

Effect of Pretreatment of Intact ‘Gala’ Apple with Ethanol Vapor, Heat, or 1-Methylcyclopropene on Quality and Shelf Life of Fresh-cut Slices

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ABSTRACT. ‘Gala’ apples [*Malus sylvestris* (L.) var. *domestica* (Borkh.) Mansf.] were treated with ethanol vapor (5 mL·kg⁻¹ fruit for 24 hours at 25 °C), heat (4 days at 38 °C and >98% RH), or 1-methylcyclopropene (1-MCP; 1 or 0.625 μL·L⁻¹ for 18 hours at 20 °C) before processing into slices, then dipped in anti-browning solutions or coatings, drained, and packaged in perforated polyethylene bags. Residual effects of pretreatments on fresh-cut slice physiological and quality attributes were investigated during storage for up to 19 days at 5.5 °C. Ethylene production was reduced by ethanol, heat, and 1-MCP pretreatments, while ethanol and heat also reduced slice respiration. Heat and 1-MCP pretreatments inhibited slice texture changes, while ethanol had no effect on instrumental texture measurements but reduced sensory firmness. Ethanol pretreatment increased the contents of ethanol and ethyl esters in slices but reduced acidity, while heat reduced both acidity and aroma volatile levels. Both ethanol and heat pretreatments led to lower sensory scores for apple flavor and ethanol-pretreated slices also received higher scores for altered flavor, although all scores were in the acceptable range. Slice acidity was best maintained by 1-MCP pretreatment. Shelf life based on appearance was 15 to 16 days for ethanol-pretreated slices and 12 days for heat-pretreated slices compared to that of control, which was 8 to 9 days, while 1-MCP pretreatment promoted decay development on the cut surface, which reduced the shelf life to 7 to 8 days. Obvious separations were determined between ethanol- and heat-pretreated slices and untreated control by canonical discriminant analysis of headspace volatile levels determined by GC and electronic nose. Therefore, pretreatments with ethanol and heat are very effective for prolonging visual shelf life at the expense of aroma quality.

Much research has been conducted in the last decade on processing and storage of fresh-cut fruit and vegetables. One goal is to help the industry to increase sales of fresh-cut fruit products. Fresh-cut fruit usually have a much larger cut surface, more surface water activity, and a shorter shelf life than fresh-cut vegetables, which have enjoyed billions of dollars in annual sales in the United States (Garrett, 2002). Various approaches have been tried to reduce microbial growth, discoloration, and other deteriorative events. Such approaches include surface treatments

with coatings or solutions (Bai and Baldwin, 2002; Baldwin et al., 1996; Saftner et al., 2003a), use of modified atmosphere (MA) packaging to maintain high relative humidity (RH) and appropriate gas atmospheres around the cut fruit (Bai et al., 2001; O’Bieme, 1990), low temperature storage, and other special treatments, such as methyl jasmonate, ethanol, and isopropanol vapors (Wang and Buta, 2003).

An alternative method would be to treat the intact fruit before cutting. This might be easily adaptable for industry if the methods, which have been shown to improve intact fruit quality and shelf life, provide a residual influence on the subsequent cut products. It is generally easier to treat intact fruits than processed products due to the stricter sanitation requirements once the peel barrier has been broken.

Application of ethanol vapor at various maturity stages inhibited tomato fruit ripening (Kelly and Saltveit, 1988; Saltveit and Mencarelli, 1988) without affecting subsequent quality (Saltveit

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Table 1. Treatment condition of whole fruit before cutting for three experiments.

Expt. (year)	1-MCP	Heat	Ethanol	Control
I (2000)	0 d → 18 h (1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, 20 °C) → 6.25 d (3 °C) ²	1d (1 °C) → 4d (38 °C) → 2 d (3 °C)	1d (1 °C) → 1d (ethanol 5 mL·kg ⁻¹ fruit, 38 °C) → 5 d (3 °C)	1d (1 °C) → 6 d (3 °C)
II (2001)	0 d → 18 h (0.625 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, 20 °C) → 6.25 d (1 °C)	1 d (1 °C) → 4d (38 °C) → 2 d (1 °C)	1 d (1 °C) → 1 d (ethanol 5 mL·kg ⁻¹ fruit, 38 °C) → 5 d (1 °C)	7d (1 °C)
III (2001)	0 d → 18 h (0.625 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, 20 °C) → 38.25 d (1 °C)	1 d (1 °C) → 4 d (38 °C) → 34 d (3 °C)	1 d (1 °C) → 1 d (ethanol 5 mL·kg ⁻¹ fruit, 38 °C) → 37 d (1 °C)	39 d (1 °C)

²Days before pretreatment, in pretreatment, and postpretreatment before cutting of whole fruit.

and Sharaf, 1992). For apple, ethanol vapor was used to reduce superficial scald, a postharvest physiological disorder mainly in Granny Smith apples (Chervin et al., 2001; Ghahramani and Scott, 1998; Scott et al., 1995) by inhibiting α -farnesene production and oxidation (Ghahramani and Scott, 1998). Apple is a climacteric-type fruit, and sensitive to ethylene. Wound ethylene, induced by cutting, would accelerate the deterioration of the cut products. Therefore, ethanol vapor pretreatment, as an effective method of ethylene inhibition, was considered a good candidate for an intact fruit treatment.

Heat has been used as a means to control surface microbes, as an insect disinfestation treatment, to delay ripening, and to alleviate pathological disorders for fruits and vegetables (Conway et al., 1994; Lurie, 1998; Lurie and Klein, 1992a; Lurie and Nussinovich, 1996). Ethylene synthesis by apple and tomato fruits was inhibited by heat treatment (Atta Aly, 1992; Biggs et al., 1988; Klein, 1989). Furthermore, fruit treated with heat did not respond to exogenous ethylene (Seymour et al., 1987; Yang et al., 1990). The softening of plum (Tsuji et al., 1984), pear (Maxie et al., 1974), avocado (Eaks, 1978), tomato (Biggs et al., 1988), and apple (Conway et al., 1994; Porritt and Lidster, 1978) was delayed by heating at 30 to 40 °C. A disadvantage of heat treatment is marked loss of volatiles; however, in apple fruit, volatile levels were recovered after an extended refrigerated storage (Fallik et al., 1997).

1-Methylcyclopropene (1-MCP) is an ethylene action inhibitor that reportedly binds to the cellular ethylene receptors and effectively inhibits ethylene responses in plants. 1-MCP inhibits ripening and senescence of many climacteric fruits such as apple (Baritelle et al., 2001; Fan and Mattheis, 1999; Rupasinghe et al., 2000; Saftner et al., 2003b; Watkins et al., 2000), apricot (Fan et al., 2000), banana (Golding et al., 1998; Sisler and Serek, 1997), pear (Baritelle et al., 2001; Lelievre et al., 1997), plum (Abdi et al., 1998), and tomato (Nakatsuka et al., 1997; Sisler and Serek, 1997). A potential disadvantage is that 1-MCP might adversely affect disease resistance (Bent et al., 1992; Ku et al., 1999; Porat et al., 1999) and ethylene-associated flavor volatile production (Rupasinghe et al., 2000). Since 1-MCP has been recently approved by the Environmental Protection Agency (2002) for postharvest use on apples, it is likely to be used commercially in the near future. 1-MCP has been shown to be beneficial for intact fruit, but its potential for fresh-cut products has not been explored.

In this research, we investigated the residual influence of three pretreatments (ethanol vapor, heat, and 1-MCP) for intact fruit on the subsequent fresh-cut product quality and shelf life.

Materials and Methods

'Gala' apples were harvested from a commercial orchard located in Wenatchee, Wash., in early Sept. 2000 and 2001, at the preclimacteric stage based on internal ethylene concentrations of

1.97 ± 1.38 (2000) and 0.96 ± 0.47 $\mu\text{L}\cdot\text{L}^{-1}$ (2002) (Watada and Massey, 1981). Defect-free fruit were randomly divided into four batches representing four treatments. Each batch had 30 fruit (Expt. I) for 2000, and 60 fruit (Expts. II and III) for 2001. The batch for 1-MCP pretreatment was immediately treated at USDA Tree Fruit Research Laboratory in Wenatchee, Wash., while other batches were stored in regular air at 1 °C. All fruit were shipped by refrigerated truck the next day to the USDA Citrus and Subtropical Products Laboratory in Winter Haven, Fla., with separate boxes for the 1-MCP-treated fruit. Fruit were then kept in regular air at 3 °C (2000) or 1 °C (2001), except during pretreatments, until processed (Table 1).

1-MCP pretreatment was performed by exposing fruit to initial concentrations of 1 (2000) or 0.625 $\mu\text{L}\cdot\text{L}^{-1}$ (2001) 1-MCP (EthylBloc, Floralife, Waltersboro, S.C.) at 20 °C for 18 h. The concentration of 1-MCP was analyzed using a gas chromatograph (GC) (HP 5880A; Hewlett Packard, Avondale, Pa.) fitted with an 80/100 mesh Parapak Q column (30 cm × 3.2 mm) and a flame ionization detector. The calibration gas was 1-butene (Gong et al., 2002). These levels were determined to be effective for intact apples and the maximum allowed by the U.S. Environmental Protection Agency (EPA) is 1 $\mu\text{L}\cdot\text{L}^{-1}$ (EPA, 2002).

Ethanol vapor pretreatment was applied by incubating fruit in a 30-L glass cylinder, sealed at the top and bottom with glass plates for 24 h at 25 °C. Five milliliters ethanol per kilogram of fruit was placed in an open beaker at the bottom of the jar. A strip of Whatman filter paper was extended within a perforated PVC pipe for the length of the glass cylinder with one end submerged in the beaker of ethanol to help diffuse the vapor throughout the enclosed container.

Heat pretreatment was accomplished by placing fruit at 38 °C with >98% RH for 4 d (Conway et al., 1994), using a controlled RH chamber (Blue M, Blue Island, Ill.). This would be considered a vapor heat treatment as opposed to other forms of heat treatments such as hot-water dip and hot air (Lurie, 1998).

Since the pretreatment of intact fruit was carried out in different places and took different times for each pretreatment, the subsequent processing of apples into slices was performed 7 d after harvest for Expt. I (2000) and Expt. II (2001) (Table 1). For Expt. III (2001), pretreatments were carried out at same time as Expt. II; however, the intact fruit were kept 32 d longer at 1 °C after pretreatment before processing into slices (and so, fruit were processed 39 d after harvest, Table 1) to determine if storage after pretreatment would minimize the pretreatment effect on the cut slices.

Processing of fruit into slices was carried out at 5.5 °C in a clean room where the processing area, tools, and gloved hands were cleaned by 70% ethanol. Apples (30) in each treatment were separated into three groups representing three replications. Each replicate of 10 apples was washed with 200 $\mu\text{L}\cdot\text{L}^{-1}$ hypochlorous acid (CIO supplied as sodium hypochlorite at pH 6.5) solution for 2 min, drained for ≈10 min, and hand-cut to 8 to 12 wedges with a

sharp stainless steel knife. After removal of the seed cavity, the slices, 20 to 25 g each, were placed into colanders and immediately dipped for 30 s in a soybean oil emulsion (Expt. I, year 2000) containing 0.8% isoascorbate, 0.8% calcium propionate, 0.4% acetylcysteine, 0.02% 4-hexyl resorcinol, 16.8% soybean oil (The Hain Food Group, Uniondale, N.Y.), 4.9% polyoxyethylenesorbitan monostearate (Tween 60; Sigma Chemical Co, St. Louis), and 3.6% sorbitan monostearate (Uniqema, Wilmington, Del.), all balanced with water. In 2001, a simplified aqueous dipping solution containing 0.8% isoascorbate, 0.8% calcium propionate, and 0.4% acetylcysteine was used because it had been found to be equally effective for retarding browning and maintaining firmness as the soybean oil emulsion used in Expt. I, when compared in another study (Bai and Baldwin, 2002). The slices were then allowed to drain for 1 h at 5.5 °C before placement into nine perforated polyethylene bags (20 × 18 cm, thickness 30 μm, with ten 1.5-mm holes), 10 slices per bag, and stored at 5.5 °C for up to 20 d. The headspace gas in the bags was monitored and CO₂ and O₂ partial pressures were similar to ambient atmosphere.

A subsample (one out of nine bags per replication) was observed daily for visual quality up to 19 d; six out of the nine bags were prepared for instrumental measurements and panel tests on day 0, 7, and 14 of storage (two bags for each sampling day); two out of the nine bags were prepared for some instrumental attributes at day 3 and 10. Three replicates were performed for all three experiments.

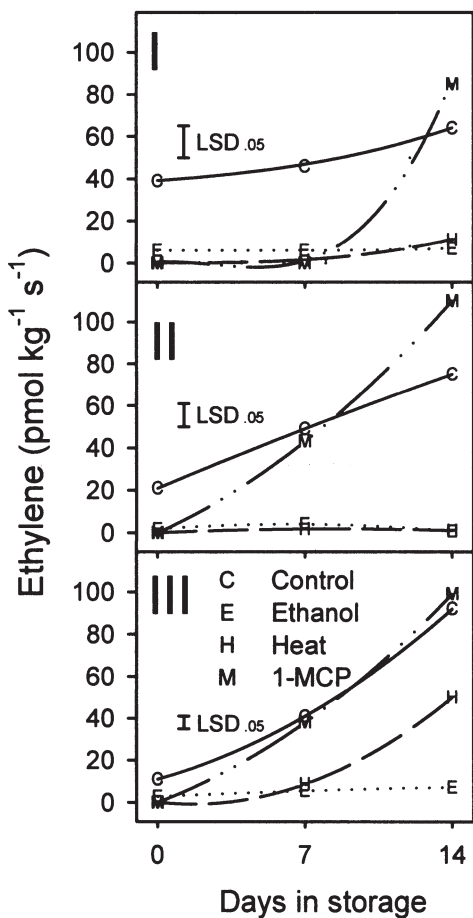


Fig. 1. Changes of ethylene evolution of fresh-cut apple during storage at 5.5 °C over three experiments (I, II, III) (n = 3). The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated before cutting.

Sensory analysis was carried out by an experienced panel of 15 members using a hedonic scale. Randomly coded samples were scored for visual quality, firmness, acidity, sweetness, apple-like flavor, altered flavor, and preference using a 9-point scale with anchor points (1 = low intensity or preference; 9 = high intensity or preference) at each end. For visual quality scales, a score of 5 (out of 10) is generally considered in postharvest studies to be the threshold level of acceptability. Obvious color change on cut surface and/or any visual microbial colony was evaluated as unacceptable. Altered flavor was interpreted as different from typical apple flavor, but not necessarily off-flavor. A score of 5 for preference or altered flavor was considered to be the threshold level for acceptability, with lower than 5 for preference and higher than 5 of altered flavor being considered unacceptable. The fruit treated by 1-MCP were not sampled by the panelists because the compound had not yet received EPA approval at the time of this experiment.

For respiration and ethylene production rates, 10 apple slices were removed from a bag to a 1-L sealed glass jar, and incubated for 30 min. Well-mixed headspace gas samples were obtained from the jar, and analyzed by GC (model HP 5890; Hewlett-Packard, Avondale, Pa.). The GC column was a CTR 1 (Alltech Associates, Deerfield, Ill.) with a thermal conductivity detector for O₂ and CO₂, and an activated alumina column with a flame ionization detector for ethylene.

Color of the cut surface was based on CIE L*, a*, b* and hue angle (H_{ab}) values using a white tile calibrated chromameter (model

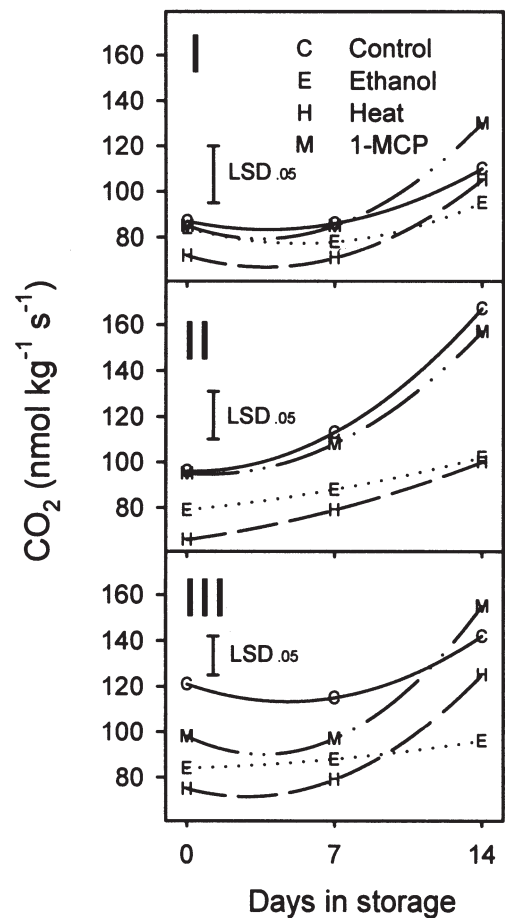


Fig. 2. Changes of respiration rate of fresh-cut apple during storage at 5.5 °C over three experiments (I, II, III) (n = 3). The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated before cutting.

CR-300; Minolta, Tokyo) with five slices per replication.

Firmness was determined using a texture analyzer (model XT2i; Stable Micro Systems, Godalming, U.K.), calibrated with a 5-kg weight and equipped with a 3-mm-diameter probe. A 10-mm-thick piece was obtained from the equatorial part of the wedge. The insert distance was 5.0 mm, with a stroke speed of 5.0 mm·s⁻¹. Four slices were measured per replicate bag, and the firmness was expressed in Newtons (N).

For headspace GC volatile and electronic nose analysis, 50-g apple slices were homogenized with 25 mL deionized water and 25 mL saturated NaCl solution. The homogenate were transferred to glass vials sealed with a crimp-topped Teflon-silicone septum, flash frozen in liquid nitrogen and stored at -80 °C before analysis. The sample prepared for headspace volatile analysis by GC consisted of 2 mL of homogenate in a 6-mL vial, and that for electronic nose consisted of 3 mL in a 10-mL vial. Sample vials were thawed under running tap water immediately before analysis.

For headspace GC analysis, sample vials were incubated at 80 °C for 15 min by a heating block in a headspace sampler (HS-6; Perkin Elmer, Norwalk, Conn.) before the headspace sample was injected into the GC. The analysis was carried out using a gas chromatograph (model 8500, Perkin Elmer) equipped with a 0.53 mm × 30 m polar Stabilwax capillary column (1.0-µm film thickness; Restek, Bellefonte, Pa.) and a flame ionization detector. Oven temperature was held 40 °C for 6 min, then raised to 180 °C at a rate of 6 °C/min. The compounds were identified by comparison of retention times with those of authenticated standards and by enrichment of

apple homogenate with authentic compounds. Concentrations were calculated by using regression equations, obtained by five different concentrations of each standard (Bai et al., 2002).

For electronic nose analysis, a Fox 4000 system (Alpha MOS, Toulouse, France) was used, fitted with 18 metal-oxide sensors, some with coated surfaces. The electrical output from the sensors was measured at 0.5-s intervals. Samples were incubated in an agitator at 500 rpm and 40 °C for 2 min before the headspace sample (500 µL) was taken from the vial and injected into the electronic nose. The carrier gas was pure air with a flow rate of 150 mL·min⁻¹. The electronic nose data acquisition program was a 2-min sampling time followed by an 18-min delay between samples for sensor recovery.

SAS version 8 (SAS Institute, Cary, N.C.) was used for analysis of data (SAS Institute, 1999). Sensory, cut surface color, firmness, respiration rate, ethylene production, and volatile concentrations were analyzed using the analysis of variance (PROCANOVA), mean separation was determined by the Duncan's multiple range test for each experiment. Electronic nose and headspace GC volatile data were analyzed using the canonical discriminant analysis (PROC CANDISC) for the three experiments combined.

Results and Discussion

In general, heat and ethanol pretreatments inhibited slice ethylene production (Fig. 1), respiration (Fig. 2) and discoloration [lower a* values (Fig. 3) and higher L* values (Fig. 4)] during 2 weeks

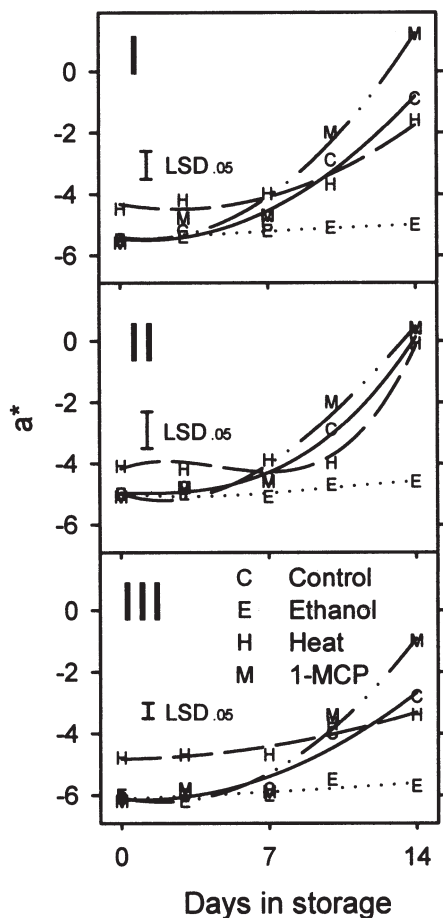


Fig. 3. Changes of CIE a* value on cut surface of fresh-cut apple during storage at 5.5 °C over three experiments (I, II, III) (n = 3). The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated before cutting.

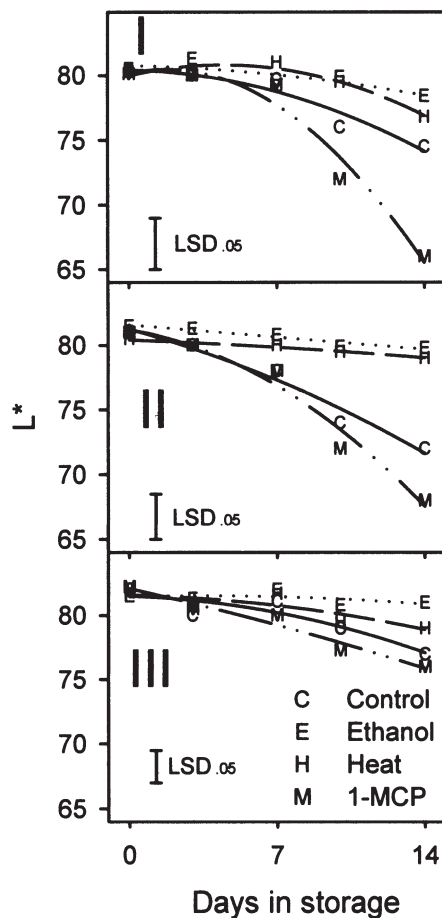


Fig. 4. Changes of CIE L* value on cut surface of fresh-cut apple during storage at 5.5 °C over three experiments (I, II, III) (n = 3). The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated before cutting.

of storage compared to nonpretreated control slices and 1-MCP pretreated slices. Titratable acidity and firmness generally declined during storage (Figs. 5 and 6), especially in nonpretreated control fruit and there were differences between treatments as denoted by LSD on figures. Initial acidity levels, however, were lower in ethanol and heat pretreated slices, and heat pretreated slices exhibited the lowest acidity during 2 weeks of storage. Nonpretreated control slices were generally less firm than pretreated slices, and 1-MCP-treated slices tended to be more firm, especially in Expt. III.

ETHYLENE. All of the pretreatments decreased ethylene evolution by cut slices to some extent (Fig. 1). The initial ethylene evolution immediately after cutting was low to undetectable in 1-MCP pretreated slices, however, ethylene synthesis increased sharply during storage, reaching control levels by day 7 for Expts. II and III and day 14 for Expt. I. 1-MCP has been reported to extend the shelf life of apple and many other climacteric fruits by inhibiting ethylene synthesis and action (Baritelle et al., 2001; Fan and Mattheis, 1999; Golding et al., 1998; Rupasinghe et al., 2000; Saftner et al., 2003b; Sisler and Serek, 1997). Heat-pretreated slices, however, also had very low ethylene evolution immediately after cutting (undetectable in Expts. I and II, and $\approx 1 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{s}^{-1}$ in Expt. III), which remained low throughout storage for Expts. I and II, but increased by day 14 in Expt. III, where the intact fruit were stored after pretreatment and before cutting for 32 d (Fig. 1). Apparently, the slices recovered some ability to produce ethylene in this case.

The mechanisms of heat inhibition of ethylene production are reportedly due to the heat sensitivity of ACC oxidase (Chan, 1986;

Paull and Chen, 1990) and ACC synthase (Biggs et al., 1988). ACC oxidase synthesis and mRNA also decreased due to heat treatment (Lurie et al., 1996). The inhibition of ethylene formation was reversed when the fruit are removed from heat (Biggs et al., 1988; Chan, 1991; Dunlap et al., 1990). In this study, the intact fruit used in Expt. III were removed from heat pretreatment and stored for 32 d at 1°C before processing (Table 1), therefore, ethylene evolution of the subsequent cut slices was higher than in Expts. I and II by day 14 (Fig. 1). Lurie and Klein (1992b) reported that the level of ethylene evolution from heat-treated apples could rise to higher levels than in nonheated fruit, given enough recovery time after removal from heat.

Ethanol pretreatment inhibited ethylene production to <20% of control levels during 14 d of storage at 5.5 °C (Fig. 1). Previous reports maintained that ethanol vapor pretreatment inhibited ethylene synthesis and action in tomato (Saltveit and Mencarelli, 1988; Yanuriati et al., 1999), although it could promote ethylene production at low concentrations (Beaulieu and Saltveit, 1997). A possible mechanism proposed by Beaulieu and Saltveit (1997) is that endogenous concentration of acetaldehyde is the biologically active factor that affects ethylene production, such that if high concentrations and/or long doses of ethanol are applied, there is a conversion of the excess ethanol to acetaldehyde. Ripening is then inhibited due to a reduction in activity of ACC oxidase. Conversely, if less-than-inhibitory concentrations of ethanol are applied, it appears to induce the synthesis of ACC (Mencarelli et al., 1991), or increase ACC synthase activity.

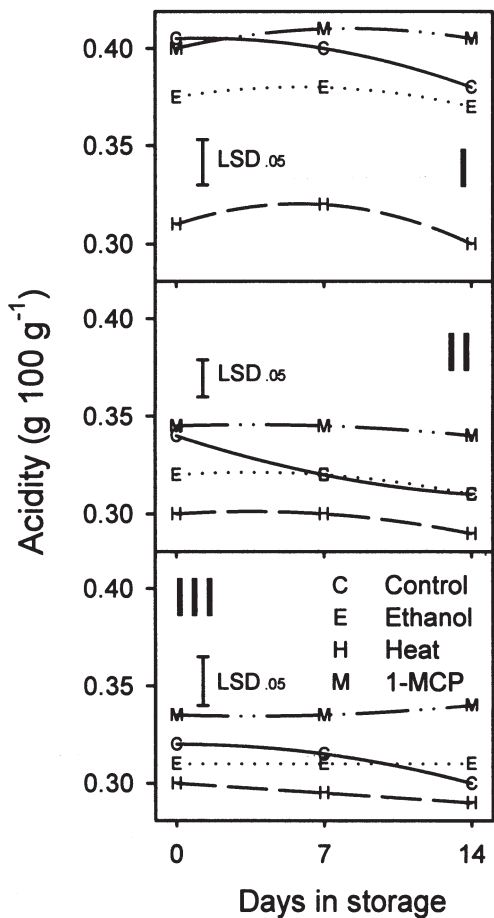


Fig. 5. Changes of titratable acidity content of fresh-cut apple during storage at 5.5 °C over three experiments (I, II, III) (n = 3). The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated before cutting.

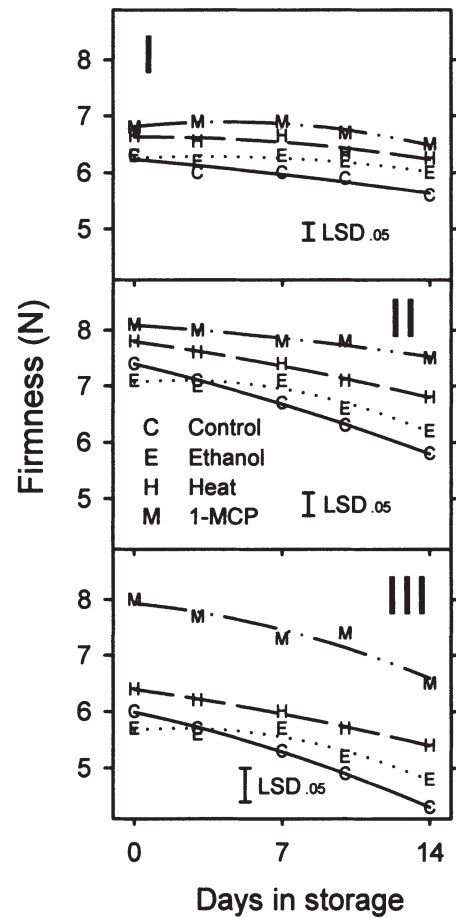


Fig. 6. Changes of flesh firmness of fresh-cut apple during storage at 5.5 °C over three experiments (I, II, III) (n = 3). The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated before cutting.

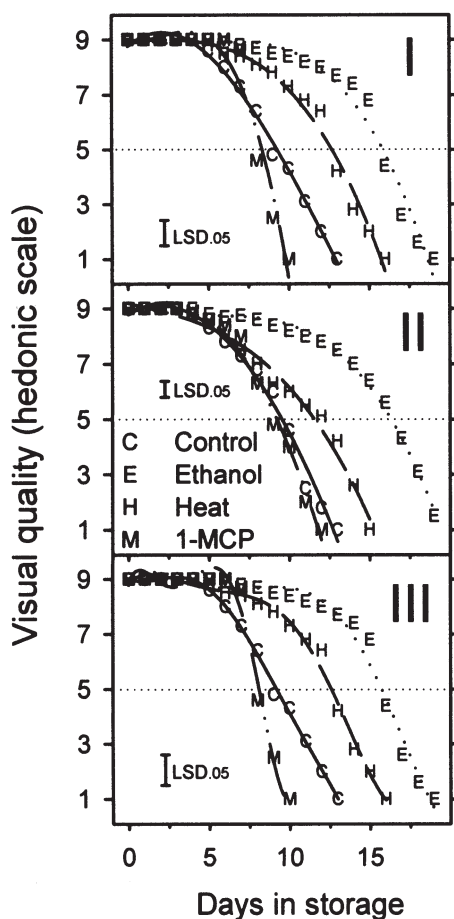


Fig. 7. Changes of visual quality of fresh-cut apples kept at 5.5 °C for 19 d over three experiments (I, II, III). The fruit were pretreated by ethanol vapor, heat, 1-MCP, or left untreated before cutting.

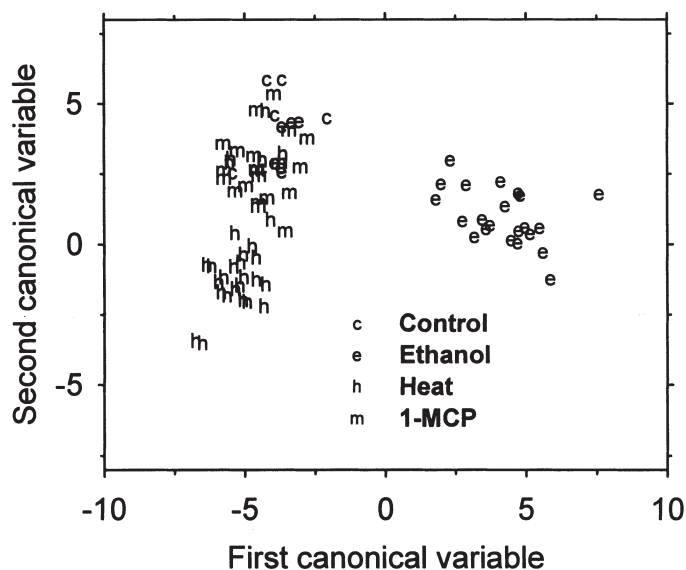


Fig. 8. Canonical discriminant analysis of headspace GC volatile concentration in fresh-cut apple. The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated (control) before cutting, and were represented in the figure as e, h, m and c, respectively [n = 3 experiments × 3 to 6 replications × 2 storage times (0 and 7 d at 5.5 °C)].

RESPIRATION. Respiration rates (Fig. 2) were significantly decreased by ethanol and heat pretreatments throughout the storage period. However, 1-MCP pretreatments generally did not significantly affect respiration rate (Fig. 2). Since 1-MCP has been reported to inhibit respiration rate in whole apple (Fan et al., 1999; Saftner et al., 2003b), a possible reason for the high respiration rate observed in the cut fruit could be due to the wound response. Wound-induced respiration, as opposed to ripening-induced respiration, might somehow circumvent the need for ethylene action or effects of 1-MCP.

APPEARANCE AND SHELF LIFE. The shelf life of ethanol- and heat-pretreated slices were 15 to 16 and 12 d, which was 7 (78% to 88%) and 3 to 4 d (33-50%) longer than that of the nonpretreated controls (8 to 9 d), respectively, based on visual observance (Fig. 7). All slices eventually became unacceptable due to discoloration and gray mold growth on the cut surface. The pretreatment of intact fruit with 1-MCP, however, shortened the shelf life to 7 to 8 d, which was 1 day shorter than controls (Fig. 7), and resulted in more serious discoloration and decay of the cut surface than observed in control slices. This appeared to be due to secondary browning, or discoloration caused by microbial growth. There is concern that decreases in ethylene sensitivity (such as with 1-MCP treatment) might adversely affect plant tissue disease resistance (Bent et al., 1992). Ethylene has been correlated with induction of a wide range of resistance responses, however, pretreatment of apples with ethanol or heat, which inhibited ethylene production, decreased disease incidence and severity compared to 1-MCP treated fruit and controls. Therefore, either only a small amount of ethylene is required to induce disease resistance, or additional factors about the 1-MCP treatment increased decay in the slices. Alternatively, both the heat and ethanol vapor treatments could be considered anti-microbial and may have reduced microbial loads on the fruit surface, which resulted in reduced microbial loads on the surface of the cut slices. This, however, does not explain why 1-MCP-pretreated slices had more decay than controls. Another possibility is that 1-MCP impaired the apple tissue defense system by some mechanism independent

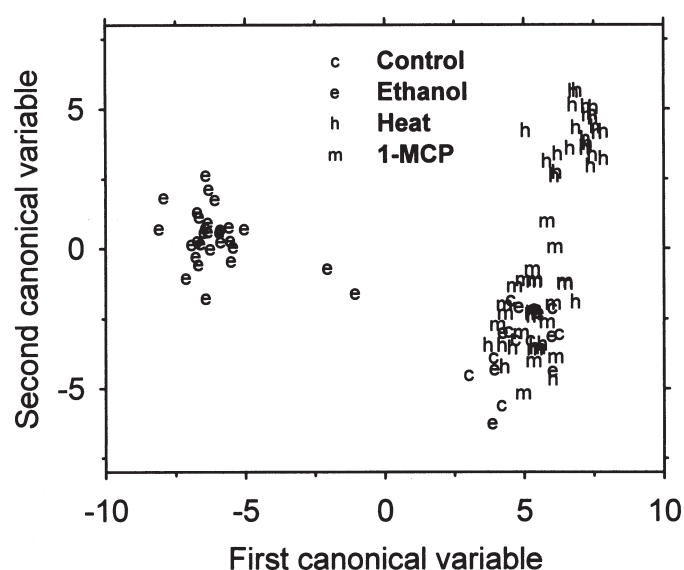


Fig. 9. Canonical discriminant analysis of electronic nose data of fresh-cut apple. The fruit were pretreated by ethanol vapor, heat, 1-MCP or left untreated (control) before cutting, and were represented in the figure as e, h, m, and c, respectively [n = 3 experiments × 3 replicates × 2 storage time (0 and 7 d at 5.5 °C)].

of ethylene synthesis and action. Itai et al. (2000) reported that, in Japanese pear, 1-MCP reduced expression of one out of three plant defense-related proteins evaluated. Interestingly, in a separate study in New Zealand with 'Braeburn' and 'Pacific Rose' apples pretreated with 1-MCP and then sliced, the results were similar for reduced ethylene with little effect on respiration (respiration slightly reduced for 'Braeburn' and no difference for 'Pacific Rose'), however, no increased decay susceptibility was observed in these varieties (Perera et al., 2003).

The severity of slice deterioration corresponded with chromameter measurements. Higher a^* (green to red) indicates more browning, whereas higher L^* values indicate lighter (whiter) color. No obvious browning occurred, due to the use of antibrowning agents (Bai and Baldwin, 2002), and discoloration was mostly due to mold growth (secondary browning), as 1-MCP pretreated slices lost shelf life after 7 to 8 d of storage (Fig. 7). Cut surface color did not change much in the first 7 d but remarkably discolored in the second week of storage with increasing a^* (Fig. 3) and decreasing L^* (Fig. 4) values for 1-MCP-pretreated and nonpretreated control slices. However, ethanol- and heat-pretreated slices maintained visual acceptance for 12 to 16 d (Fig. 7) which was reflected in fairly stable values for a^* (ethanol) and L^* (heat and ethanol, Figs. 3 and 4, respectively). Ethanol pretreated slices generally showed lower a^* and ethanol and heat pretreated slices showed higher L^* values than 1-MCP pretreated on nonpretreated control slices (Figs. 3 and 4). Meanwhile, 1-MCP-pretreated slices displayed similar or higher a^* and similar or lower L^* compared with untreated controls (Figs. 3 and 4). Slices from heat-pretreated fruit exhibited higher a^* (Fig. 3) and lower b^* (data not shown) initial values than control slices or slices from the other pretreated fruit, however, no color difference was visually observed. Lurie and Klein (1990) reported a decrease in chlorophyll content in apple peel due to heat treatment, and a similar decrease was supposed to have occurred in the flesh, although the flesh contains much less chlorophyll than that in peel.

TITRATABLE ACIDITY. 1-MCP-pretreated slices and nonpretreated controls exhibited the highest initial levels of titratable acidity, but control slices showed the greatest rate of acidity decline during 2 weeks of storage, while acidity levels were generally maintained in 1-MCP-pretreated slices. Initial levels for titratable acidity were lower for ethanol- and heat-pretreated slices compared to 1-MCP-pretreated and nonpretreated controls (Fig. 5). It is possible that the acids were used as respiratory substrate during pretreatment at 25 °C, and/or acids were used in ester synthesis due to metabolism of the excess ethanol, which is an ester precursor. Heat-pretreatment resulted the lowest initial acidity levels in slices compared to all other pretreatments and nonpretreated controls. Both ethanol and heat-treated slices showed a slight decline in acidity levels in the second week of storage (Fig. 5). None of the pretreatments had any effect on soluble solids levels in the subsequent slices (data not shown). Similar results were observed by Klein et al. (1990), Liu (1978), and Porritt and Lidster (1978), for heat-treated intact fruit.

AROMA VOLATILES. Overall, after 1 week of storage, ethanol-pretreated slices displayed the highest level of total volatiles, followed by nonpretreated controls and 1-MCP-treated slices (which were similar), with heat-pretreated slices having the lowest total volatile levels (Table 2). This agrees with earlier data published in a review by Beaulieu and Baldwin (2002), where slices dipped in ethanol did not have the same effect (reduced browning) as slices from fruit subjected to ethanol vapor. Ethanol pretreatment led to accumulation of ethanol and some ethyl esters in fruit

slices, especially ethyl acetate and ethyl propionate (Table 2). Storage of intact fruit for 32 d at 1 °C after heat treatment (Expt. III) did not result in volatile recovery (Table 2). Ethyl acetate, ethyl propionate, and ethyl butanoate increased during the first week of storage for nonpretreated controls, ethanol, heat, and 1-MCP-pretreated slices in some cases. For nonpretreated control slices, butyl acetate, 2-methylbutyl acetate, hexyl acetate, butyl butanoate, 2-methylbutyl-3-methylbutanoate, and butanol (seven out of the 12 volatiles measured) generally declined during the first week of storage, while ethyl hexanoate, butyl hexanoate, and ethanol levels generally did not change. For ethanol-pretreated slices, four out of the 12 volatiles measured declined, including hexyl acetate, butyl butanoate, 2-methylbutyl-3-methylbutanoate, and butyl hexanoate as well as ethyl hexanoate and 2-methylbutyl acetate (Expts. I and III). No volatiles declined over 1 week of storage for heat-pretreated fruit (except butyl butanoate and butanol in Expt. I), and in fact many increased. Nevertheless, butyl butanoate and 2-methylbutyl-3-methylbutanoate were only found at trace levels in Expts. I (methylbutanoate only) and II, and volatiles were generally low compared to the other pretreated slices. Many volatiles actually increased in the first week and 1-MCP-pretreated fruit exhibited a similar pattern of volatile trend during the first week of storage as did nonpretreated controls. The mechanism by which heat inhibits apple volatiles, especially the major apple aroma-esters, has not been reported. However, the disappearance of mRNA for fruit ripening genes and the accumulation of heat shock proteins (Lurie et al., 1996; Picton and Grierson, 1988) can affect volatile production. In addition, ethylene has been associated with volatile production in apples (Mattheis et al. 1991), and the heat pretreatment resulted in the lowest ethylene levels of all the pretreatments. This may be the reason that heat-pretreated slices exhibited the lowest volatiles levels as well.

Canonical discriminant analysis of headspace GC volatiles and electronic nose data over three experiments separated the ethanol-pretreatment slices from nonpretreated control slices and those from other pretreatments in the first canonical variable (Figs. 8 and 9), which had the most discriminatory power (77% for GC volatiles and 78% for electronic nose data). The second canonical variable, representing 22% of the variation for both GC and nose data, separated nonpretreated control slices from heat-pretreated slices based on GC headspace volatiles (Fig. 8) and electronic nose data (Fig. 9). The squared canonical correlation (R^2) values between first canonical variable and the actual GC and nose data was 0.94 for the headspace GC volatiles, and 0.97 for the electronic nose data. The squared canonical correlation (R^2) value between second canonical variable the GC and nose data was also high: 0.80 for the headspace GC volatiles, and 0.89 for the electronic nose data. The square distances between treatments were significant (Table 3). This information indicates that there should be flavor differences between ethanol-pretreated slices and the other pretreated and nonpretreated slices and between the heat-pretreated slices and the 1-MCP and nonpretreated control slices, based on aroma volatile profiles. This was somewhat supported by the sensory data, where apple-like rating was highest in nonpretreated controls and lowest in heat-pretreated slices, while altered flavor was highest in ethanol-pretreated slices (Table 4).

FIRMNESS. Instrumental measurement of slice tissue firmness revealed that slices from 1-MCP- and heat-pretreated fruit had higher firmness and softened more slowly over the storage period than nonpretreated controls, and in some cases, ethanol pretreated slices (Fig. 6). The same phenomenon was reported by previous

Table 2. Concentration of volatile components ($\mu\text{mol}\cdot\text{L}^{-1}$) in fresh-cut 'Gala' apples kept at 5.5 °C for 7 d. Intact fruit were treated with ethanol vapor, heat, 1-MCP, or left untreated before cutting.^z

Component	Pretreatment							
	Control		Ethanol		Heat		1-MCP	
	Day 0 ^y	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Expt. I								
Ethyl acetate	0.8 e ^x	77.9 c	257.4 a	185.0 b	0.4 a	9.8 d	1.8 e	14.7 d
Ethyl propionate	42.1 c	72.1 b	250.3 a	194.4 a	36.3 c	44.3 c	79.5 b	63.3 bc
Ethyl butanoate	1.3 c	12.2 a	1.5 c	9.4 a	1.3 c	4.0 b	1.4 c	7.1 ab
Ethyl hexanoate	0.08 b	0.09 ab	0.22 a	0.06 b	0.04 b	0.07 b	0.18 a	0.01 c
Butyl acetate	21.7 a	6.4 bc	9.7 ab	4.1 c	0.6 d	0.7 d	17.0 a	3.9 c
2-methylbutyl acetate	8.1 ab	3.8 c	15.1 a	3.5 c	1.4 d	1.0 d	5.6 bc	1.3 d
Hexyl acetate	1.1 a	0.2 b	0.8 a	0.1 b	0.01 c	0.01 c	0.9 a	0.03 c
Butyl butanoate	0.9 bc	0.3 d	2.6 a	0.6 c	1.2 b	0.6 c	1.3 b	0.1 d
2-methylbutyl								
3-methylbutanoate	1.0 a	0.2 b	0.7 a	0.1 b	tr	tr	0.8 a	0.1 b
Butyl hexanoate	0.27 ab	0.17 c	0.57 a	0.22 bc	0.13 cd	0.13 cd	0.27 ab	0.11 d
Ethanol ($\times 10^3$)	9.0 c	11.3 bc	50.6 a	49.1 a	6.9 d	10.7 c	18.6 b	11.4 bc
Butanol	24.0 a	4.5 c	13.0 b	2.3 d	5.8 c	2.1 d	46.1 a	3.9 cd
Total volatile abundance (peak height, $\times 10^4$)	5.2 c	7.0 b	24.0 a	21.4 a	3.2 c	5.0 c	8.6 b	6.8 bc
Expt. II								
Ethyl acetate	0.8 c	17.0 b	396.4 a	763.7 a	0.5 c	0.9 c	1.2 c	36.2 b
Ethyl propionate	0.2 e	0.5 de	192.3 b	289.1 a	0.7 d	2.2 d	1.2 d	15.9 c
Ethyl butanoate	1.3 cd	16.6 a	1.7 c	17.1 a	1.2 d	4.3 b	1.4 cd	10.8 ab
Ethyl hexanoate	0.59 a	0.67 a	0.56 a	0.55 a	0.21 b	0.47 ab	0.54 ab	0.43 ab
Butyl acetate	25.5 a	18.8 ab	6.8 c	14.7 b	0.1 d	0.1 d	22.0 a	15.0 b
2-methylbutyl acetate	6.0 ab	5.6 ab	8.3 a	10.7 a	0.5 c	1.0c	5.1 b	3.2 bc
Hexyl acetate	1.61 a	0.43 b	0.58 b	0.18 c	0.04 d	0.05 d	1.03 ab	0.17 c
Butyl butanoate	0.86 a	0.19 b	0.86 a	0.13 b	tr	tr	0.57 a	0.02 c
2-methylbutyl								
3-methylbutanoate	1.40 a	0.40 b	0.53 b	0.19 c	tr	tr	0.91 a	0.18 c
Butyl hexanoate	0.25 a	0.16 ab	0.25 a	0.15 ab	0.12 b	0.12 b	0.21 a	0.14 b
Ethanol ($\times 10^3$)	4.7 b	3.9 bc	54.0 a	58.4 a	1.1 d	2.4 cd	2.4 cd	4.8 b
Butanol	74.8 a	19.0 b	19.4 b	23.7 b	2.2 c	2.0 c	81.7 a	18.1 b
Total volatile abundance (peak height, $\times 10^4$)	3.6 b	4.2 b	25.6 a	33.6 a	0.7 d	1.7 cd	2.6 bc	4.0 b
Expt. III								
Ethyl acetate	1.1 c	20.5 b	394.6 a	403.4 a	0.8 c	1.4 c	0.9 c	15.8 b
Ethyl propionate	1.3 d	24.4 b	115.8 a	105.2 a	0.4 e	0.3 e	0.8 d	12.8 c
Ethyl butanoate	2.0bcd	14.6 a	2.2 bcd	13.1 a	1.3 d	3.5 bc	1.3 d	5.6 ab
Ethyl hexanoate	0.60 a	0.12 cd	0.38 ab	0.16 c	0.10 d	0.22 bc	0.26 b	0.11 cd
Butyl acetate	35.7 a	17.6 ab	18.5 ab	18.1 ab	0.2 c	0.6 c	17.1 ab	11.8 b
2-methylbutyl acetate	23.9 a	8.0 bc	22.8 a	11.1 b	0.2 f	0.7 e	6.7 c	2.6 d
Hexyl acetate	3.19 a	0.21 bc	0.53 b	0.12 c	0.01 e	0.03 e	0.46 b	0.09 d
Butyl butanoate	2.34 a	0.06 c	0.83 b	0.03 c	0.01 d	0.01 d	0.50 b	0.01 d
2-methylbutyl								
3-methylbutanoate	2.73 a	0.21 bc	0.48 b	0.14 c	0.02 d	0.01 d	0.43 b	0.04 cd
Butyl hexanoate	0.45 a	0.14 bcd	0.25 ab	0.14 bcd	0.11d	0.11 d	0.20 bc	0.12 cd
Ethanol ($\times 10^3$)	4.4 b	3.6 b	30.1 a	21.5 a	0.6 d	0.7 d	1.9 c	2.9 bc
Butanol	28.4 a	11.5 b	21.9 ab	13.5 b	4.0 c	3.6 c	32.7 a	12.4 b
Total volatile abundance (peak height, $\times 10^4$)	5.5 b	4.8 b	18.4 a	16.1 a	0.9 d	1.3 d	2.4 cd	3.0 c

^zPretreatments: ethanol vapor, 5 mL·kg⁻¹ at 25 °C for 24 h; Heat, static air with 38 °C and >98% RH for 4 d; 1-MCP, 1 (Expt. I) or 0.625 (Expts. II and III) $\mu\text{L}\cdot\text{L}^{-1}$ at 20 °C for 18 h.

^ySamples for day 0 were obtained 2 h later after cutting and antibrowning-sanitizing dipping.

^xAverage of three replications. Mean separation by Duncan's multiple range test at the 5% level in same row.

Table 3. Squared Mahalanobis distances between pretreatment means by GC headspace volatile and electronic nose data using cononical discriminant analysis.^z

Paarameter	Ethanol		Heat		1-MCP	
	D ²	Prob	D ²	Prob	D ²	Prob
GC headspace						
Control	75.7	<0.0001	27.5	<0.0001	7.2	0.0355
Ethanol			95.3	<0.0001	79.4	<0.0001
Heat					16.3	<0.0001
Electronic nose						
Control	136.6	<0.0001	60.6	<0.0001	7.0	<0.0001
Ethanol			183.2	<0.0001	137.2	<0.0001
Heat					35	<0.0001

^zPretreatments include ethanol vapor, 5 mL·kg⁻¹ at 25 °C for 24 h; heat, static air with 38 °C and >98% RH for 4 d; 1-MCP, 1 (Expt. I) or 0.625 (Expts. II and III) μL·L⁻¹ at 20 °C for 18 h.

D²: Pairwise squared distance to pretreatments.

Prob: probability > Mahalanobis distance for squared distance to pretreatments.

Table 4. Hedonic ratings for sensory attributes of of fresh-cut apples kept at 5.5 °C for 7 d. Intact fruit were treated with ethanol vapor, heat or left untreated and were cold-stored before cutting.^z

Expt.	Attribute	Pretreatment					
		Control		Ethanol		Heat	
		Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
I	Apple-like flavor	7.6 a ^y	7.1 a	6.0 b	6.1 b	4.9 c	5.6 bc
	Alter flavor	3.3 b	3.0 b	4.3 c	4.4 c	2.6 ab	1.5 a
	Acidity	4.2 a	4.8 a	4.3 a	4.5 a	3.8 a	4.6 a
	Firmness	8.1 a	7.6 a	7.4 a	7.4 a	6.2 b	5.9 b
	Preference	7.5 a	7.1 a	5.6 c	5.2 c	6.7 b	6.4 b
II	Apple-like flavor	6.9 a	7.1 a	6.4 b	5.9 b	5.7 b	5.7 b
	Alter flavor	3.3 a	3.5 a	4.3 b	4.4 b	3.3 a	2.8 a
	Acidity	5.2 a	5.2 a	4.5 b	3.9 b	3.8 b	4.3 b
	Firmness	6.7 a	7.0 a	5.4 b	4.0 b	6.3 ab	6.1 ab
	Preference	7.6 a	7.1 a	5.2 c	5.3 c	6.0 b	6.3 b
III	Apple-like flavor	8.1 a	7.5 a	5.2 b	5.8 b	5.6 b	5.7 b
	Alter flavor	1.3 a	2.4 a	4.8 b	3.4 ab	2.3 a	2.5 a
	Acidity	4.9 a	5.2 a	4.1 b	3.7 b	4.2 b	2.6 b
	Firmness	7.6 a	6.8 a	5.9 b	4.0 b	5.4 b	4.9 b
	Preference	8.1 a	7.1 a	5.2 b	6.0 b	5.9 b	5.5 b

^zPretreatments: ethanol vapor, 5mL·kg⁻¹ at 25 °C for 24 h; Heat, static air with 38 °C and >98% RH for 4 d; 1-MCP, 1 (Expt. I) or 0.625 (Expts. II and III) μL·L⁻¹ at 20 °C for 18 h.

^yAverage of 15 replications (panelists). Mean separation by Duncan's multiple range test at the 5% level in same row.

researchers for heat-treated intact fruit (Conway et al., 1994; Klein et al., 1990; Porritt and Lidster, 1978). Cell wall studies found less soluble pectin and more insoluble pectin in heat-treated fruit (Klein et al., 1990). Less calcium was present in the soluble pectin and more was bound to the cell wall in heat-treated fruit (Lurie and Klein, 1992a).

SENSORY. Only ethanol-pretreated, heat-pretreated, and nonpretreated control slices were tested for taste/ flavor since 1-MCP had not yet been approved at the time of the panel test, and testing was only done after 7 d of storage. By 14 d, only the ethanol-pretreated slices were still visually acceptable. The panel test results showed that the slices of nonpretreated controls were preferred over slices from the pretreated fruit, and that there was no change in any quality attribute during the first week of storage for any of the treatments (Table 4). This is interesting because levels of many important aroma components, such as the ethyl esters (Ueda et al., 1993; Young et al., 1996) changed during this time period (Table 2), as well as titratable acidity for nonpretreated controls (Fig. 5).

Ethanol pretreatment of intact fruit led to generally lower

ratings for apple-like flavor, acidity, and firmness (Expts. II and III) in the subsequent slices compared to nonpretreated control slices, and generally higher ratings for altered flavor than control and heat-pretreated slices (Table 4). The titratable acidity for ethanol-pretreated slices was lower than that of controls initially, but by day 7, control slices showed a similar level of acidity (Fig. 5). Aroma compounds were overall higher in ethanol-pretreated slices (especially ethanol and some ethyl esters) compared to controls (Table 2), explaining differences in apple-like flavor and altered flavor from control slices. Interestingly, even though firmness rating of the ethanol-pretreated slices was lower than that of controls in the sensory test (Table 3), instrumental analysis of firmness showed no difference or slightly lower firmness for ethanol-pretreated slices initially and slightly higher firmness after 7 d of storage compared to nonpretreated controls (Fig. 6).

Heat-pretreated slices were also generally scored lower for apple-like flavor, acidity, and preference than controls and these slices were, in fact, found to be lower in both aroma volatiles (Table 2) and titratable acidity (Fig. 5) compared to controls. It is interesting that the panelists rated heat-pretreated slices less firm

or the same as nonpretreated control slices when instrumental compression tests indicated that heat-pretreated slices were firmer. Conway et al. (1994) using compression tests found the heated apples to be tougher, while Lurie and Nussinovitch (1996), using Instron compression and shearing measurements, found heated intact apples to be crisper than nonheated control. These results indicate that the firmness, as felt by teeth and mouth, is a sensitive and complex trait that requires more than simple compression analysis to measure. In addition, panelists may confuse crispness and firmness. However, marketability of slices was determined to have a threshold level of 5, thus, all slices were still marketable after 1 week of storage.

In conclusion, our data show that pretreating intact 'Gala' apple with ethanol and heat prolongs shelf life of the fresh-cut products by slowing down respiration and ethylene production of cut slices, and inhibiting decay development on the cut surface, despite aroma loss and/or flavor changes. Pretreating intact apples with 1-MCP, while delaying acidity and firmness loss, promoted decay development and shortened the shelf life of 'Gala' apple slices.

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