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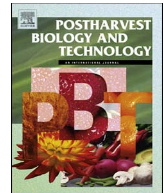

Effects of melatonin treatment on the postharvest quality of strawberry fruit

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ABSTRACT

The effects of exogenous melatonin on postharvest life and quality in strawberry fruit after harvest were evaluated. To explore the optimum concentration of melatonin treatment, strawberry fruit were treated with 0, 0.01, 0.1, 1 and 10 mmol L^{-1} melatonin for 5 min and then stored at 4 °C and 90% RH for 12 d. The results showed that application of melatonin at 0.1 or 1 mmol L^{-1} was notably effective in reducing decay and weight loss of fruit. Senescence of strawberry fruit was clearly delayed by the 0.1 or 1 mmol L^{-1} melatonin treatment, as disclosed by the color, firmness, the total soluble solids content and titratable acidity of the fruit. Melatonin treatment at 0.1 or 1 mmol L^{-1} significantly reduced the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), but increased the total phenolics and flavonoid contents, resulting in the higher antioxidant capacity. Nevertheless, melatonin treatment had a negative impact on the ascorbic acid content. The optimum concentration of melatonin for extending the postharvest life and improving the quality of strawberry fruit was 0.1 or 1 mmol L^{-1}. Moreover, melatonin treatment at 0.1 mmol L^{-1} enhanced the expression of melatonin biosynthetic genes including *FaTDC*, *FaT5H*, *FaSNAT*, and *FaASMT* and consequently increased the content of endogenous melatonin. These findings suggested that melatonin treatment may be a useful technique to extend the postharvest life and improve quality in strawberry fruit.

1. Introduction

Strawberry (*Fragaria* × *ananassa*, Duch.) fruit is one of the most commonly consumed berries both in fresh and processed forms. It is a rich source of a wide variety of nutritive compounds such as sugars, vitamins and minerals, as well as bioactive compounds such as ascorbic acid, carotenoids, phenolic compounds and folates, most of which are natural antioxidants and contribute to the high nutritional quality of the fruit (Tulipani et al., 2011; Giampieri et al., 2015). All of these compounds exert a synergistic and cumulative effect on human health promotion and in disease prevention. Strawberry fruit is a non-climacteric fruit and should be harvested at full maturity stage in order to get the maximum marketing quality. This fruit is also highly perishable, due to high respiration rate, low mechanical resistance, and high susceptibility to the pathogen attack (Hashmi et al., 2013; Neri et al., 2014). Undesirable changes observed during postharvest include desiccation, loss of flesh firmness, mechanical injury, and *Botrytis cinerea* induced decay (Charles et al., 2009; Pombo et al., 2009). Thus, there is an urgent need for reducing decay and extending the postharvest life of strawberry fruit. To date, various postharvest treatments including coating (Fan et al., 2009), chitosan coating combined with calcium treatment (Hernandez-Muñoz et al., 2008), salicylic acid treatment (Babalar et al., 2007), UV-C irradiation (Nigro et al., 2000; Charles et al., 2009; Pombo et al., 2011), and ultrasonic treatment (Cao et al., 2010) have been applied into postharvest preservation of the strawberry fruit. Nevertheless, some of these methods are not commercially reasonable due to low customer preference or need for verifying the effectiveness. The use of different fungicides is probably the most commonly used method to control postharvest decay, but it leaves residues that have potential risks to humans and the environment. Thus, it is urgent to develop new and effective methods to extend postharvest life and improve quality of strawberry fruit.

Melatonin (*N*-acetyl-5-methoxytryptamine) is an endogenously produced indoleamine in all plant species (Reiter et al., 2015). As a healthy ingredient contained in the diet, many fruits and vegetables, including tomato, apple, cherry, banana, and strawberry provide natural melatonin (Stürtz et al., 2011; Feng et al., 2014; Sun et al., 2015). As a safe and beneficial indoleamine, melatonin acts not only as a signaling molecule for enhancing the resistance of plants to biotic and abiotic stresses, but also as a powerful free-radical scavenger and has a direct antioxidant activity (Tan et al., 1993; Arnao and Hernández-Ruiz, 2015). Recently, exogenous melatonin treatment has been tested as an

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effective postharvest treatment to promote ripening and improve quality of tomato fruit (Sun et al., 2015), delay postharvest senescence and increase chilling tolerance of peach fruit (Cao et al., 2016; Gao et al., 2016), attenuate postharvest decay and maintain nutritional quality of strawberry fruit (Aghdam and Fard, 2017), and attenuate postharvest physiological deterioration of cassava storage roots (Ma et al., 2016). However, little information is available regarding the effects of melatonin as a postharvest treatment on the postharvest life and quality of strawberry fruit.

Therefore, the aim of the present study was to assess the effects of postharvest melatonin treatment on the postharvest life and quality of strawberry fruit during storage at 4 °C. This study may promote the application of melatonin on the postharvest quality in strawberry fruit as well as other fruits and vegetables in the future.

2. Materials and methods

2.1. Strawberry fruit handling and treatment

Fresh strawberry (*Fragaria* × *anannasa* cv. Hongyan) fruit at commercial ripeness (75% red stage of ripening) were harvested from a greenhouse in Hefei, China. Healthy fruit of uniform size and free from apparent disease or injuries were selected, and immediately transported to the laboratory. The strawberry fruit were rinsed in tap water before treatment and then immersed in solution for 5 min at 20 °C. For melatonin treatment, 1200 fruit were selected and grouped into 5 lots (240 fruit per lot) for the following treatments in triplicate (80 fruit per replicate). The five solutions made for treatments were: control (distilled water), M0.01 (0.01 mmol L^{-1} melatonin), M0.1 (0.1 mmol L^{-1} melatonin), M1 (1 mmol L^{-1} melatonin), and M10 (10 mmol L^{-1} melatonin). Following immersion, the fruit were dried in air at room temperature for approximately 30 min. Then, all fruit were stored at 4 °C and 90% RH for 12 d. 20 fruit of each replicate were randomly taken at 3, 6, 9 and 12 d after treatment for measurements of decay incidence, severity of decay, weight loss, color, firmness and total soluble solid contents, and then the rest of the fruit were immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Determination of decay incidence and weight loss

Decay incidence of strawberry fruit was the number of fruit showing decay symptoms (rot, lesions or visible fungal growth) relative to the total number of fruit and expressed in percentage (%). The severity of decay was evaluated by observing the decay area on the fruit surface using a 1–5 scale: 0, healthy fruit; 1, 1–20% fruit surface infected; 2, 21–40% fruit surface infected; 3, 41–60% fruit surface infected; 4, 61–80% fruit surface infected; 5, ≥ 81% fruit surface infected and showing sporulation according to Aghdam and Fard (2017). Each treatment contained 3 replicates with 20 fruit per replicate, and the experiment was repeated 3 times. Weight of each fruit was measured following the treatment at day 0 and at the different sampling day. Weight loss was expressed as the percentage loss of the initial weight.

2.3. Determination of color, firmness, total soluble solids content and titratable acidity

Fruit external color was measured using a chromometer (Chroma Meter WSC-S, Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China) to obtain L^*, a^* and b^* values by the CIE color system. L^* value represents lightness or darkness, a^* value represents redness or greenness (−greenness to +redness) and b^* value represents blueness or yellowness (−blueness to +yellowness). The a^* and b^* values were converted to hue angle ($H = \tan^{-1}\frac{b^*}{a^*}$) and chroma ($C = \sqrt{a^{*2} + b^{*2}}$). Firmness of fruit was measured by performing a penetration test with a 5 mm cylindrical probe on the skin of whole fruit using a TA-XT2i texture analyzer (Stable Micro Systems, Guildford, UK). The penetration depth was 10 mm and the speed of the probe was 1.0 mm s^{-1}. Firmness was defined as the maximum penetration force (N). Total soluble solid (TSS) content of fruit was measured with a portable refractometer (WYT-32, Quanzhou Optical Co. Ltd., China). Result was expressed in percentage (%). The titratable acidity was measured titrimetrically with 0.1 mol L^{-1} NaOH solution using an automatic titrator (Mettler Toledo V20 volumetric titrator). The result was expressed as g of citric acid per kg of fruit fresh weight basis.

2.4. Determination of ascorbic acid

Ascorbic acid was determined by the phenolindo-2,6-dichlorophenol (DPIP) solution titration procedure (Jagadeesh et al., 2011). Frozen fruit sample was added to 20 mL of buffer solution containing 4 g L^{-1} of anhydrous sodium acetate and 1 g L^{-1} of oxalic acid. The fruit extract was titrated with a calibrated DPIP solution and the titration was repeated using standard ascorbic acid solution (0.10 g L^{-1} ascorbic acid in buffer solution) in place of the fruit extract. Result was expressed as g kg^{-1} of fruit fresh weight basis.

2.5. Determination of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents

The H_2O_2 and MDA contents were measured by using the method described by Ma et al. (2016) with a slight modification. For H_2O_2 content assay, frozen fruit samples were homogenized with 1 mL of 0.1% cold trichloroacetic acid (TCA) solution and then incubated in an ice bath for 10 min. After centrifugation at 12 000 rpm for 15 min at 4 °C, 0.5 mL supernatant was mixed with 0.5 mL of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) and 1 mL of 1 mol L^{-1} potassium iodide, and the absorbance was measured at 390 nm in a UV/VIS spectrophotometer (Genesys 10S, ThermoFisher). The H_2O_2 content was calculated using H_2O_2 as a standard and expressed as μmol g^{-1} of fruit fresh weight basis.

For MDA content assay, 0.1 g fruit sample was homogenized with 0.9 mL of 10% cold TCA and then incubated in an ice bath for 10 min. After centrifugation at 12 000 rpm for 15 min at 4 °C, 50 μL supernatant was mixed with 1 mL of 10% TCA-containing 0.67% thiobarbituric acid. Then the mixture was incubated at 95 °C for 20 min and centrifuged at 12 000 rpm for 10 min at 4 °C. The absorbance was measured at 450, 532 and 600 nm. The MDA content was calculated using the formula and expressed as nmol g^{-1} of fruit fresh weight basis: MDA content (nmol g^{-1}) = 6.45 × (OD_{532} − OD_{600}) − 0.56 × OD_{450}.

2.6. Determination of total phenolics and flavonoid contents

Total phenolics and flavonoid contents were measured according to the method of Toor and Savege (2005), with little modifications. For the total phenolics assay, the ethanol extract of fruit was added to 2.0 mL of 1 mol L^{-1} Folin-Ciocalteau reagent. After mixing thoroughly for 5 min, 1.5 mL of 7.5% Na_2CO_3 solution was added into the mixture. The mixtures were then incubated for 1 h at room temperature in the dark and the absorbance was measured at 765 nm. Total phenolic content was expressed as g of gallic acid per kg of fruit fresh weight basis.

For total flavonoid assay, the ethanol extract of fruit was mixed with 0.3 mL of 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $Al(NO_3)_3$ was added and after 5 min, 1.5 mL of 1 mol L^{-1} NaOH was added to the mixture and adjusted to 4 mL with distilled water. The absorbance was measured at 510 nm and the result was expressed as g of rutin per kg of fruit fresh weight basis.

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