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Influence of wounding and aging on 1-MCP sorption and metabolism in fresh-cut tissue and cell-free homogenates from apple fruit

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ABSTRACT

Non-receptor sorption of 1-MCP has been reported for a number of materials including woods and cardboard, as well as fruit and vegetables. Sorption rates and capacity are enhanced in response to tissue processing (cutting), suggesting that surface area, wounding and epidermis removal are factors that potentially contribute to depletion of gaseous 1-MCP in static systems. A processing-induced increase in 1-MCP sorption has been studied using fresh-cut apple and apple cell-free homogenates (CFH) as model systems. Fresh-cut tissue from four apple cultivars showed high rates and capacities for 1-MCP sorption, and cultivar rankings for sorption were maintained in CFH. Apple tissue subjected to short-term aging (6 h) showed marked declines in 1-MCP sorption. Trimming the surface tissue of aged tissue resulted in a 90% recovery of sorption properties. CFH prepared from progressively aged apple tissue showed no decline in 1-MCP sorption, whereas direct aging of CFH resulted in declines. The data from aged apple tissue and CFH suggested that loss in tissue sorption was due to processes occurring at the cut surfaces. Anoxia (0.02 kPa O_2) suppressed sorption rates of fresh-cut apple tissue and apple CFH by 74 and 68%, respectively. Ascorbate reduced sorption rates in apple tissue and CFH by 82 and 65%, respectively. Sorption rates of tissue and CFH were reduced by 88 and 73%, respectively, by hypotaurine, an antioxidant that targets the hydroxyl radical. The data suggest that 1-MCP sorption by fresh-cut apple tissue is due to oxidative metabolism in response to wound-induced production of reactive oxygen species.

1. Introduction

1-Methylcyclopropene (1-MCP) is a potent inhibitor of ethylene action (Sisler, 2006) and has been applied to a diverse range of horticultural commodities to extend shelf-life and maintain postharvest quality (Watkins, 2006; Huber, 2008). The ability of 1-MCP to reverse the ethylene-induced triple response in *Arabidopsis* and to compete with [^{14}C]ethylene for binding to yeast-expressed *ETR1* or *ERS1 Arabidopsis* receptors provided direct evidence for 1-MCP interaction with ethylene receptors (Hall et al., 2000). The strong and reversible influence of elevated internal ethylene levels in reducing 1-MCP efficacy with fruit and vegetative systems provides additional evidence for interaction with common binding sites (Sisler et al., 2003; Apelbaum et al., 2008; Zhang et al., 2009).

Aside from interactions with ethylene receptors, 1-MCP has been shown to bind to non-target material including woods and corrugated cardboards through adsorption or physical entrapment (Vallejo and Beaudry, 2006; Ambaw et al., 2011). In plant systems, avocado fruit and oil (Dauny et al., 2003) and intact fruit and vegetables (Nanthachai et al., 2007; Ambaw et al., 2011) bind 1-MCP in quantities that exceed those expected based on numbers of ethylene receptors. The differential sorption of 1-MCP to different tissues of fruit and vegetables has been attributed to hydrophobic targets including lignin and high-methoxy pectin, both of which showed high 1-MCP sorption *in vitro* (Choi and Huber, 2009). High non-specific sorption of 1-MCP to fresh-cut tissue and cell-free homogenates (CFH) from apple fruit led to speculation that 1-MCP can be metabolized, possibly in response to wounding (Huber et al., 2010).

The objective of the present study was to further examine the potential role of 1-MCP metabolism as a mechanism for 1-MCP sorption to fresh-cut tissue and cell-free homogenates from a number of apple cultivars.

2. Material and methods

2.1. Plant material

'Honeycrisp', 'McIntosh', 'Cameo', and 'Jonagold' apple fruit (*Malus x domestica* Borkh.) were harvested from mature trees

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growing at the Cornell Research Orchard, Ithaca, at the preclimacteric stage (internal ethylene <0.05 $\mu L\,L^{-1}$). Fruit were packed in foam protection and shipped within 24 h to the postharvest facilities at the University of Florida. 'Delicious' apple fruit obtained from local retail sources were used for analyses of the effects of aging and oxidation on 1-MCP sorption to fresh-cut tissue and CFH.

2.2. 1-MCP sorption to fresh-cut tissue

Fruit were peeled using a vegetable peeler and the flesh tissue without core processed into cubes (23 mm × 23 mm × 23 mm) of approximately 10 g. Additional tissue was stored at −80 °C until analyzed for 1-MCP sorption to CFH. Processed fresh-cut tissue was placed in 244 mL Mason jars (one tissue piece per jar) and sealed with lids containing septa. A volume of stock gaseous 1-MCP was injected to yield a final concentration of around 841 $\mu mol\,m^{-3}$ (20 $\mu L\,L^{-1}$). Headspace 1-MCP concentrations were measured at hourly intervals for up to 6 h at 20 °C using gas chromatography as described below. Jars containing 1-MCP without fruit tissue were monitored as controls.

Rate data for 1-MCP consumption reflect values determined over the initial 2 h reaction period. The percentage of initial headspace 1-MCP consumed over the 6 h incubation represents 1-MCP sorption capacity.

2.3. 1-MCP sorption to cell-free homogenates

Cell-free homogenates (CFH) were prepared from fresh-cut apple tissue following the method of Huber et al. (2010) with some modifications. Frozen apple tissue (30 g) was homogenized with 90 mL of 250 $mol\,m^{-3}$ sodium 2-(N-morpholino) ethanesulfonic acid (Na-MES) at pH 5.5 for 45 s using a bench-top homogenizer at maximum speed. The homogenates were filtered through two layers of Miracloth. Twenty mL of CFH (equivalent to 5 g tissue fresh weight) were added to 244 mL jars. The jars were sealed with lids fitted with septa and gaseous 1-MCP provided at approximately 420.5 $\mu mol\,m^{-3}$ (10 $\mu L\,L^{-1}$). Jars containing 1-MCP and 20 mL Na-MES buffer served as controls. Headspace 1-MCP concentrations were monitored over 6 h at 20 °C. Rate data for 1-MCP consumption reflect values determined over the initial 2 h reaction period. The percentage of initial headspace 1-MCP consumed over the 6 h incubation represents 1-MCP sorption capacity.

2.4. Effect of tissue aging on 1-MCP sorption to fresh-cut tissue and CFH

Individual tissue pieces (10 g) of 'Delicious' apple in 244 mL jars were held in sealed jars with humidified air for 0, 3, and 6 h provided via a dual septa/needle flow system. At these intervals, the flow was terminated, the needles removed and the sealed jars provided with 841 $\mu mol\,m^{-3}$ 1-MCP. In separate experiments, 1.5 mm of surface tissue (5 g fresh weight equivalent) of fresh-cut cubes (27 mm × 27 mm × 27 mm; 15 g) after 6 h aging was removed using a razor blade. Peeled and unpeeled fresh-cut tissues aged for 6 h were placed in 244 mL jars. The jars were sealed and provided with 841 $\mu mol\,m^{-3}$ gaseous 1-MCP.

Other experiments examined the effects of exposure of fresh-cut apple tissue to multiple additions of 1-MCP. In the first treatment, individual tissue pieces of either 10 or 15 g were placed in 244 mL jars and provided with 841 $\mu mol\,m^{-3}$ or 1261.5 $\mu mol\,m^{-3}$ (30 $\mu L\,L^{-1}$) gaseous 1-MCP, respectively. After monitoring headspace 1-MCP for 6 h, the jars were opened and approximately 1.5 mm of surface tissue (approximately 5 g fresh weight) was trimmed from the aged 15 g sample using a razor blade. The aged 10 g sample was left intact. The jars containing 10 g of trimmed/untrimmed tissue were resealed and provided with 841 $\mu mol\,m^{-3}$ gaseous 1-MCP.

1-MCP sorption of CFH from aged fresh-cut tissue was examined with 'Delicious' apple. Fruit were processed into 10 g fresh-cut pieces (23 mm × 23 mm × 23 mm) and aged for 0, 3, and 6 h. CFH were prepared by homogenizing 30 g (three tissue pieces) of fresh-cut tissues with 90 mL of 250 $mol\,m^{-3}$ Na-MES, pH 5.5. Twenty mL of CFH were placed in 244 mL jars. The jars were sealed and provided with 420.5 $\mu mol\,m^{-3}$ 1-MCP. Headspace 1-MCP concentrations were monitored for 6 h at 20 °C. 1-MCP metabolism in aged CFH was examined by placing 20 mL of freshly prepared CFH from 'Delicious' apple tissue in 244 mL jars and exposing to humidified air for 0, 3, and 6 h. At these intervals, the jars were sealed and provided with 420.5 $\mu mol\,m^{-3}$ gaseous 1-MCP. In all of the experiments described above, headspace 1-MCP concentrations were monitored for 6 h at 20 °C.

2.5. Influence of pO_2 and antioxidants on 1-MCP sorption of fresh-cut apple tissue and CFH

Fresh-cut apple pieces ('Delicious', approximately 80 g) were placed in 244 mL jars. The jars were sealed and headspace O_2 measured for 6 h at 20 °C using gas chromatography as described below. The influence of hypoxia on 1-MCP sorption was determined for both fresh-cut tissue and CFH. Fresh-cut tissue (5 g) and CFH (20 mL, 5 g tissue equivalent) were placed in 244 mL Jars. The jars were sealed and hypoxic conditions (0.02 kPa O_2) generated by purging the jars with 100 kPa N_2 for 20 min via two needles inserted through septa in the lids. Control samples received a 20 min purge using compressed air. Oxygen in samples was measured using a sensor (CheckMate 9900; PBI Dansensor, Ringsted, Denmark). Gaseous 1-MCP was provided to each sample at 420.5 $\mu mol\,m^{-3}$. Jars containing 1-MCP without fresh-cut tissue or with boiled CFH served as controls.

The influence of antioxidants on 1-MCP sorption was determined with fresh-cut pieces and CFH from 'Delicious' apple. Freshly prepared apple tissue (10 g) was immersed for 3 min in 360 $mol\,m^{-3}$ hypotaurine (2-aminoethanesulfinic acid) or ascorbate (Sigma–Aldrich, St. Louis, MO). Control samples were immersed in distilled water. Excess liquid was removed by blotting on Whatman #4 filter paper. After transfer to 244 mL jars, the jars were sealed and provided with 841 $\mu mol\,m^{-3}$ gaseous 1-MCP. Freshly prepared CFH (20 mL) were supplemented with 1 $mol\,m^{-3}$ hypotaurine or 4 $mol\,m^{-3}$ ascorbate. The jars were sealed and provided with 420.5 $\mu mol\,m^{-3}$ gaseous 1-MCP. Boiled CFH (20 mL) served as controls. Headspace 1-MCP concentrations were monitored for 6 h at 20 °C.

2.6. 1-Methylcyclopropene and oxygen measurement

For 1-MCP analysis, headspace (1 mL) from jars containing apple tissue or CFH was analyzed using a FID gas chromatograph (Varian CP-3800, Walnut Creek, CA) equipped with a stainless steel column (2 m × 2 mm) packed with Chromosorb 103 [particle size 112–149 μm (60/80 mesh)] (Ohio Valley Specialty Chemical Inc., Marietta, OH). Injector, oven, and detector were maintained at 125, 125, and 150 °C, respectively. The flow rates of carrier gas (N_2), H_2, and air were 0.5, 0.5 and 5 $mL\,s^{-1}$, respectively. Headspace removed at each measurement was replaced with 1 mL of air. Isobutylene, which has a FID response similar to that of 1-MCP (Jiang et al., 1999) was used as a calibration standard.

For headspace O_2, samples (4 mL) were withdrawn and analyzed using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a thermal conductivity detector (TCD). Via an automated sample-loop and valve system, a 1 mL portion of the injected sample for O_2 determination passed through