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The effects of cold storage and aminoethoxyvinylglycine (AVG) on bioactive compounds of plum fruit (*Prunus salicina* Lindell cv. 'Black Amber')

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ABSTRACT

Effects of two different aminoethoxyvinylglycine (AVG) doses (100 and 200 mg L^{-1}), applied 2 weeks ahead of estimated harvest date, on fruit color (L^*, C^* and $h°$), firmness, total soluble solids content (TSSC), total phenolics (TP), total antioxidant activity (TAA) and individual phenolic compounds of 'Black Amber' plums at the time of harvest and during 4 weeks of cold storage were investigated. Color characteristics linearly decreased in all treatments during storage. Fruit firmness at the end of the storage period was significant decreased ($P < 0.05$) with AVG treatments. TSSC rates linearly increased during the storage period although the differences among treatments were not significant. Both TP and TAA increased with all treatments until the 21st day of storage and decreased by the 28th day. TP and TAA were significantly decreased ($P < 0.05$) with the 100 mg L^{-1} AVG treatment at the end of storage. While chlorogenic acid, p-coumaric acid and rutin increased during storage, epicatechin, catechin, caffeic acid, ferulic acid and kaempferol linearly decreased. AVG treatments generally had negative impacts on individual phenolic compounds.

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1. Introduction

Plums are one of the most significant stonefruit commercially produced in Turkey. They are rich in vitamins A, C, E, phytochemicals and bioactive compounds such as anthocyanins, carotenoids and phenolic compounds (Stacewicz-Sapuntzakis et al., 2001). Plums are a climacteric fruit, and can be retained in cold storage for 1–8 weeks depending on the variety (Perez-Gago et al., 2003; Crisosto et al., 2004). Storage life can be prolonged with pre-harvest treatments on the tree or postharvest pre-storage treatments. The ethylene inhibitor AVG (aminoethoxyvinylglycine) used is a common pre-harvest treatment and calcium, heat treatment, polyamines, jasmonates and 1-MCP (1-methylcyclopropene) treatments are the other common postharvest pre-storage treatments for plums (Singh and Khan, 2010).

AVG is commercially sold under the name of ReTain®. It is a human and environmentally friendly organic product registered for use for apple, pear, peach, plum and nectarine in several countries (Greene and Schupp, 2004; Rath and Prentice, 2004). Byers (1997) reported the inhibitory effect on ethylene biosynthesis and consequent suppression of ethylene production by AVG in various plant tissues. Autio and Bramlage (1982) observed that AVG treatments delayed ripening and harvest, increased fruit firmness and prolonged storage life of fruit. Fruit firmness of plums is a quality parameter directly related to fruit ripening, shelf life (De Ketelaere et al., 2006; Valero et al., 2007), and fruit quality (Jobling et al., 2003; Greene, 2005).

Physical, mechanical, chemical and bioactive characteristics of fruit are controlled by various factors such as variety, growth period, growing site, environmental conditions, plant nutrients, type of production (organic or inorganic), harvest period and other cultural practices (Awad et al., 2001; Lata, 2007; Shin et al., 2008). Growth regulators may also cause variations in these factors (Fan et al., 1997). The variation in antioxidant activity, total phenolics and especially, individual phenolic compounds of plums during cold storage has not been studied in detail. This study investigates the effects of AVG on variations in bioactive compounds and quality parameters of plums during cold storage.

2. Material and methods

2.1. Material

This study was conducted at the Research Station of Gaziosmanpaşa University Agricultural Faculty located at 40°20′02.19″N latitude, 36°28′30.11″E longitude and 623 m above sea level. As the plant material, 5-year old 9 'Black Amber' (*Prunus salicina* Lindell) trees grafted on myrobalan

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(*Prunus ceresifera* Ehrh.) rootstock were selected. The planting density was 4 m × 4 m, and trees were grouped in 3 blocks with 3 trees in each block. Each AVG dose (100 and 200 mg L^{-1}) was applied to one tree in each block and one tree in each block was considered as a control. Trees with a homogeneous fruit load were selected for experiments and trees were trained in a modified leader system.

The ethylene inhibitor, ReTain® (containing 150 mg aminoethoxyvinylglycine/g; ValentBioScience Corp. Libertyville, III) was applied two weeks before the estimated harvest date (105 days following full bloom). During the preparation of ReTain® solution, 'Sylgard 309' surfactant [0.05%, v/v (DowCorning, Canada Inc., Toronto)] was used to reduce the surface tension and increase the effectiveness of the material applied to the plant. Water (pH 6.48) + surfactant mixture was used in control treatments.

One hundred and twenty five fruit were randomly harvested from a tree in each block for each treatment at the estimated harvest date (July 25, 2011). Of these fruit, 20 samples were used for the fruit quality characteristics (geometric mean diameter, fruit firmness, TSSC), and 5 were used for bioactive compounds. The remaining fruit were placed into cardboard boxes in single rows and transferred to cold storage at 0 °C and 90 ± 5% RH for 4 weeks of storage. To evaluate color characteristics, 50 fruit from a tree in each block for each treatment were stored in cardboard boxes in single rows on the harvest date.

The fruit harvested were analyzed on the 7th, 14th, 21st, and 28th days (August 1, 8, 15 and 22, 2011) to determine changes in fruit quality parameters and bioactive compounds. For each analysis period, 25 fruit were selected and 20 were used for the fruit quality characteristics (geometric mean diameter, fruit firmness, TSSC) and 5 for bioactive compounds. Physical (geometric mean diameter and color) and mechanical (firmness) measurements were performed shortly after the fruit were taken out of cold storage. The fruit used for TSSC and bioactive characteristics were kept at 21 °C for 6 h prior to measurements.

2.2. Methods

2.2.1. Color characteristics during cold storage

Color characteristics of fruit were measured on the 7th, 14th, 21st and 28th days of the storage period. The measurements were taken from the same fruit at each time. The color characteristics were determined with a colorimeter (Minolta, model CR–400, Tokyo, Japan) from three different points on the equatorial section of fruit skin. The CIE L^*, a^* and b^* values of fruit skin color were determined.

2.2.2. Geometric mean diameter and firmness

Fruit length (L), width (W) and thickness (T) were measured with a 0.01 mm sensitive digital caliper (Model No; CD-6CSX, Mitutoyo, Japan). Geometric mean diameter (D_g) was determined using the relationship ($D_g = (LWT)^{1/3}$) described by Mohsenin (1970). The fruit skin was cut at two different points (on the cheeks) along the equatorial part of the fruit and the firmness was measured using an Effegi penetrometer (model FT–327; MoCormick Fruit Tech, Yakima, WA) with a 7.9 mm penetrating tip. The measurements were expressed in newtons (N/cm).

2.2.3. Total soluble solids content

A sample of juice was also taken from each piece of fruit (20 fruit) and the percentage total soluble solids content (% TSSC) was measured using a digital refractometer (PAL-1, McCormick Fruit Tech., Yakima, Wash).

2.2.4. Bioactive compounds

Five fruit from each tree were washed with distilled water, homogenized and kept at room temperature (21 °C) for 6 h. The fruit samples were kept in 50 mL tubes at −20 °C for biochemical analysis. A 1 g sample was taken from each fruit sample and 5 mL methanol was added to each sample. The tests were performed at 6 h intervals.

2.2.4.1. Total phenolics. A portion of 300 μL from each sample was diluted with 4.3 mL distilled water and 100 μL Folin-Ciocalteu reagent were added. After an interval of 3 min, 2% Na_2CO_3 was added to 300 μL portions, and the mixture vortexed and incubated for 30 min. Absorbances were then read on a UV–vis (PerkinElmer, Lambda–1050 spectrophotomer, CA, USA) spectrophotomer at 760 nm. Gallic acid was used as the standard. The results were expressed as mg gallic acid equivalents (GAE)/g flesh weight.

2.2.4.2. Total antioxidant activity. ABTS⁺ radical scavenging activity: 2 mM of $ABTS^+$ (2.2″-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) and 2.45 mM of $K_2S_2O_8$ solution were prepared in 0.1 M of PO_4^{-3} buffer solution (pH 7.4). The ABTS $^+$ and $K_2S_2O_8$ solutions were mixed in (1:2) ABTS–$K_2S_2O_8$ and incubated for 6 h in the dark. The absorbance of the mixture was read at 734 nm and it was diluted with PO_4^{-3} buffer if the value was greater than 0.75. Finally, 20 μL samples were taken out of the mixture into tubes, 1 mL of $ABTS^+$–$K_2S_2O_8$ solution was added to each and buffer solution was added to make the total sample volume 4 mL. Following vortexing, they were incubated for 30 min and absorbances were read at 734 nm. The results were expressed as μmol Trolox equivalent (TE)/g flesh weight.

Ferric ions (Fe^{+3}) reducing antioxidant power assay (FRAP): portions of 120 μL were taken from the samples, 0.2 M of phosphate buffer (PO_4^{-3}) (pH 6.6) was added to obtain a volume of 1.25 mL and then 1.25 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution was added. After vortexing, they were incubated at 50 °C. Afterwards, 1.25 mL of 10% TCA and 0.25 mL of 0.1% $FeCl_3$ were added to the samples. The absorbances of the resulting solution were read on an UV–vis spectrometer at 700 nm. The results were expressed as μmol TE/g flesh weight.

2.2.4.3. Individual phenolics. Instrumentation and conditions: a Perkin Elmer Series of 200 liquid chromatography systems (Perkin Elmer, USA) equipped with a quaternary solvent delivery system and UV detector was used at 280 nm. The analytes were separated on a Phenomenex Kromasil (Phenomenex Inc., USA) 100A C18 (250 mm × 4.60 mm, 5 μm) column. The column temperature was maintained at 26 °C using a water bath (Wisebath, WB-22, and Daihan Scientific, Korea). The mobile phase was consisted of acetonitrile (A) and water containing 2.5% formic acid (B). The following gradient conditions were used: initial 0–3 min, held at A–B (5:95, v/v), 3–8 min, linear change from A–B (5:95, v/v) to A–B (10:90, v/v); 8–13 min, linear change from A–B (10:90, v/v) to A–B (15:85, v/v); and 13–15 min, isocratic elution A–B (15–85, v/v); 15–22 min, linear change from A–B (15:85, v/v) to A–B (25:75, v/v); 22–37 min, linear change from A–B (25:75, v/v) to A–B (50:50, v/v); 37–40 min, isocratic elution A–B (100–0, v/v). The mobile phase flow rate was set at 1 mL/min and the injection volume was 20 μL.

Preparation of standard solutions: accurately weighed solid portions of each standard were dissolved in methanol to prepare stock solutions. Working solutions were obtained by diluting the stock solutions with methanol. The final mixed standard solution contained 100 μg/mL of each standard.

Sample preparation: all crude fruit samples were homogenized and 1000 mg slurry was accurately weighed and extracted with (5 mL) methanol in test tube for 6 h. After filtration using a syringe type filter (Chromtech, 13 mm, 0.22 μm), the filtrate was injected

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