portable refractometer (Atago PAL-1, Japan). The results were expressed as a percentage at 20 °C. TA was determined by titration with 0.025 mol L^{-1} NaOH to pH 8.2, and the results were expressed as the percentage of $[H^+]$.

2.5. Malondialdehyde (MDA) content and lipoxygenase (LOX) activity determination

MDA content was determined according to the method described by Li et al. (2006) with some modification. Fruit tissues (1 g) were extracted with 5 mL of 200 mmol L^{-1} sodium phosphate buffer (pH 6.8). Three milliliters of 0.5% thiobarbituric acid (TBA) were added to 1 mL of the extract. The solution was heated in a boiling water bath for 20 min, then immediately cooled, and finally centrifuged at 10,000 × *g* for 10 min to clarify the solution. Absorbance was measured at 450, 532 and 600 nm. MDA content was expressed as mmol kg^{-1} of fresh weight by the method of Li et al. (2006).

LOX activity was assayed by the method described as Tao et al. (2008). One unit of LOX activity was defined as 0.1 change of absorbance at 234 nm per min. Specific LOX activity was expressed as units per kilogram of fruit fresh weight.

2.6. Total anthocyanin and total phenolic contents determination

Two gram samples from each replicate were treated with liquid nitrogen, and then pulverized and extracted with 25 mL of pre-cooled 70% ethanol containing 1% hydrochloric acid (v/v) for 3 h, and centrifuged at 10,000 × *g* for 15 min (4 °C). The supernatant was adjusted to 25 mL for analysis.

Total anthocyanin content of mulberry extract was measured using the pH differential method (Yang et al., 2009). Absorbance was measured at 510 and 700 nm, respectively, in different buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$ with a molar extinction coefficient of cyaniding 3-glucoside of 29,600. Results were expressed as grams of cyaniding 3-glucoside (C 3-G) equivalents per kilogram of fresh weight. Total phenolic contents were estimated colourimetrically Folin–Ciocalteu method (Zheng et al., 2003; Yang et al., 2009). Gallic acid was used as a standard, and phenolic contents were expressed as grams of gallic acid equivalents (GAE) per kilogram of fresh weight.

2.7. Antioxidant enzyme measurements

Two grams of fruit tissues were homogenized in 10 mL of 100 mmol L^{-1} sodium phosphate buffer (pH 7.8) containing 5% polyvinylpyrrolidone and 5 mmol L^{-1} 1,4-dithiothreitol at 4 °C. The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C, and then the supernatant was collected for SOD activity assay as described by Yang et al. (2009) with some modification. The reaction medium contained 1.7 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.8), 0.3 mL of 130 mmol L^{-1} methionine, 0.3 mL of 100 μmol L^{-1} EDTA-Na, 0.3 mL of 750 μmol L^{-1} nitro-blue-tetrazolium (NBT), 0.3 mL of 20 μmol L^{-1} riboflavin, and 25 μL of enzyme extract. The reaction was initiated by switching on the light consisting of two 4000 lx fluorescent lamps; after 25 min, the light was turned off, and the absorbance at 560 nm was recorded. One unit of SOD activity was defined as the amount of