Induction of Volatile Compounds in Broccoli by Postharvest Hot-Water Dips

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Volatile emissions from heads of fresh broccoli (*Brassica oleracea* L. Italica group cv. Paragon) following hot-water treatments were studied to identify indicators of stress and causes of off-odors. Hot-water treatments of 45 °C for 10, 15, or 20 min and of 52 °C for 1, 2, or 3 min prevented yellowing of broccoli. However, the injurious treatment of 52 °C for 3 min enhanced off-odor development and caused visual damage to flower buds. Hot-water treatments increased the production of ethanol, 1-propanol, 1-hexanol, *cis*-3-hexen-1-ol, hexyl acetate, *cis*-3-hexenyl acetate, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and methyl thiocyanate (MTC) following treatment. Injury in broccoli treated at 52 °C for 3 min could be detected 2 h after treatment by 370- and 27-fold increases in headspace concentrations of ethanol and *cis*-3-hexen-1-ol. Ethanol headspace concentrations >100 μmol·m^{-3} 24 h after treatment could be used to identify phytotoxic heat treatments in broccoli. Olfactory detection of gas chromatograph effluent determined that *cis*-3-hexen-1-ol, DMTS, and DMDS were responsible for off-odors that developed following the 52 °C/3 min treatment.

Keywords: *Brassica oleracea; heat stress; ethanol; off-odor*

INTRODUCTION

Hot-water dips of fresh broccoli following harvest can extend its shelf life by delaying yellowing and the development of decay (Forney, 1995; Kazami et al., 1991; Tian et al., 1996). However, extended treatments at temperatures of 50 °C or greater may induce injury to the broccoli. This injury is characterized by water soaking (Tian et al., 1996) and the production of an off-odor by the broccoli heads (Forney, 1995). The induced odor is not characteristic of odors evolved when broccoli is subjected to anaerobic conditions or decay organisms (Forney et al., 1991) but may reflect physiological injury of the broccoli caused by the hot-water treatment.

Indicators of physiological injury to broccoli or other plant tissues would be helpful to identify injurious postharvest or quarantine heat treatments resulting in lost quality or storage life. Chlorophyll fluorescence of broccoli florets was affected by heat treatments (Tian et al., 1996). The ratio of variable fluorescence to maximum fluorescence decreased immediately following hot-water treatments, but measurements did not differentiate between treatments that were considered optimum to delay yellowing and those that induced injury. Characterization of volatile compounds induced by hot-water treatments may provide insights into the physiological and organoleptic changes that may occur in treated broccoli. The objectives of this study were to (1) characterize volatile compounds produced by fresh broccoli following injurious and noninjurious hot-water treatments, (2) determine if induced volatile production could be used as an indicator of physiological injury, and (3) identify compounds responsible for the heat-induced off-odor in treated broccoli.

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MATERIALS AND METHODS

Plant Material. Broccoli (*Brassica oleracea* L. [Italica group] cv. Paragon) was obtained from a commercial grower on the day of harvest. Heads were trimmed to a length of 100−120 mm, and only heads free of decay were used.

Heat Treatments. In a previous study, hot-water treatments at 45 °C for 10−20 min and at 52 °C for 1 or 2 min delayed senescence and improved postharvest storage life of broccoli, whereas treatments at 52 °C for 3 min induced an off-odor (Forney, 1995). In this study, broccoli heads were immersed in water at 25 °C for 10 min (control), at 45 °C for 10, 15, or 20 min, or at 52 °C for 1, 2, or 3 min. Three heads of broccoli for each time/temperature combination were placed into a stainless steel cage with a mesh lid. The cage was placed into a 35 L insulated container filled with 25 L of distilled water. Temperature of the water was controlled by an immersion circulator (model E8; Haake, Berlin). Following treatment, excess water was removed from the heads by centrifugation at 500*g* for 2 min. To standardize storage conditions, heads then were placed in plastic bags perforated with four 4-mm holes and held at 20 °C in the dark. The experiment was replicated four times.

Quality Evaluation. Broccoli quality was evaluated daily following hot-water treatments. Hue angle as a measure of head color was determined using a Chromometer (model CR-200; Minolta, Japan) with an 8-mm measuring aperture as described by Forney (1995). Subjective rankings of odor were made using the criteria of Wang and Hruschka (1977), where 0 = normal and 10 = nauseating. In addition, observations of water-soaked flower buds were made as an indication of hot-water damage.

Collection of Volatiles. Volatiles were collected from each broccoli head 2, 24, and 72 h after treatment. To collect volatiles, each head was placed into a 4-L glass jar and sealed with a Teflon lid. The headspace over the broccoli was allowed to equilibrate for 1 h under a 100 mL·min^{-1} flow of purified

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air. A 100-mL sample of the headspace in the jar was then trapped onto 120 mg of Tenax GR 20/35 (Alltech Associates, Inc., Deerfield, IL) in a 100 mm × 6.4 mm (o.d.) glass tube. Volatiles for sensory analysis also were trapped on Tenax as described above except 6 L rather than 100 mL of headspace was trapped.

Volatile Analysis. Samples were analyzed on a Magnum gas chromatograph/mass spectrometer (GC/MS) system (Finnigan MAT, San Jose, CA) equipped with an LSC 2000 purge and trap concentrator (Tekmar, Cincinnati, OH). The valve and transfer line on the LSC 2000 were held at 170 °C. Traps were placed in the Tekmar 2000 and desorbed at 250 °C for 4 min directly onto a Supelcowax 10, 60 m × 0.53 mm column with a film thickness of 1 μm (Supelco Inc., Bellefonte, PA). The column flow rate was 10 mL·min^{-1} of helium. The temperatures of the transfer line from the GC to the ion trap and the ion trap were 180 and 220 °C, respectively. The column temperature was held at 40 °C for 2 min, increased to 120 °C at a rate of 16°·min^{-1}, increased to 240 °C at a rate of 15°·min^{-1}, and held at 240 °C for 3 min. Quantitation was done using single ions of external standards. All peak areas were normalized using the peak area of a 4-ng dodecane standard that was run on the day of the analysis. Retention indices (RI) of identified compounds were determined by analysis of an alkane series (C_8−C_{22}) by injection using the same GC/MS conditions described for the broccoli sample analysis. The retention times were fitted to RI (carbon number of alkane standard × 100) using a spline function (n − 15) (Halang et al., 1978) fitted with Genstat, version 5 (Payne et al., 1993). Alkane data bracketed most volatile compounds analyzed from the broccoli. The RI of acetaldehyde and dimethyl sulfide were determined by extrapolation from the fitted curve. Corrections were made for the retention time difference for low-boiling compounds sampled with the purge and trap concentrator versus normal liquid injection of the alkane standards.

Sensory Analysis. Compounds responsible for the aroma of the broccoli were identified using a GC equipped with an olfactory detector outlet (SGE, Austin, TX). Trapped volatiles were desorbed and analyzed under the same conditions described above for GC/MS analysis. However, the effluent from the GC column was split, allowing one-third to go to a flame ionization detector (FID) and the remainder to pass through the olfactory detector, where it was mixed with 100 mL·min^{-1} of humidified air. Peaks of odor active compounds were identified through effluent sniffing by the authors. Qualitative descriptors were assigned to odor active peaks. Retention index was calculated for both sniffed peaks and GC/MS peaks and used to identify mass spectra of odor active compounds.

Experimental Design. The experiment was conducted using a completely randomized block design. Blocks consisted of four harvests that occurred ~1 week apart. The effects of treatments on the log transformations of volatile chemical concentrations were analyzed separately for each sampling time using ANOVA (Payne et al., 1993). Means were separated using least significant difference (LSD) at $P < 0.05$.

RESULTS

Broccoli Quality. All hot-water treatments used in this study inhibited yellowing of the broccoli. Hue angle of treated broccoli remained between 120° and 130°, which represents a green color during 4 days at 20 °C following treatment (Figure 1). Control broccoli became yellow after 3 days as seen by the decrease in hue angle to 110°. Yellowing continued in controls, and after 4 days, the hue angle reached 100°.

Off-odor development in fresh broccoli was enhanced by hot-water treatments at 52 °C for 2−3 min (Figure 1). "Green, floral" odors not typical of fresh broccoli developed in these treatments. Broccoli treated at lower temperatures or for shorter times developed off-odors at a slower rate than broccoli treated at 52 °C for 2 or 3 min.

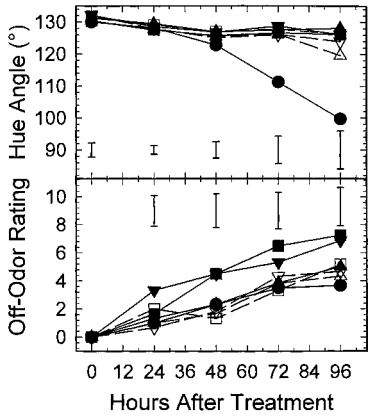

Figure 1. Changes in hue angle and off-odor rating in broccoli held at 20 °C for 96 h following dipping in water at 25 °C for 10 min (●, control), at 45 °C for 10 min (△), at 45 °C for 15 min (▽), at 45 °C for 20 min (□), at 52 °C for 1 min (▲), at 52 °C for 2 min (▼), or at 52 °C for 3 min (■). Vertical bars represent the LSD at $P < 0.05$.

Table 1. RI and Major Ion Peaks with Relative Abundance of Compounds Analyzed in the Headspace of Hot-Water-Treated Fresh Broccoli

RI[a]	compound	ions m/z (rel area)
748	acetaldehyde	43, 45 (92), 32 (65), 44 (19)
793	dimethyl sulfide	62, 47 (87), 45 (76), 46 (46)
950	ethanol	31, 45 (73), 47 (50), 46 (18)
1047	1-propanol	31, 32 (39), 43 (32), 45 (26)
1091	dimethyl disulfide	94, 45 (78), 79 (40), 46 (24)
1100	hexanal	44, 56 (82), 41 (65), 43 (53)
1213	limonene	67, 93 (64), 32 (63), 39 (54)
1246	*trans*-2-hexenal	41, 55 (83), 69 (70), 43 (56)
1266	hexyl acetate	43, 56 (59), 62 (40), 41 (39)
1295	methyl thiocyanate	73, 45 (91), 72 (61), 46 (43)
1329	*cis*-3-hexenyl acetate	43, 67 (63), 82 (23), 45 (22)
1341	*trans*-2-hexenyl acetate	43, 82 (60), 67 (49), 83 (46)
1354	1-hexanol	84, 56 (61), 41 (42), 69 (38)
1387	*cis*-3-hexen-1-ol	67, 41 (77), 39 (58), 82 (43)
1410	*trans*-2-hexen-1-ol	32, 57 (28), 41 (18), 45 (15)
1411	dimethyl trisulfide	126, 45 (56), 79 (39), 47 (22)

[a] Compounds were analyzed on a 60 m Supelcowax column with a GC/MS.

No visual damage from the hot-water treatments was observed in any of the broccoli except that treated at 52 °C for 3 min. In this treatment small numbers of water-soaked flower buds were observed 1 or 2 days after treatment. Damaged buds never exceeded 10% of the broccoli head.

Induced Volatiles. Postharvest hot-water treatments altered the volatile composition of fresh broccoli. Volatile content increased as treatment temperature or duration increased. Of 16 compounds that were monitored in all samples (Table 1), 10 compounds were identified that increased in concentration following treatment in hot water. These included four alcohols (ethanol, 1-propanol, 1-hexanol, and *cis*-3-hexen-1-ol), two esters (hexyl acetate and *cis*-3-hexenyl acetate), three sulfides [dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS)], and methyl thiocyanate (MTC). All 10 compounds were found at low concentrations in the control broccoli that was treated at 25 °C for 10 min, and concentrations of these compounds did not change significantly in controls during 72 h at 20 °C. The headspace concentrations of acetaldehyde, hexanal, *trans*-2-hexenal, and limonene

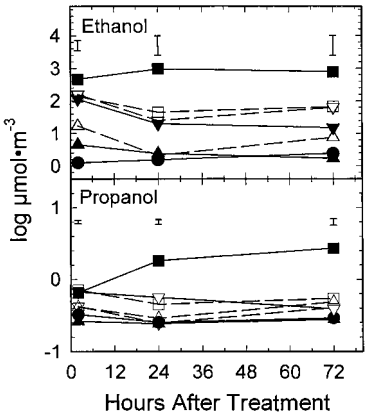

Figure 2. Headspace concentrations of ethanol and 1-propanol in broccoli held at 20 °C for 72 h following dipping in water at 25 °C for 10 min (●, control), at 45 °C for 10 min (△), at 45 °C for 15 min (▽), at 45 °C for 20 min (□), at 52 °C for 1 min (▲), at 52 °C for 2 min (▼), or at 52 °C for 3 min (■). Vertical bars represent the LSD at $P < 0.05$.

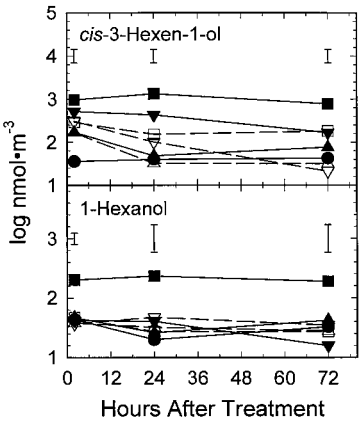

Figure 3. Headspace concentrations of *cis*-3-hexen-1-ol and 1-hexanol in broccoli held at 20 °C for 72 h following dipping in water at 25 °C for 10 min (●, control), at 45 °C for 10 min (△), at 45 °C for 15 min (▽), at 45 °C for 20 min (□), at 52 °C for 1 min (▲), at 52 °C for 2 min (▼), or at 52 °C for 3 min (■). Vertical bars represent the LSD at $P < 0.05$.

were not affected by hot-water treatments, and *trans*-2-hexenyl acetate and *trans*-2-hexen-1-ol were not detected in any sample.

Ethanol content increased the most of all compounds analyzed in the heat-treated broccoli. Two hours after the injurious treatment of 52 °C for 3 min, the headspace concentration of ethanol was 370-fold greater than that of the control broccoli (Figure 2). Ethanol concentrations peaked at 24 h, being 620-fold greater than the control. Ethanol production was stimulated by other noninjurious hot-water treatments but to a lesser extent than the 52 °C/3 min treatment. After 2 h, the headspace concentration of ethanol was ~100-fold greater than the control in broccoli treated at 52 °C for 2 min, at 45 °C for 20 min, and at 45 °C for 15 min. Even the milder treatments of 45 °C/10 min and 52 °C/1 min had 13- and 4-fold more ethanol than the control, respectively. However, the increase in alcohol concentration in these noninjurious treatments was reduced after 24 h. Ethanol concentrations in broccoli treated at 52 °C for 2 min, at 45 °C for 20 min, and at 45 °C for 15 min dropped to 13-, 29-, and 16-fold greater than the control, respectively, whereas elevated concentrations of ethanol were no longer detected in the 45 °C for 10 min and 52 °C for 1 min treatments. Acetaldehyde, the precursor of ethanol, was detected at low concentrations but was not significantly affected by the hot-water treatments. Propanol also increased due to heat treatment but not as dramatically as the increase observed for ethanol (Figure 2). Propanol content in the 52 °C/3 min-treated broccoli was 2-fold greater than in the control after 2 h and continued to increase to 9-fold greater after 72 h at 20 °C.

Hot water treatments increased the production of the C_6 alcohols *cis*-3-hexen-1-ol and 1-hexanol (Figure 3). *cis*-3-Hexen-1-ol, also known as leaf alcohol, was the most abundant of these alcohols in the broccoli. Concentrations of *cis*-3-hexen-1-ol in broccoli treated at 52 °C for 3 min was 27-fold greater than in the control after 2 h and peaked at 33-fold after 24 h. In addition, concentrations of *cis*-3-hexen-1-ol in the injurious 52 °C/3 min treatment were 2- and 3-fold greater than all other treatments after 2 and 24 h, respectively. Less severe treatments also increased *cis*-3-hexen-1-ol. Concentrations after 2 h were 14-fold greater in broccoli treated at 52 °C for 2 min and 8-fold greater in broccoli treated at 45 °C for 20 min and at 45 °C for 15 min than in the control. Even the milder treatments of 45 °C/10 min and 52 °C/1 min increased *cis*-3-hexen-1-ol content 5-fold greater than the control. However, these increases in the noninjurious treatments were reduced 24 h after treatment, similar to that observed for ethanol. Heat-induced production of 1-hexanol was similar to that of *cis*-3-hexen-1-ol, except concentrations were ~5-fold less. 1-Hexanol concentration in the 52 °C/3 min-treated broccoli was 5-fold greater than the control after 2 h and increased to 12-fold greater after 24 h. In addition, concentrations of 1-hexanol in the 52 °C/3 min treatment were 4- and 5-fold greater than the noninjurious treatments after 2 and 24 h, respectively. Other heat treatments had no effect on 1-hexanol concentration after 2 h but did increase it by as much as 3-fold after 24 h. *trans*-2-Hexen-1-ol was not detected in any of the broccoli samples.

The C_6 aldehydes, *cis*-3-hexenal, *trans*-2-hexenal, and hexanal are potential C_6 alcohol precursors. *trans*-2-Hexenal was detected at concentrations of 1.5 μmol·m^{-3} 2 h after treatment and decreased to 0.7 μmol·m^{-3} after 72 h. Similarly, hexanal concentrations averaged 0.75 μmol·m^{-3} 2 h after treatment and decreased to 0.53 μmol·m^{-3} after 72 h. However, neither *trans*-2-hexenal nor hexanal concentrations were affected by the hot-water treatments. *cis*-3-Hexenal was not detected in the control or treated broccoli. The instability of *cis*-3-hexenal under the analysis conditions used in this study makes it difficult to conclude if it was present in broccoli samples.

C_6 acetate esters were found in the broccoli, and their production was enhanced by the hot-water treatments. *cis*-3-Hexenyl acetate concentrations were 2−6-fold greater in the hot-water-treated broccoli than in the control 2 h after treatment (Figure 4). This difference increased to >200-fold after 72 h primarily due to a decrease in the concentration in the control treatment over time. Unlike other heat-induced volatiles, the highest concentrations were not found in the broccoli treated at 52 °C for 3 min. Heat treatments also increased hexyl acetate concentrations (Figure 4). Concentrations were 0.3-fold greater in the 52 °C/3 min and 52 °C/2 min treatments after 2 h than in the control. After 72 h, hexyl acetate concentrations in the broccoli treated at 52 °C for 3 min and at 45 °C for 15 min were 2-fold greater than in the control. All heat-treated

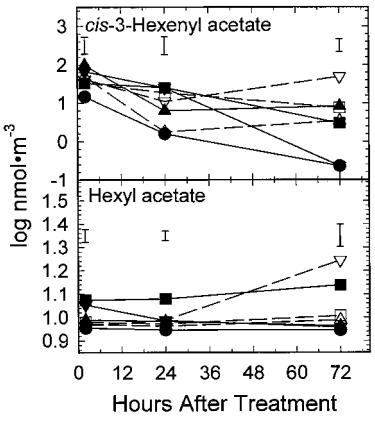

Figure 4. Headspace concentrations of *cis*-3-hexenyl acetate and hexyl acetate in broccoli held at 20 °C for 72 h following dipping in water at 25 °C for 10 min (●, control), at 45 °C for 10 min (△), at 45 °C for 15 min (▽), at 45 °C for 20 min (□), at 52 °C for 1 min (▲), at 52 °C for 2 min (▼), or at 52 °C for 3 min (■). Vertical bars represent the LSD at $P < 0.05$.

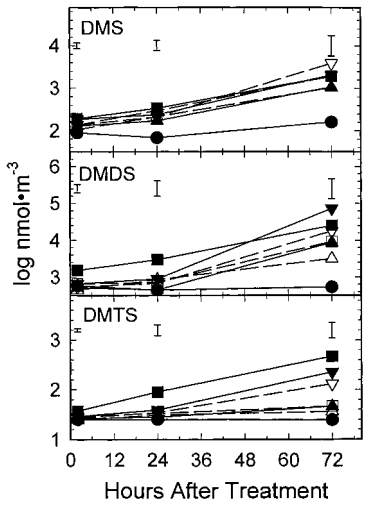

Figure 5. Headspace concentrations of DMS, DMDS, and DMTS in broccoli held at 20 °C for 72 h following dipping in water at 25 °C for 10 min (●, control), at 45 °C for 10 min (△), at 45 °C for 15 min (▽), at 45 °C for 20 min (□), at 52 °C for 1 min (▲), at 52 °C for 2 min (▼), or at 52 °C for 3 min (■). Vertical bars represent the LSD at $P < 0.05$.

broccoli maintained higher concentrations of hexyl acetate throughout the experiment than the controls. *trans*-2-Hexenyl acetate was not detected in broccoli samples.

The hot-water treatments had less of an effect on the sulfides, DMS, DMDS, and DMTS, 2 or 24 h following treatment than on the alcohols (Figure 5). Unlike the rapid increase of the alcohols following treatment, the sulfides increased steadily during the 72 h holding period following treatment. After 72 h, DMS concentrations in broccoli treated at 45 °C for 15 min were ~25-fold greater than the control, whereas concentrations in the broccoli treated at 52 °C for 3 min, at 52 °C for 2 min, and at 45 °C for 20 min were 12-fold greater. DMDS concentrations increased ~140-fold in the 52 °C/2 min treatment and from 15- to 50-fold in the 52 °C/1 min, 45 °C/20 min, 45 °C/15 min, and 52 °C/3 min treatments. DMTS concentrations were 20-, 10-, and 6-fold greater in the 52 °C/3 min, 52 °C/2 min, and 45 °C/15 min treatments than the control, respectively.

MTC concentration was ~4-fold greater in the broccoli treated at 52 °C for 3 min 2 h after treatment and ~9-fold greater 72 h after treatment than in the control (Figure 6). The remainder of the treated broccoli had MTC concentrations similar to those of the controls after 2 and 24 h. After 72 h, MTC concentration in the broccoli treated at 52 °C for 2 min, at 45 °C for 20 min, and at 45 °C for 15 min was 3-fold greater than that in the control.

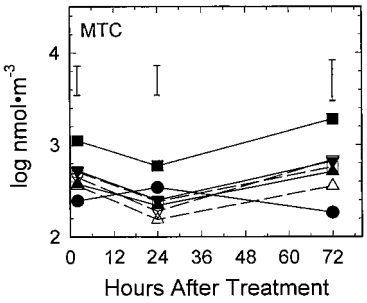

Figure 6. Headspace concentrations of MTC in broccoli held at 20 °C for 72 h following dipping in water at 25 °C for 10 min (●, control), 45 °C for 10 min (△), 45 °C for 15 min (▽), 45 °C for 20 min (□), 52 °C for 1 min (▲), 52 °C for 2 min (▼), or 52 °C for 3 min (■). Vertical bars represent the LSD at $P < 0.05$.

Sensory Properties of Volatiles. The most intense odors detected through sniffing volatiles were from broccoli that had been treated at 52 °C for 3 min. The most intense compound had an RI of ~1390 and was described as "floral", "green", and "hay-like". The second most intense compound had an RI of ~1410 and was described as "sulfury" and "cooked or processed". The third most intense compound had an RI of ~1085 and was described as "floral", "sulfury", and "green". None of these odors were detected through sniffing samples collected from control broccoli treated at 25 °C for 10 min.

DISCUSSION

Physiological Significance of Volatiles. Ethanol was the best indicator of physiological injury of all the volatiles that were induced by hot-water treatments. Enhanced production of ethanol was observed within 2 h after treatment, and production increased with severity of the treatment. The 52 °C/3 min treatment, which caused injury to the broccoli, induced 3-fold more ethanol 2 h after treatment than the noninjurious treatments tested. This concentration difference continued to increase, as ethanol increased in the injurious treatment and decreased in the noninjurious treatments, becoming >20-fold after 24 h.

Ethanol concentrations >100 μmol•m^{-3} in broccoli 24 h after treatment could provide a good indicator of permanent physiological injury to broccoli. Templeton and Colombo (1995) measured headspace ethanol concentrations in bags containing heat-stressed black spruce seedlings and reported that ethanol concentration correlated with loss of root growth, foliage viability, and terminal bud viability. They suggested that ethanol headspace concentrations >41.6 mmol•m^{-3} (1000 ppm, v/v) could be a good parameter to assess seedling quality prior to planting. The lower concentration of headspace ethanol measured in broccoli could be a result of lower rates of ethanol synthesis in herbaceous plants compared to woody plants (Harry and Kimmerer, 1991). In addition, headspace concentration in this study was measured under a dynamic system in which equilibrium headspace was sampled under a flow of 100 mL•min^{-1},

whereas Templeton and Colombo (1995) sampled static headspace from tree seedlings after overnight incubation. S. J. Colombo (Ontario Forest Research Institute, Sault Ste. Marie, Ontario, personal communication, 1998) suggests that anaerobic conditions experienced by seedlings during storage prior to incubation also may have contributed to these higher ethanol concentrations. The relatively rapid and large increase in ethanol production in broccoli damaged by hot-water treatment could make ethanol a useful indicator of physiological injury and could be used to identify injurious heat treatments. This would be useful in developing nonphytotoxic heat treatments to inhibit senescence and decay or to meet quarantine requirements.

The production of ethanol by plants may be induced by many different stresses in addition to heat including anaerobiosis, SO_2 fumigation, freezing, crushing, and water stress (Forney et al., 1991; Hansen et al., 1992; Kimmerer and Kozlowski, 1982). The mechanism by which ethanol production is induced by these stresses may be mediated by a common mechanism involving the mitochondrial membranes and oxidative phosphorylation. Under anaerobic conditions, the lack of oxygen inhibits oxidative phosphorylation resulting in the conversion of pyruvate to acetaldehyde and ethanol. Hot-water treatments could induce anaerobic conditions by increasing oxygen depletion through increased respiration rates and decreased oxygen solubility in the cells. Other stresses also may inhibit oxidative phosphorylation by damaging mitochondrial membranes. The primary responses of plant cells to heat is the denaturation of proteins and membrane perturbations (Ho, 1987). Similarly, other stresses may result in chemical or physical alterations of mitochondrial membranes resulting in ethanol formation.

Acetaldehyde is the precursor to ethanol in fermentation. However, in this study very low levels of acetaldehyde were detected in control and treated broccoli and treatments had no significant effect on its concentration. This could be due to a rapid conversion of acetaldehyde to ethanol not allowing its accumulation. In addition, due to the high volatility of acetaldehyde, it may not have been efficiently retained by the Tenax traps, resulting in inaccurate estimations of actual acetaldehyde content.

Elevated concentrations of *cis*-3-hexen-1-ol and 1-hexanol also indicated physiological injury induced by hot-water treatments and could be an indicator of heat-induced membrane damage. *cis*-3-Hexen-1-ol is produced by a wide variety of plants, and rough handling or cutting increased the amount of emission (Arey et al., 1991). *cis*-3-Hexen-1-ol is a product of membrane breakdown and oxidation in which linolenic acid is hydrolyzed from membrane lipids by lipid acyl hydrolase, oxidized to form hydroperoxylinolenic acid by lipoxygenase, and cleaved into *cis*-3-hexenal and 12-oxo-*cis*-9-dodecenoic acid by hydroperoxide lyase; *cis*-3-hexenal is then reduced to *cis*-3-hexen-1-ol by alcohol dehydrogenase (Anderson, 1989; Charron et al., 1995; Hatanaka and Harada, 1973). Similarly, when linoleic acid is metabolized through this pathway, 1-hexanol is formed (Hatanaka et al., 1987). Linolenic and linoleic acids comprise about 47 and 20%, respectively, of the fatty acid composition of phospholipids in broccoli flower buds (Makhlouf et al., 1990). The 10-fold or greater difference in 1-hexanol and *cis*-3-hexen-1-ol concentrations reflects this difference in substrate concentration and may also be influenced by differences in enzyme specificity. Linolenic acid is a preferred substrate over linoleic acid by lipoxygenase in tea leaves (Hatanaka et al., 1987). Environmental stress including anoxia in broccoli (Hansen et al., 1992), bruising of lettuce (Arey et al., 1991) and tea leaves (Saijo and Takeo, 1975), homogenizing *Brassica* bud and leaf samples (Tollsten and Bergsröm, 1988), and high light levels and temperatures in tea leaves (Hatanaka et al., 1987) induced increased concentrations of C_6 alcohols and C_6 aldehydes. Alcohol dehydrogenase activity that is apparent from the rapid production of ethanol could rapidly convert hexanal and *cis*-3-hexenal produced by lipid oxidation to 1-hexanol and *cis*-3-hexen-1-ol (Harry and Kimmerer, 1991; Hatanaka and Harada, 1973) and explain why little C_6 aldehyde accumulated in the stressed broccoli tissue.

Acetate esters of the C_6 alcohols that were found in the hot-water-treated broccoli were present in quantities similar to those of the alcohols. *cis*-3-Hexenyl acetate was the most abundant ester, followed by hexyl acetate. Arey et al. (1991) reported that *cis*-3-hexenyl acetate was the most dominant volatile emitted from many crops grown in California's Central Valley. Cauliflower and other *Brassica* vegetables produced *cis*-3-hexenyl acetate and hexyl acetate, and quantities increased when plant tissues were homogenized (Wallbank and Wheatley, 1976). It is believed that these C_6 acetates are products of the lipid breakdown by the lipoxygenase system (Charron et al., 1995).

The sulfides did not increase dramatically in concentration until 72 h after treatment. This may indicate that their accumulation was a secondary reaction resulting from altered metabolism or cellular disruption caused by the heat treatments. Chin and Lindsay (1993, 1994) demonstrated that DMS, DMDS, and DMTS concentrations increased at a linear rate following homogenization of fresh cabbage. They suggest that the slow appearance of these compounds could be attributed to the fact that they are the secondary enzymic reaction products of the primary C−S lyase action on *S*-methylcysteine sulfoxide. Maceration of cauliflower florets (Wallbank and Wheatley, 1976) and *Brassica napus* buds and leaves (Tollsten and Bergsröm, 1988) stimulated the production of DMDS. Concentrations of DMTS also increased when buds of several *Brassica* species were macerated (Tollsten and Bergsröm, 1988), indicating that cellular disruption stimulated sulfide production. In addition, these sulfides accumulated when broccoli was held under anaerobic atmospheres (Forney et al., 1991; Hansen et al., 1992).

DMS is a major component in cooked cabbage (MacLeod and MacLeod, 1968) but is found in low concentrations in fresh cabbage (Chin and Lindsay, 1993). The heat treatments in this study may have helped to induce the production of DMS, which was at low concentrations in the control treatment.

MTC also demonstrated a rapid increase following heat treatment and may be useful as a stress indicator for broccoli and other *Brassica* plants. The relative increase in concentration in the 52 °C/3 min-treated broccoli was similar to that of *cis*-3-hexen-1-ol, except its concentration was less after 24 h. MTC has been reported in broccoli, and its synthesis increased when broccoli was held under anaerobic conditions (Hansen et al., 1992). The mechanism for the formation of MTC has not been determined. Organic and inorganic thio-

cyanates are formed when glucosinolate-containing plant tissues are crushed (Larson, 1981). Indol-3-ylmethylglucosinolates comprise ~20% of the glucosinolates in fresh broccoli (Hansen et al., 1995) and are believed to release thiocyanate ions when cells are disrupted. Heat also has been reported to break down glucosinolates in *Brassica* vegetables, causing a release of thiocyanate ions (Slominski and Campbell, 1989). An accumulation of thiocyanate ions could become toxic to the plant cell, making it necessary for the plant to remove these free ions. This could occur through the methylization of the thiocyanate ions to form MTC.

Sensory Properties of Volatiles. Hot-water treatments of 52 °C for 3 min were reported to induce a nontypical "floral-like" odor (Forney, 1995). The most intense odor detected through effluent sniffing was described as having a "floral" and "green" type odor and had an RI similar to that of *cis*-3-hexen-1-ol. The odor of *cis*-3-hexen-1-ol was described as a green, herbaceous, leafy odor (Fenaroli, 1975) and may be a major contributor to the off-odor induced by injurious hot-water treatments. In addition, compounds with RI similar to those of DMTS and DMDS were also more intense during sniffing of GC effluent. DMTS is described as having a penetrating odor reminiscent of fresh onions and is a major component in the aroma of cooked *Brassica* vegetables (Fenaroli, 1975). DMDS has a sulfury odor that is reminiscent of rotting cabbage. These compounds appear to be responsible for the heat-induced off-odor that is formed when tissue is damaged.

Conclusion. Injurious heat treatments of fresh broccoli induce the production of numerous volatile compounds, 10 of which were identified in this study. The most prominent of these compounds is ethanol. Ethanol could be used as a rapid indicator of physiological injury in broccoli or other crops following heat treatments. Other compounds that could serve as stress indicators include *cis*-3-hexen-1-ol, 1-hexanol, and MTC. Compounds contributing to off-odors of heat-treated broccoli include *cis*-3-hexen-1-ol, DMTS, and DMDS. Volatile emissions could be used as rapid indicators of physiological injury when heat treatments are developed to extend the storage life of fresh produce or to use as quarantine treatments.

ABBREVIATIONS USED

DMDS, dimethyl disulfide; DMS, dimethyl sulfide; DMTS, dimethyl trisulfide; MTC, methyl thiocyanate.

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