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Postharvest hexanal vapor treatment delays ripening and enhances shelf life of greenhouse grown sweet bell pepper (*Capsicum annum* L.)

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ABSTRACT

Aqueous compositions of hexanal, an inhibitor of phospholipase D, has been shown to enhance the shelf life and quality of fruits and vegetables. In the present study, sweet bell pepper fruit were exposed to hexanal vapor and its effect on quality attributes, shelf-life, and antioxidant enzyme activities were evaluated during storage at 7 day intervals for 21 d. Peppers subjected to hexanal vapor treatments (0.005, 0.01, and 0.02%, w/w) showed a significant ($p < 0.05$) delay in ripening process and preservation of postharvest qualities than untreated peppers, even at 21 d of storage. Treated fruit were characterized by increased firmness, a reduction in physiological water loss and lower electrical conductivity than control fruit, which indicated better membrane preservation. These treatments also resulted in an increase in the levels of antioxidant enzyme activities, specifically that of superoxide dismutase, catalase, glutathione reductase and guaiacol peroxidase. Evidences from the present study indicate that postharvest hexanal vapor treatment at optimal levels can effectively enhance the quality and shelf life of sweet bell peppers.

1. Introduction

Bell pepper (*Capsicum annuum* L.) is one of the commercially important horticultural crops grown in greenhouses worldwide. Bell pepper fruit is a popular vegetable consumed fresh or used in a variety of processed food products. Peppers are generally considered as a good source of essential vitamins including C, E, A, and B and also rich in many health benefitting antioxidant phytochemicals such as flavonoids, phenolic acids, carotenoids *etc*., that may reduce the risk of developing chronic degenerative diseases (Marin et al., 2004; Knekt et al., 1996). Bell pepper production comprises approximately 500 ha of area in Canadian greenhouse industry, with an annual production of about 150,000 tons of pepper, which is worth nearly $500 million CAD (Statistics Canada, 2012). Postharvest losses of sweet bell peppers are estimated as 25–35% of the total production.

Bell pepper fruit are highly perishable and need appropriate handling and adequate care to maintain postharvest quality. The principal physiological factors that negatively impact the postharvest quality of peppers during transportation, storage and marketing are water loss (Lownds et al., 1993) and chilling injury (Hardenburg et al., 1986; Paull, 1990). Currently, there are no targeted practices to prevent these from occurring. The storage life of pepper fruit is also limited by pathological deterioration (Ceponis et al., 1987) caused by *Botrytis cinerea* and *Alternaria alternata* (Barkai-Golan, 1981). *Botrytis* can grow even at the recommended pepper storage temperatures. Refrigerated storage at optimal temperature can help to prolong the shelf life of peppers considerably, though this does not completely prevent the development of fungal infections. Temperatures above the optimal range can encourage postharvest diseases such as soft rot and water loss while low temperature can often cause chilling injury in bell peppers (Meir et al., 1995).

Maintenance of an efficient antioxidant system can delay the senescence process even though anti-oxidative activity in fruits decreases with aging (Zheng et al., 2007). Stress and senescence enhances the generation of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) in various plant cell compartments (Paliyath and Droillard, 1992; Noctor and Foyer, 1998). ROS are lethal and can induce oxidative damage to the cellular components. Plants have developed efficient ROS scavenging systems including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX), and glutathione reductase (GR) that are able to

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eliminate ROS (Noctor and Foyer, 1998; Foyer and Noctor, 2011).

Excessive fruit softening resulting in the loss of crispness and firm texture of pepper fruit is a major postharvest concern that can affect consumer preference and market value. Softening is a natural process associated with fruit ripening, and occurs due to turgor loss (Paliyath and Droillard, 1992; Chen et al., 2011). Depolymerization and disintegration of cell wall components particularly pectin by the action of enzymes such as polygalacturonase, pectin methyl esterase, and β-galactosidase can further hasten fruit softening (Handa et al., 2007; Ogasawara et al., 2007; Chuni et al., 2010). Studies have shown that activity of the enzyme phospholipase D (PLD) can initiate membrane deterioration during ripening and senescence, and the membrane deterioration is also enhanced by reactive oxygen species (ROS) produced during stress conditions associated with cold storage and postharvest handling. (Paliyath and Droillard, 1992). Ways to slow down or reduce the disintegration of cell wall and membrane structures could delay senescence process to some extent. Many biologically active volatile compounds, including hexanal, a natural plant volatile C_6 aldehyde, and an inhibitor of PLD (Paliyath et al., 2003; Tiwari and Paliyath, 2011) with antimicrobial properties have been reported to improve shelf life of fruits and vegetables (Cheema et al., 2014). Hexanal is an FDA approved food additive with GRAS (generally regarded as safe) status and has an ORL-MAM LD50 of 3.7 g kg^{-1} (EAFUS, 2006). Extensive trials were conducted to study the effect of hexanal/hexanal containing formulations as preharvest sprays, postharvest dips and vapor, on postharvest quality and ripening in a number of fruits and vegetables including sweet cherry, apple, peach, guava, and tomato (Sharma et al., 2010; Cheema et al., 2014; Paliyath et al., 2015; Gill et al., 2016). These experiments were successful in improving the shelf life and visual attributes of produce without impairing fruit color development or compromising the flavour characteristics, while delaying ripening and senescence (Paliyath et al., 2003; Paliyath and Murr, 2007; Misran et al., 2015). One advantage of hexanal is that, being volatile, it can be applied as a vapor with great ease and convenience. The antimicrobial properties of hexanal vapor against major postharvest fungal pathogens in particular *Penicillium expansum, Botrytis cinerea, Alternaria alternata, Sclerotinia sclerotiorum, Colletotrichum gloeosporioides,* and *Monilinia fructicola* have been proven (Song et al., 1996, 2007; Fan et al., 2006; Thavong et al., 2010; Utto et al., 2008). Previously, hexanal vapor has also been tried to prolong the postharvest quality of longan (Thavong et al., 2010), apple (Lanciotti et al., 1999) and sweet cherry (Sharma et al., 2010).

The principal objective of the present study was to assess the feasibility of postharvest hexanal vapor treatments in extending the postharvest shelf life and quality of bell pepper fruit. The effectiveness of hexanal as vapor application at different concentrations on shelf life and fruit quality of bell peppers were evaluated by analyzing various fruit quality parameters including color, texture, firmness, respiration, weight loss, and the activities of various antioxidant enzymes.

2. Materials and methods

2.1. Plant material

Sweet bell pepper (*Capsicum annuum* L. cv. Felicitas) plants were hydroponically grown (7.4 plants m^{-2} on rock wool growing media) under natural light in a commercial greenhouse (Veri Hydroponics Inc., Exeter, Ontario) following standard production practices (OMAFRA, 2005). Fruits of uniform size and color at mature green stage were harvested and used for various experiments.

2.2. Post-harvest hexanal vapor treatment of bell pepper fruit

The effect of hexanal vapor treatments on storage life and ripening related-quality parameters of bell pepper fruit was investigated in laboratory- and commercial-scale experimental trials. The present study consisted of two experiments; a small laboratory-scale and a commercial-scale.

Experiment 1: Hexanal vapor treatment was carried out according to standard procedure with some modifications (Sharma et al., 2010). Peppers were treated with three different concentrations of hexanal vapor, 0.005%, 0.01% or 0.02% (w/w) and the control fruit were left untreated. Peppers were weighed and placed in 6 mil plastic bags prior to treatment and the bags were closed and sealed immediately after placing the required amount of hexanal (60 μL kg^{-1} for 0.005%, 120 μL kg^{-1} for 0.01% and 240 μL kg^{-1} for 0.02% respectively) measured into a beaker along with a strip of Whatman 3 filter paper to facilitate fast evaporation (~2 h for complete evaporation). Peppers were exposed to hexanal vapor for 18 h at room temperature. Untreated peppers were also stored for 18 h enclosed in plastic bags under similar conditions. After 18 h of exposure, plastic bags were removed, and thereafter control and treated fruit were stored separately at 12 °C and 90–95% relative humidity in refrigerated incubators for 21 d. For weight loss study, peppers were stored separately in containers under the same conditions. Fruit samples were withdrawn at 1, 7, 14 and 21 d of storage for analysis of various quality parameters such as fruit color, firmness, electrical conductivity, and respiration rate as outlined below. Fruit pericarp and flesh tissues were stored at −40 °C for antioxidant enzymes assays. Experiment was performed in a completely randomized design having three replications of 3 fruit each per treatment with a total of 180 fruit.

Experiment 2: In this experiment, fruit were treated with 0.01% (w/w) hexanal vapor as described above and compared with untreated control. Both treatment and control had three replications each and each replicate had 5 kg of peppers. After treatment, peppers were stored in a walk-in cold storage at 12 °C with 90–95% relative humidity for 21 d. Samples were withdrawn on 1, 7, 14 and 21 d for quality analysis.

2.3. Evaluation of shelf life quality during storage

Samples were removed on 1, 7, 14 and 21 d of storage and fruit were visually evaluated based on overall physical appearance. Fruit were also inspected for the presence of spoilage, microbial infections and injury or decay.

2.4. Fruit color and texture

Pepper fruit color was recorded (Minolta Chromameter, C*R*-300, Ramsay, N.J.) from three points at the equator region of each fruit and fruit firmness was measured using a penetrometer (Model FT-327, QA Supplies, Norfolk, VA, 7 mm probe) by pushing the penetrometer probe into the locular space of the whole fruit at the basal end of the pepper lobes.

2.5. Measurement of respiration

Treated and untreated fruit (3 fruit per container per replicate) were placed separately in 4 L glass bottles at room temperature and the bottles were sealed for an hour. The amount of carbon dioxide in the head space was measured by injecting 3 mL of head-space gas samples into an ADC infrared gas analyzer (Nortech Control Equipment Inc., Etobicoke, ON). The carbon dioxide produced was quantified by comparing to commercial standards.

2.6. Electrical conductivity

Electrical conductivity (EC) was measured from pericarp discs collected from 3 fruit each per replicate according to standard methods (Ahmed et al., 2010) with some modifications. Discs (10 mm diameter, 5 discs per fruit) were excised from fruit pericarp, washed in distilled water and incubated at room temperature for 2 h in 40 mL of distilled water with an initial 5 min agitation at 60 rpm. EC of the bathing