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Effect of 1-methylcyclopene on senescence and quality maintenance of green bell pepper fruit during storage at 20 °C

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ABSTRACT

The effect of 1-methylcyclopropene (1-MCP) on senescence and quality maintenance of green bell pepper fruit was investigated. To explore the optimum concentration of 1-MCP to delay senescence, pepper fruit were treated with 0.5, 1.0 or 1.5 μL L^{-1} 1-MCP and then stored for 10 d at 20 °C. The results showed that the application of 1-MCP at 1.0 μL L^{-1} was most effective in delaying senescence, manifested as chlorophyll degradation and increase in weight loss. 1-MCP treated peppers had higher levels of chlorophyll, protein and vitamin C and lower respiration rates and ethylene production compared with that in control peppers. In addition, 1-MCP enhanced activities of superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase and levels of polyamines including putrescine, spermidine and spermine. These results suggested that the delaying senescence of green bell pepper by 1-MCP treatment is associated with enhanced antioxidant enzyme activities and polyamine contents.

1. Introduction

Green peppers are usually harvested before fully ripe, at which stage the pericarp wall becomes thick and the fruit reaches a typical size and seed viability (Sargent, 2000). Therefore, green bell peppers are able to withstand transport and have a relatively long postharvest life (Salkadaş and Kaynaş, 2010). However, after detachment from the plant, bell peppers commonly encounter postharvest problems, such as pathological deterioration, rapid water loss, and rapid senescence, which often cause serious commercial losses (Du et al., 2007). Effective postharvest treatments that can delay fruit senescence and control quality deterioration therefore are crucial for the development of the pepper industry. Several methods (e.g. edible coating, UV-C) have been reported to delay development of senescence, but most treatments provide only partial control (Lerdthanangkul and Krothta, 1996; Vicente et al., 2005). Thus, there is still a need for more effective techniques for green bell pepper storage.

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action, has been shown to strongly block ethylene perception, preventing ethylene effects in plant tissues for extended periods (Sisler and Serek, 1997). 1-MCP provides commercial potential for controlling ethylene-dependent processes such as ripening, senescence, yellowing and softening (Blankenship and Dole, 2003) and extending shelf life of some vegetables including broccoli (Ku and Wills, 1999), coriander (Jiang et al., 2002), chrysanthemum (Able et al., 2003), cucumber (Nilsson, 2005) and parsley (Lomaneic et al., 2003). Green pepper fruit is generally classified as a non-climacteric fruit (Watkins, 2002), although varying patterns of ethylene and carbon dioxide production have been observed (Villavicencio et al., 1999). Recently, Ilić et al. (2011) found that 1-MCP treatment had a pronounced effect on delaying ripening as shown by inhibiting color change, decreasing decay, and maintaining quality of non-climacteric green pepper fruit. However, the mode of action of 1-MCP in delaying senescence and quality deterioration has not been clearly elucidated.

Plant senescence is often associated with increased oxidative damage to cellular macromolecules by reactive oxygen species (ROS). Effective destruction of ROS requires the action of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) (Shewfelt and Purvis, 1995). Meanwhile, polyamines, ubiquitous in plants and known to be associated with cell division and growth, have also been shown to act as anti-senescence agents in a number of tissues (Pradey et al., 2000). Therefore, the objective of this study was to determine whether a 1-MCP-induced modification in antioxidant enzymes and polyamines is linked to delayed senescence and quality maintenance of green bell pepper during storage at 20 °C.

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2. Materials and methods

2.1. Selection of 1-MCP optimal dose

Green bell peppers (*Capsicum annuum* cv. Sujiao 13; ever-green cultivar) were grown in a shade net house during the winter season in a commercial farm at Nanjing in Jiangsu Province, China. Land preparation, planting, and plant protection for the crop were as is the standard in this area. Peppers were hand-harvested and transported, within 2 h, to the laboratory. Fruit of uniform size and colour, and without mechanical damage were divided randomly into four lots, each containing 150 fruit. The fruit were placed in sealed 40 L plastic tanks and treated with 0 (control), 0.5, 1.0 or 1.5 $\mu L\,L^{-1}$ 1-MCP for 12 h in the dark at 20 °C and 80–85% RH. 1-MCP was released from a commercial powder formulation (SmartFresh™, Agrofresh Ltd., Philadelphia, USA) by adding distilled water according to the manufacturer's instructions. Each treatment was replicated three times. Following treatment, the tanks were opened, ventilated for 30 min, and then the fruit were stored at 20 °C and 80–85% RH for 10 d. There were three replicates of 15 fruit each per treatment, and the experiment was conducted twice. Fruit samples were taken before 1-MCP treatment (time 0) and at 2 d intervals during storage for measurements of respiration rate, ethylene production, weight loss, ion leakage and levels of chlorophyll, protein, amino acids and vitamin C.

2.2. Experiments using 1.0 $\mu L\,L^{-1}$ 1-MCP treatment

Fifty green bell peppers were treated as described above in order to obtain a dose of 1.0 $\mu L\,L^{-1}$ 1-MCP. After treatment, the tanks were opened, ventilated for 30 min, and then the fruit were stored at 20 °C and 80–85% RH for 10 d. Seventy-five peppers without 1-MCP treatment were directly stored at 20 °C and used as controls. There were three replicates of 10 fruit each per treatment, and the experiment was conducted twice. Fruit samples were taken before 1-MCP treatment (time 0) and at 2 d intervals during storage for measurements of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), POD (EC 1.11.1.7) and APX (EC 1.11.1.11), and polyamines such as putrescine (Put), spermidine (Spd) and spermine (Spm).

2.3. Measurement of respiration rate, ethylene production and weight loss

Five fruit for each of three replicates before 1-MCP treatment and at 2, 4, 6, 8, and 10 d were enclosed in 1 L glass jars at 20 °C. Ten milliliters of headspace gas was taken with a syringe from each jar at the end of 2 h. CO_2 was measured with an infrared gas analyzer (GXH-305, Beijing Analysis Instrument Factory, Beijing, China). Headspace gas was pumped into the detector at a rate of 0.5 L min^{-1} and CO_2 generated by the fruit in the effluent air stream was quantified. Results were expressed as mg CO_2 kg^{-1} fresh weight (FW) h^{-1}. Ethylene was analyzed with a gas chromatograph (GC-14B, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector with the temperature of 140 °C and an alumina column (3 mm × 2 m) held at 60 °C. Results were expressed as μL ethylene kg^{-1} FW h^{-1}. Weight loss was measured on 15 fruit per treatment, with the results expressed as a percentage.

2.4. Determination of levels of chlorophyll, protein, amino acid and vitamin C

Chlorophyll content was determined according to Arnon (1949). The protein content was measured according to Bradford (1976). Amino acid content was determined by the ninhydrin method (Yemm and Cocking, 1954). The results of chlorophyll, protein and amino acid content were all expressed as mg g^{-1} FW. Vitamin C contents of green bell pepper were measured using 2,6-dichloro-indophenol titration as described by Jones and Hughes (1983). Fresh samples (10 g) were homogenized with 10 mL of 3% (v/v) metaphosphoric acid. The extract was made up to a volume of 100 mL and centrifuged at 3000 × *g* for 15 min at 25 °C. Ten milliliters of supernatant were titrated against standard 2,6-dichloro-indophenol, which had already been standardized against standard ascorbic acid and the result was expressed in mg g^{-1} FW.

2.5. Measurement of enzyme activities

All enzyme extraction procedures were conducted at 4 °C. For SOD, 1 g of green pepper tissue was ground in 5 mL of 50 mM sodium phosphate buffer (pH 7.8). For CAT, 1 g of tissue was ground in 5 mL of 50 mM sodium phosphate buffer (pH 7.0). For APX, 1 g of tissue was ground in 50 mM sodium phosphate buffer (pH 7.0), containing 0.1 mM EDTA, 1 mM ascorbic acid and 1% (w/v) polyvinyl-pyrrolidone. For POD, 1 g of tissue was ground with 50 mM sodium borate buffer (pH 8.7). The extracts were then homogenised and centrifuged at 10,000 × *g* for 20 min at 4 °C and the supernatants were used for enzyme assays.

SOD activity was determined as described by Rao et al. (1996). The reaction medium contained 50 mM sodium phosphate buffer, pH 7.8, 14 mM methionine, 3 μM EDTA, 1 μM nitro blue tetrazolium (NBT), 60 μM riboflavin and 0.1 mL crude enzyme extract. The formation of blue formazan was monitored by taking the absorbance at 560 nm. One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of NBT.

CAT activity was assayed according to Chance and Maehly (1955). One unit of CAT activity was defined as the amount of enzyme that decomposed 1 μmol H_2O_2 min^{-1} at 30 °C. APX activity was measured as described by Nakano and Asada (1989). One unit of APX activity was defined as the amount of enzyme that oxidised 1 μmol ascorbate min^{-1} at 25 °C. POD was assayed according to Kochba et al. (1977). The assay mixture contained 50 mM sodium acetate (pH 5.4), 0.75% (v/v) H_2O_2, 20 mM guaiacol, and 0.1 mL crude enzyme extract. POD activity was determined by measuring the absorbance at 460 nm. One unit of POD activity was defined as the amount of enzyme causing an increase in A_{460} of 0.01 unit min^{-1}. The specific activities of all enzymes were expressed as U g^{-1} FW.

2.6. Analysis of polyamines

Flesh tissue (2 g) were homogenized in a chilled mortar with 3 mL of 0.2 M $HClO_4$ and 1 mL of 0.6 mM 1,6-hexane-diamine as an internal standard. The homogenate was then centrifuged at 12,000 × *g* for 20 min at 4 °C. Polyamines in the supernatant were dansylated, as previously described (Gonzalez-Aguilar et al., 2000), then extracted with 500 μL of toluene. The organic phase was dried under a stream of N_2 at 70 °C and the residue was resuspended in 200 mL of acetonitrile (HPLC grade) and filtered using a 0.2 μm pore filter before HPLC analysis. Polyamines were eluted through a 200 mm × 4.6 mm reverse-phase C_{18} column packed with 5 μm Hypersil ODS resin. Elution was performed at a flow rate of 1.5 mL min^{-1} with a gradient of 60–90% acetonitrile for 25 min at 35 °C. Dansylated polyamines in the extracts were detected by fluorescence at an emission wavelength of 447 nm and quantified by comparing peak areas using 1,6-hexanediamine as the internal standard, and with standard curves for Put, Spd and Spm.

2.7. Statistical analyses

Experiments were performed using a completely randomized design. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The data were analysed by one-way