EFFECT OF 1-MCP PRETREATMENT, CA STORAGE AND SUBSEQUENT MARKETING TEMPERATURE ON VOLATILE PROFILE OF ‘GALA’ APPLE

JINHE BAI AND ELIZABETH A. BALDWIN
USDA/ARS Citrus & Subtropical Products Lab.
600 Avenue S, N.W.
Winter Haven, FL 33881

JAMES P. MATTHEIS
USDA/ARS, Tree Fruit Research Lab.
Wenatchee, WA 98801

Abstract. ‘Gala’ apples pretreated or non-treated with 1-methylcyclopropene (1-MCP, 0.625 µL·L⁻¹) were stored under controlled atmosphere (CA, 2 kPa O₂ + 2 kPa CO₂) or regular air (RA) for 6 months at 1 °C. Aroma compounds (measured by gas chromatograph and electronic nose) were analyzed every month directly and after transferring to 20 °C for one week to simulate marketing conditions. The effect of 1-MCP was stronger than CA after transfer of fruit to room temperature. For electronic nose data, canonical discriminant analysis separated the storage treatments (1-MCP + CA, 1-MCP, CA, RA), indicating that the aroma profile was different in apples from each treatment. This was confirmed by GC analysis. Fruit lost most of the esters but not alcohols after transferring apples from 1 °C to 20 °C for all treatments. 1-MCP + CA inhibition of volatile production was greater, and did not show any benefit for maintaining firmness and acidity, compared to 1-MCP alone. Therefore, 1-MCP alone was the best treatment for storing ‘Gala’ apples with minimal loss of volatiles, while maintaining firmness and acidity.

Controlled atmosphere (CA) storage has been used for apple commercial storage for decades, and had been recognized as the second most practical and effective method, in addition to proper temperature management, for extending the shelf life of intact and fresh-cut fruit and vegetables (Schlimme and Rooney, 1994). Recommended CA conditions were given as 0 °C with 1-3 kPa O₂ and 1-5 kPa CO₂, with about 50% of U.S. apple production being stored under CA conditions (Kader, 1989).

One of the major effects of CA storage for prolonging shelf life of commodities is inhibition of ethylene production and decreased sensitivity when products are exposed to ethylene. Although most of the ethylene inhibitors, such as aminoxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG), have been found effective for prolonging storage life of commodities, but they can be toxic to fruit tissue and expensive. Sisler and coworkers (1996, 1999) have discovered a very effective ethylene action inhibitor—1-methylcyclopropene (1-MCP) during their searches for the ethylene receptor in plants. 1-MCP can be synthesized easily (Magid et al., 1971), and has been recently approved by Environmental Protection Agency (2002) for postharvest use on apples.

Researches using 1-MCP on apples showed positive results in inhibiting ripening and senescence through decreased ethylene production, respiration, softening, loss of titratable acidity, color change and other senescence-related metabolic events (Baritelle et al., 2001; Fan and Mattheis, 1999; Rupasinghe et al., 2000; Saftner et al., 2003; Song et al., 1997; Watkins et al., 2000).

In this study we report on the effect of 1-MCP on volatile profile of ‘Gala’ apple in comparison to apples stored in air or CA.

Materials and Methods

‘Gala’ apples (Malus × domestica Borkh.) were harvested from a commercial orchards located in Wenatchee, Wash. on 4 Sept. 2001. Defect-free fruit were randomly divided into two groups, one for 1-MCP treatment and one for a non-treated control. Pretreatment with 1-MCP was immediately per-
formed after harvest at 0.625 µL·L⁻¹ 1-MCP (EthylBloc™, Floralife, Inc., Waltersboro, SC) at 20 °C for 18 h at the USDA Tree Fruit Research Laboratory in Wenatchee, Wash. The initial concentration of 1-MCP was determined by gas chromatography (GC) (Gong et al., 2002). Both the 1-MCP-treated and non-treated groups were further divided into two batches, one for CA storage and another for regular air (RA) storage at 1 °C and 95% RH. The CA conditions were 2 kPa CO₂ and 2 kPa O₂.

Immediately after 1-MCP treatment, and also after each month of storage, fruit were shipped by refrigerated truck to the USDA Citrus and Subtropical Products Laboratory in Winter Haven, Fla., overnight with separate boxes for the 1-MCP-treated and non-treated fruit. Every shipment included 40 fruit for each treatment, 20 for immediate analysis of quality attributes, and another 20 fruit were transferred to 20°C and held for a week at that temperature, to simulate the marketing conditions.

Flesh firmness was measured on 20 individual fruit per treatment, with a penetrometer (FT 327, McCormick, Facchini, Alfonsine, Italy), equipped with a cylindrical plunger 11 mm in diameter. The measurement was obtained from equatorial area with the peel removed.

Titratable acidity (TA), headspace GC volatile and electronic nose analysis were determined using 5 composite replicates of 4 fruit each.

For TA content, 50 g of fruit tissue was blended with 50 mL deionized water at speed 4 for 45 s with a homogenizer (Model PT 10/35, Brinkmann Instruments, Co., Switzerland). The homogenate was vacuum-filtered through 25-50 µm Büchner funnel. The filtrates were titrated to pH 8.1 with 0.1N NaOH, and the acidity was calculated as malic acid on weight basis.

For headspace GC volatile and electronic nose analysis, 50-g apple slices were homogenized with 25 mL deionized water and 25 mL of a saturated NaCl solution. The homogenate was transferred to glass vials sealed with a crimp-topped Teflon-silicone septum, flash frozen in liquid nitrogen and was transferred to glass vials sealed with a crimp-topped Teflon-silicone septum, flash frozen in liquid nitrogen and stored at -80 °C prior to analysis. The sample prepared for enrichment of apple homogenate with authentic compounds.

For electronic nose analysis, a Fox 4000 system (Alpha MOS, Toulouse, France) was used, fitted with 18 metal oxide sensors, some with coated surfaces (SV/LG, SY/G, SY/AA, SY/Gh, SY/gCTl, SY/gCT, T30/1, P10/1, P10/2, P40/1, T70/2, PA2, P30/1, P40/2, P30/2, T40/2, T40/1, and TA2). 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 set as 2 min sampling time followed by an 18 min delay between samples for sensor recovery.

Data were analyzed using the general linear model (PROC GLM) and the canonical discriminant (PROC CANDISC) program, and mean separation was determined by the Scheffe’s test (SAS Version 8, SAS Institute, Cary, N.C.).

Results and Discussion

Total volatile abundance in GC FID response in RA control fruit increased slightly in the first two months and then declined consistently throughout storage at 1 °C (Fig. 1). The increase of volatiles indicates that the fruit continued ripening during storage, and the subsequent decrease indicates that the fruit was undergoing senescence. Matthes et al. (1998) reported similar pattern of ‘Gala’ volatile productions during storage.

However, there were significant drops of total volatile abundance after transferring fruit from 1 °C to 20 °C for one week (Fig. 1). The average decrease in total volatiles, caused by the increased storage temperature compared to volatile levels averaged over 6 months storage at 1 °C was 50% (Table 1) for RA control. The shorter the storage of fruit at 1 °C, the greater decrease of total volatile abundance after transferring to 20 °C (Fig. 1). Volatile accumulation, as measured in this research, depends on the production of volatiles by the fruit in relation to loss of volatiles due to off-gassing from the fruit. Since transferring apples from 1 °C cold storage to 20 ºC room temperature usually increases metabolism and the production of volatiles, the decreased volatile levels at 20 °C may have been due to increased evaporation.

There was much less total volatile abundance in the fruit from treatments of 1-MCP and CA compared to that of control (RA-stored fruit, Fig. 1). The magnitude of inhibition of 1-MCP was similar to that of CA during storage at 1 °C. Average total volatile abundance in apples from CA, and 1-MCP

![Fig. 1. Total volatile abundance (FID response peak height) of ‘Gala’ apples pretreated or not with 1-MCP and stored in CA or RA at 1 °C (Solid line), sub-samples were subsequently transferred to 20 °C for an additional week (Dotted line).](image-url)
treatments over the chilled storage period were 35% and 40% of that detected from RA fruit, respectively (Table 2). However, total volatile abundance in 1-MCP-pretreated fruit was lower than those stored in CA after transferring to 20 °C (Fig. 1), being 16% compared to 75% remaining after transfer to 20 °C, respectively (Table 1). Inhibition of total volatile abundance by the 1-MCP + CA combination treatment was the most severe compared to either treatment alone (Fig. 1, Tables 1 and 2).

CA and modified atmosphere (MA) is reported to decrease volatile production in most fruits, including apple (Guadagni et al., 1971; Mattheis et al., 1998; Patterson et al., 1974; Saftner et al., 2003), because metabolic activity is reduced. Production of lipid-derived volatiles is inhibited by low O₂, while volatiles that arise from amino acid catabolism are negatively impacted by high CO₂ (Brackmann et al., 1993; Fellman et al., 2000). The mechanism of how 1-MCP inhibits volatile production is not yet clear, but in many cultivars of apple, 1-MCP inhibits production of volatiles (Fan and Mattheis, 1999, 2001; Lurie et al., 2002; Rupasinghe et al., 2000) perhaps through inhibition of ethylene production and related events. Aroma production during fruit ripening is considered to be an ethylene-mediated response (Abeles et al., 1992), as evidenced by reduced aroma in 1-MCP-treated plums, and by the fact that propylene application restored aroma (Abdi et al., 1998).  

In apple fruit, esters are the most significant contributors to aroma (Mattheis et al., 1998; Plotto et al., 2000; Ueda et al., 1993). Of these, three esters (butyl acetate, hexyl acetate and 2-methylbutyl acetate) have been identified as characteristic aroma compounds of ‘Gala’ apples (Mattheis et al., 1998; Plotto et al., 2000; Young et al., 1996). Normally butyl acetate is the most abundant ester in RA stored ‘Gala’ apple. This volatile increased during first two months storage, then kept relatively consistent until a sharp drop was observed at the end of six months storage at 1 °C (Fig. 2); at same time, the flesh firmness declined to 48 N (Table 3), which is considered below commercial acceptability (Bai et al., 2002a; Ueda et al., 1993). Similar to the total volatile abundance, transfer of fruit to 20 °C caused butyl acetate to decline sharply to 27% of the amount present when the fruit were in cold storage (Fig. 2, Table 1). Butyl acetate levels in apples from CA, 1-MCP and 1-MCP + CA were 29%, 32% and 12% of the amount detected in RA fruit averaged over the 6 months cold storage, respectively (Table 2). Transferring the fruit to 20 °C for a week caused a further decline (Fig. 2, Table 1) in butyl acetate levels.

The effect of CA and/or 1-MCP treatments and subsequent storage temperature increase (from 1 to 20 °C) on hexyl acetate was similar to butyl acetate (Tables 1 and 2). The inhibition of 1-MCP treatment on butyl acetate and hexyl acetate levels was stronger than that of CA after transferring the fruit from 1 °C to 20 °C storage. The strongest inhibition was

### Table 1. Relative amount (%) of major esters, alcohols and total volatile abundance of ‘Gala’ apples after transferring from 1 °C to 20 °C for 1 wk compared to levels exhibited immediately after removal from cold (1 °C) storage (as 100%). Volatiles were analyzed every month directly from cold storage and after transferring to 20 °C for 1 wk, within storage period of 6 months at 1 °C. Averaged data over the 6 months were used.

<table>
<thead>
<tr>
<th>Component</th>
<th>RA</th>
<th>CA</th>
<th>1-MCP</th>
<th>1-MCP+CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyl acetate</td>
<td>27 ab**</td>
<td>48 a**</td>
<td>15 b**</td>
<td>31 ab**</td>
</tr>
<tr>
<td>2-methylbutyrate acetate</td>
<td>57 a*</td>
<td>55 a**</td>
<td>14 b**</td>
<td>14 b**</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>19 b**</td>
<td>55 a*</td>
<td>10 c**</td>
<td>63 a*</td>
</tr>
<tr>
<td>Butanol</td>
<td>119 a</td>
<td>102 a</td>
<td>21 b**</td>
<td>32 b**</td>
</tr>
<tr>
<td>2-methylbutanol</td>
<td>163 a**</td>
<td>163 a**</td>
<td>45 b**</td>
<td>62 b**</td>
</tr>
<tr>
<td>Hexanal</td>
<td>173 a**</td>
<td>159 a**</td>
<td>49 b**</td>
<td>43 b**</td>
</tr>
<tr>
<td>Total volatile abundance</td>
<td>50 a**</td>
<td>75 a*</td>
<td>16 b**</td>
<td>24 b**</td>
</tr>
</tbody>
</table>

*Mean values (n = 30) in same row that are not followed by the same letter are significantly different (p < 0.05).

### Table 2. Relative amount (%) of major esters, alcohols and total volatile abundance of ‘Gala’ apples from three treatments compared with that of the RA-stored control (as 100%) averaged over 1-6 months storage at 1 °C.

<table>
<thead>
<tr>
<th>Component</th>
<th>CA</th>
<th>1-MCP</th>
<th>1-MCP+CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyl acetate</td>
<td>75 a</td>
<td>67 a</td>
<td>67 a</td>
</tr>
<tr>
<td>2-methylbutyrate acetate</td>
<td>109 ab</td>
<td>109 ab</td>
<td>89 b</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>119 a</td>
<td>119 a</td>
<td>89 a</td>
</tr>
<tr>
<td>Butanol</td>
<td>157 a</td>
<td>157 a</td>
<td>123 b</td>
</tr>
<tr>
<td>2-methylbutanol</td>
<td>173 a</td>
<td>173 a</td>
<td>147 b</td>
</tr>
<tr>
<td>Hexanal</td>
<td>194 a</td>
<td>194 a</td>
<td>194 a</td>
</tr>
<tr>
<td>Total volatile abundance</td>
<td>113 a</td>
<td>113 a</td>
<td>113 a</td>
</tr>
</tbody>
</table>

*Mean values in same column that are not followed by the same letter are significantly different (p < 0.05).

### Table 3. Firmness and titratable acidity content of ‘Gala’ apples after 6 months storage at 1 °C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Firmness (N)</th>
<th>TA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (before storage)</td>
<td>75 a</td>
<td>0.33 a</td>
</tr>
<tr>
<td>RA</td>
<td>48 c</td>
<td>0.17 c</td>
</tr>
<tr>
<td>CA</td>
<td>58 b</td>
<td>0.26 b</td>
</tr>
<tr>
<td>1-MCP</td>
<td>67 a</td>
<td>0.28 ab</td>
</tr>
<tr>
<td>1-MCP+CA</td>
<td>72 a</td>
<td>0.31 a</td>
</tr>
</tbody>
</table>

*Mean values in same column that are not followed by the same letter are significantly different (p < 0.05).
the 1-MCP + CA treatment combination at 1 °C, with a relatively small loss after transferring the fruit to 20 °C probably because the volatile levels were already minimum, and there was nothing left to lose (Fig. 2, Tables 1 and 2).

CA and/or 1-MCP influence on 2-methylbutyl acetate showed a different pattern compared to butyl acetate and hexyl acetate. Sometimes 2-methylbutyl acetate was higher in fruit treated with CA and/or 1-MCP, and at other times, it was lower during storage at 1 °C (Fig. 3). There was no difference between the average 2-methylbutyl acetate levels in RA fruit and the treated fruit at 1 °C averaged over the cold storage period (Table 2), although transferring the fruit to 20 °C resulted in a greater loss of 2-methylbutyl acetate in 1-MCP-treated fruit, with or without CA, than those in RA and CA alone (Fig. 3, Table 1).

These results indicate that there was a difference in which 1-MCP and CA affected straight- and branch-chain esters. 2 kPa O\(_2\) + 2 kPa CO\(_2\), the CA condition in this research, offered a low enough O\(_2\) level to reduce lipid-derived esters (straight chain); but the CO\(_2\) level was not high enough to reduce amino acid-derived (branch-chain) ester production (Brackmann et al., 1993). On the other hand, 1-MCP influenced ester production in a similar way to low O\(_2\) at 1 °C.

Alcohols also play an important part in fruit aroma, as volatile compounds in their own right, and as substrates of ester volatiles. Figure 4 shows the changes of butanol concentra-


Fig. 7. Plots of canonical discriminant analysis from electronic nose data. 'Gala' apples were pretreated or not with 1-MCP and stored at CA or RA at 1 °C for 6 months, and subsequently transferred to 20 °C for 1 week. R = regular air; C = CA; M = 1-MCP; X = 1-MCP + CA.

First canonical variable (x 10^5) First canonical variable (x 10^5)
1°C 1 month 1°C 2 month + 20°C 1 week
First canonical variable (x 10^5) First canonical variable (x 10^5)
1°C 6 months 20°C 1 week

First canonical variable (x 10^5) First canonical variable (x 10^5)
1°C 6 months + 20°C 1 week