# Changes in Headspace Volatiles during Physiological Development of Bisbee Delicious Apple Fruit[†]

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Volatile compounds produced by Delicious apple fruit during the 8-week transition period from pre- to postclimacteric were identified using headspace sampling of intact fruit and GC–MS. As apple development progressed, concentrations of butanal, pentanal, (E)-2-hexenal, and heptanal declined. Concentrations of most alcohols were variable throughout the same period, but 2-methyl-1-butanol was present only in samples from the late harvests. Ketones lacked a discernible pattern, while some esters, most notably 2-methylbutyl acetate, appeared in samples before the onset of ethylene production. Acetic acid was present in the samples throughout the harvest period, indicating alcohol substrate availability may be the limiting factor in ester formation. Production of several acetate esters preceded the increased ethylene levels associated with the onset of apple ripening.

## INTRODUCTION

Optimum apple fruit quality following extended controlled atmosphere (CA) storage depends upon a number of factors including fruit developmental stage at harvest. To achieve optimum quality for apples after long-term (5–9 months) CA storage, fruit should be harvested as close as possible to the end of the preclimacteric period (Fidler et al., 1973). For commercial harvest to be initiated at this stage of development, maturity indices have been developed that attempt to identify progression through the maturation and ripening processes. These indices are based in part on fruit quality attributes (i.e., starch index, percent soluble solids, titratable acidity, flesh firmness, internal and external color) (Olsen, 1982; Lau, 1989). Because these quality attributes change gradually over a several week period, interpretation of the progression of ripening can be difficult.

Measurement of ethylene synthesis provides additional information pertaining to fruit physiological status. Ideally, apples are harvested as close as possible to the attainment of physiological maturity where ripening will progress normally after detachment from the tree (Watada et al., 1984). Measurement of fruit ethylene synthesis provides a physiological index that can indicate fruit development is too advanced to assure optimal storage performance (Blanpied, 1969).

Over 300 compounds that contribute to apple flavor and aroma have been identified from many different cultivars (Dimick and Hoskin, 1983). As fruit development progresses and ripening is initiated, the major volatiles produced by apples change. Aldehydes are the main group of volatile compounds detectable from intact immature apples (DePooter, 1987), whereas mature, ripening apples produce primarily esters and aliphatic alcohols (Flath et

al., 1967). Major volatiles are thought to change in conjunction with the initiation of the climacteric rise in respiration and ethylene synthesis (Brown et al., 1966). Because of the temporal association with attainment of physiological maturity, detection of changes in apple volatile synthesis may be useful as another index of maturity that reflects the current physiological stage of development (Heinz et al., 1965; Romani and Ku, 1966; Golias, 1986).

This study identifies volatiles other than ethylene produced by Delicious apple fruit during the transition from immature to mature stages of development. Along with data on volatile compounds, commercially important indices of maturity were also used for comparison.

## MATERIALS AND METHODS

**Plant Material.** Bisbee Delicious apples were harvested at weekly intervals in two orchards near Wenatchee, WA, beginning in mid-August 1990. Rootstock–scion combinations were Bisbee Delicious on seedling rootstock, planted in 1959, in orchard 1 and Bisbee Delicious on MM106 rootstock, planted in 1977, in orchard 2. Fruits were transported to the laboratory, where fruit quality analyses, volatile sample collection, and measurement of internal ethylene concentrations were performed within 6 h of harvest.

**Ethylene Analyses.** An 18-gauge stainless steel syringe covered with a rubber septum was inserted through the calyx end of the apple into the core cavity (Williams and Patterson, 1962). One-milliliter headspace samples collected using a gastight syringe inserted through the septum of the stainless steel syringe were analyzed by gas chromatography using a Hewlett-Packard 5880 GC with a glass column (30 cm, 0.32 cm i.d.) packed with 80–100-mesh Porapak Q. The column was held isothermally at 35 °C. Gas flows for $N_2$ carrier, $H_2$, and air were 30, 30, and 300 mL min$^{-1}$, respectively. Ethylene averages reported were calculated from analyses of 30 apples.

**Fruit Quality Analyses.** Apple firmness was measured using an EPT-1 electronic pressure tester (Lake City Technical Products, Kelowna, BC, Canada) equipped with an 11-mm tip. The extent of fruit starch hydrolysis was estimated after an apple horizontal section was stained with a 0.5% iodine solution. Total soluble solids and titratable acidity were measured on samples of freshly homogenized apple juice prepared using a Champion juicer. Total soluble solids content was measured using a hand-held refractometer (Atago N1) and titratable acidity calculated

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**Table I. Changes in Fruit Weight, Flesh Firmness (FF), Starch Index (SI), Total Soluble Solids (TSS), and Titratable Acidity (TA) of Bisbee Delicious Apples during Fruit Growth and Maturation in 1990[a]**

| harvest date | wt, g | FF, N | SI, 1–5 | TSS, % | TA, % malic acid |
|---|---|---|---|---|---|
| | | | Orchard 1 | | |
| Aug 14 | 134.1 ± 1.5 | 97.0 ± 1.5 | 1.2 ± 0.1 | 7.78 ± 0.06 | 0.338 ± 0.008 |
| Aug 21 | 166.1 ± 6.0 | 94.8 ± 1.4 | 1.2 ± 0.1 | 8.56 ± 0.09 | 0.330 ± 0.008 |
| Aug 28 | 149.7 ± 5.9 | 89.2 ± 1.3 | 1.3 ± 0.1 | 8.55 ± 0.06 | 0.296 ± 0.007 |
| Sept 4 | 178.2 ± 6.9 | 87.8 ± 1.1 | 1.3 ± 0.1 | 8.62 ± 0.08 | 0.304 ± 0.008 |
| Sept 11 | 191.9 ± 4.1 | 83.0 ± 1.0 | 1.5 ± 0.1 | 9.23 ± 0.08 | 0.294 ± 0.006 |
| Sept 18 | 211.5 ± 6.3 | 82.0 ± 1.0 | 1.6 ± 0.1 | 9.25 ± 0.09 | 0.299 ± 0.006 |
| Sept 25[b] | 227.2 ± 5.6 | 81.0 ± 1.0 | 1.8 ± 0.1 | 9.78 ± 0.13 | 0.280 ● 0.006 |
| Oct 2 | 218.4 ± 6.3 | 77.4 ± 0.7 | 2.3 ± 0.1 | 9.83 ± 0.11 | 0.286 ± 0.009 |
| | | | Orchard 2 | | |
| Aug 15 | 144.0 ± 4.2 | 90.1 ± 1.0 | 1.2 ± 0.1 | 8.06 ± 0.09 | 0.375 ± 0.013 |
| Aug 22 | 146.0 ± 4.9 | 89.8 ± 0.9 | 1.4 ± 0.1 | 8.49 ± 0.10 | 0.369 ± 0.012 |
| Aug 29 | 168.8 ± 3.3 | 84.1 ± 0.9 | 1.4 ± 0.1 | 8.83 ± 0.08 | 0.296 ± 0.003 |
| Sept 5 | 175.1 ± 4.5 | 81.7 ± 0.7 | 1.4 ± 0.1 | 8.73 ± 0.07 | 0.307 ± 0.008 |
| Sept 12 | 164.9 ± 6.7 | 79.4 ± 0.9 | 1.3 ± 0.1 | 8.40 ± 0.11 | 0.288 ± 0.007 |
| Sept 19[b] | 202.4 ± 6.1 | 79.5 ± 0.9 | 1.8 ± 0.1 | 9.67 ± 0.13 | 0.264 ± 0.006 |
| Sept 26 | 201.9 ± 4.6 | 77.0 ± 0.7 | 1.8 ± 0.1 | 9.97 ± 0.13 | 0.252 ± 0.005 |
| Oct 3 | 210.9 ± 4.8 | 75.6 ± 1.1 | 2.3 ± 0.1 | 10.90 ± 0.13 | 0.237 ± 0.007 |
| Oct 10 | 253.0 ± 6.3 | 72.9 ± 0.5 | 3.1 ± 0.1 | 11.51 ± 0.11 | 0.234 ± 0.005 |

[a] Values are means ± SE for analyses of 30 fruit. [b] Optimum harvest date for long-term CA storage based on starch and ethylene values.

following titration of 10 mL of juice to a malic acid endpoint (pH 8.2).

**Analysis of Headspace Volatile Compounds.** Three replicate samples (1 kg each, typically four–six apples) were placed into 4-L glass jars and the jars sealed using Teflon lids with two gas ports. Compressed air, purified via a column containing potassium permanganate, activated charcoal, calcium hydroxide, and molecular sieve, was passed through the jars at 150 mL min⁻¹ for 30 min and then decreased to 100 mL min⁻¹ for 5 min prior to sampling. Fruits were held at 21 °C during sampling. The volume of dynamic headspace collected from each replicate was 1 L.

Volatile compounds were adsorbed onto 50 mg of Tenax TA (35–60 mesh) packed into glass tubing (15 cm, 0.64 cm i.d.). Tenax was conditioned prior to use by Soxhlet extraction with methanol for 8 h and subsequent heating at 250 °C for 30 min with a flowing stream of nitrogen at 60 mL min⁻¹. After collection, volatile compounds were introduced into a GC–MS system (Farwell et al., 1979). Traps were inserted into a carrier gas loop constructed from Teflon-lined stainless steel tubing, Cajon screw-type fittings, and a six-port Hamilton switching valve. A 1-m section of fused silica capillary glass tubing connected between the switching valve and the analytical column served as a retention gap (Grob, 1982) and a cryoloop. Volatiles were desorbed from the trap with a hot air gun at 200 °C and cryofocused by inserting a portion of the retention gap into a Dewar containing liquid N₂. Compounds were introduced to the GC by exchanging the liquid N₂ Dewar for a container of hot (100 °C) water.

A Hewlett-Packard 5890A–5971A GC–MSD system equipped with a DB-Wax column (J&W Scientific, 60 m, 0.25 mm i.d., 0.25-μm film thickness) was used for analysis. Conditions for chromatography were as follows: initial oven temperature, 35 °C held for 5 min, increased to 50 °C at 2 °C min⁻¹, increased to 200 °C at 5 °C min⁻¹, and held for 5 min. Linear velocity of He carrier gas was 30 cm s⁻¹. Mass spectra were obtained by electron ionization at 70 eV. Transfer line and ion source temperatures were 280 and 180 °C, respectively. Spectra were recorded with a Hewlett-Packard 59970C Chemstation. Initial identification was made by matching against the Wiley/NBS library. Confirmation of identification was made by comparison of sample retention times and spectra with those of standards. Quantification was accomplished using selected ion monitoring for base peaks, and values were calculated using response factors generated with standards.

## RESULTS AND DISCUSSION

During the harvest period, fruit weight, firmness, starch index, soluble solids and titratable acidity changed gradually (Table I). In Washington state, starch values for Delicious apples in the 1.5–1.9 range (scale 1–5) are

**Table II. Internal Ethylene Concentration of Bisbee Delicious Apples during Fruit Growth and Maturation in 1990[a]**

| harvest date | ethylene concn (orchard 1), μL L⁻¹ | harvest date | ethylene concn (orchard 2), μL L⁻¹ |
|---|---|---|---|
| Aug 14 | 0.00 ± 0.00 | Aug 15 | 0.00 ± 0.00 |
| Aug 21 | 0.00 ± 0.00 | Aug 22 | 0.00 ± 0.00 |
| Aug 28 | 0.01 ± 0.01 | Aug 29 | 0.13 ± 0.08 |
| Sept 4 | 0.00 ± 0.00 | Sept 5 | 0.26 ± 0.17 |
| Sept 11 | 0.01 ± 0.01 | Sept 12 | 0.18 ± 0.14 |
| Sept 18 | 0.01 ± 0.01 | Sept 19[b] | 0.71 ± 0.24 |
| Sept 25[b] | 0.78 ± 0.48 | Sept 26 | 2.20 ± 0.94 |
| Oct 2 | 2.27 ± 0.73 | Oct 3 | 3.41 ± 1.44 |
| | | Oct 10 | 9.32 ± 2.54 |

[a] Values are means ± SE for analyses of 30 fruit. [b] Optimum harvest date for long-term CA storage.

considered acceptable for CA storage depending on values of the other quality factors (Olsen, 1982).

The increase in internal ethylene concentration during the final weeks of harvest in fruits from both orchards indicated the ripening phase of development had been initiated (Table II). Ripening began a week earlier in orchard 2 than in orchard 1 as indicated by the increase in internal ethylene concentration the week of September 19 in orchard 2 and the week of September 25 in orchard 1. The scion–rootstock combination is known to affect the timing of climacteric onset, with apples from seedling rootstocks ripening earlier (Drake et al., 1988).

Volatile samples collected in mid-August were primarily composed of aldehydes and alcohols (Tables III and IV). Ethanol had the highest concentration in the initial samples, but the amount of ethanol decreased as the harvest season progressed. Aldehydes having from 3 to 10 carbons were identified in samples from both orchards. Trace amounts of acetaldehyde were also found in these samples, but due to considerable variability amounts were not reported. Acetaldehyde is poorly entrained on Tenax (DePooter et al., 1987). Acetaldehyde was previously reported as a component of Delicious apple essence (Flath et al., 1967). Total headspace aldehyde concentration increased until the first week of September in fruit from both orchards; however, concentrations of many of the individual aldehydes declined thereafter. Concentrations of butanal, pentanal, (E)-2-hexenal, and heptanal declined to nondetectable levels by the end of the harvest period. These changes may reflect the increase in fruit ester

**Table III. Headspace Volatiles (pL kg⁻¹ h⁻¹) of Bisbee Delicious Apples Harvested from Orchard 1 during Fruit Growth and Maturation in 1990[a]**

| | harvest date | | | | | | | |
| | Aug | | | Sept | | | | Oct |
| volatile | 14 | 21 | 28 | 4 | 11 | 18 | 25[b] | 2 |
|---|---|---|---|---|---|---|---|---|
| | | | Aldehydes | | | | | |
| propanal | 47.6 | 92.4 | 76.2 | 10.1 | 16.7 | 14.6 | 10.4 | 15.2 |
| butanal | 46.1 | 335.2 | 402.5 | 17.0 | 73.1 | 24.0 | 0 | 0 |
| pentanal | 88.2 | 244.6 | 351.9 | 8.6 | 93.9 | 0 | 0 | 0 |
| hexanal | 355.8 | 1043.7 | 1541.4 | 228.4 | 348.7 | 260.0 | 61.9 | 148.8 |
| heptanal | 161.1 | 870.0 | 1882.2 | 52.8 | 248.8 | 74.1 | 21.7 | 0 |
| (E)-2-hexenal | 12.6 | 6.6 | 39.1 | 0 | 7.4 | 0 | 0 | 0 |
| octanal | 229.2 | 660.5 | 1671.9 | 91.1 | 130.5 | 133.3 | 32.4 | 141.9 |
| nonanal | 435.5 | 1474.5 | 3288.0 | 222.6 | 361.2 | 274.8 | 83.6 | 206.1 |
| decanal | 449.5 | 437.6 | 992.7 | 104.7 | 102.8 | 208.3 | 77.9 | 910.3 |
| benzaldehyde | 59.2 | 62.5 | 76.1 | 21.7 | 16.1 | 14.9 | 7.5 | 17.3 |
| | | | Alcohols | | | | | |
| 2-propanol | 800.4 | 198.5 | 60.1 | 544.1 | 438.4 | 370.7 | 239.9 | 55.7 |
| ethanol | 4866.3 | 2522.7 | 527.9 | 192.9 | 163.8 | 180.5 | 1932.8 | 153.6 |
| 1-butanol | 23.6 | 35.1 | 33.1 | 8.6 | 13.4 | 17.7 | 29.3 | 34.7 |
| 2-methyl-1-butanol | 0 | 0 | 0 | 0 | 0 | 0 | 61.4 | 65.4 |
| 1-hexanol | 7.3 | 13.2 | 5.5 | 5.2 | 4.0 | 4.5 | 3.8 | 4.0 |
| 2-ethyl-1-hexanol | 26.7 | 54.4 | 41.2 | 19.4 | 13.0 | 24.6 | 10.9 | 24.6 |
| 2-methyl-2,4-pentanediol | 101.8 | 477.4 | 1005.7 | 154.5 | 24.1 | 32.8 | 0 | 0 |
| | | | Esters | | | | | |
| ethyl acetate | 658.4 | 2842.2 | 8.8 | 6.6 | 14.8 | 13.5 | 19.3 | 6.7 |
| propyl acetate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| propyl propanoate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| butyl acetate | 0 | 0 | 0 | 0 | 0 | 0 | 89.2 | 216.7 |
| 2-methylbutyl acetate | 0 | 0 | 0 | 11.9 | 12.3 | 14.7 | 898.0 | 1489.0 |
| butyl propanoate | 0 | 0 | 0 | 0 | 0 | 0 | 6.0 | 48.6 |
| pentyl acetate | 0 | 0 | 0 | 0 | 0 | 0 | 17.0 | 26.3 |
| butyl butyrate | 0 | 0 | 0 | 0 | 0 | 0 | 16.3 | 95.1 |
| butyl 2-methylbutyrate | 0 | 0 | 0 | 0 | 0 | 0 | 15.6 | 83.0 |
| 3-methyl-2-buten-1-yl acetate | 0 | 0 | 0 | 0 | 0 | 0 | 14.1 | 0 |
| hexyl acetate | 18.2 | 4.8 | 4.4 | 4.2 | 12.9 | 6.5 | 85.2 | 168.3 |
| 2-methylbutyl 2-methylbutyrate | 0 | 0 | 0 | 0 | 0 | 0 | 19.9 | 53.5 |
| propyl hexanoate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58.2 |
| 2-hexenyl acetate | 26.8 | 0 | 0 | 0 | 8.2 | 0 | 5.3 | 0 |
| hexyl propanoate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| hexyl 2-methylbutyrate | 0 | 0 | 0 | 0 | 0 | 0 | 70.6 | 184.0 |
| hexyl hexanoate | 26.7 | 13.9 | 13.0 | 6.0 | 7.4 | 2.9 | 14.4 | 73.6 |
| | | | Ketones | | | | | |
| 2-propanone | 600.7 | 719.2 | 515.9 | 171.7 | 155.3 | 134.5 | 110.6 | 271.0 |
| 6-methyl-5-hepten-2-one | 99.2 | 76.7 | 99.4 | 41.0 | 38.4 | 44.4 | 15.5 | 31.1 |
| | | | Acid | | | | | |
| acetic acid | 452.7 | 3505.0 | 2655.9 | 161.3 | 106.8 | 61.9 | 25.6 | 70.5 |

[a] Values are means of three replicate 1-kg samples. [b] Optimum harvest date for long-term CA storage based on starch and ethylene values.

synthesis as ripening began. Apple fruits reduce aldehydes to alcohols that are subsequently esterified with carboxylic acids (DePooter et al., 1987; Knee and Hatfield, 1981). Concentrations of higher molecular weight aldehydes, particularly octanal, nonanal, and decanal, remained high throughout the harvest period.

Amounts of several alcohols other than ethanol remained relatively constant throughout the sampling period; however, 2-methyl-1-butanol, missing from the initial samples, was present 1 week after 2-methylbutyl acetate was found in orchard 2 (Table IV) and was detected the same week 2-methylbutyl acetate was found in samples from orchard 1 fruits (Table III). Differences in amounts of 2-propanol and 2-methyl-2,4-pentanediol were considerable between fruits from the two orchards through much of the sampling period.

Large amounts of ethyl acetate were detected in headspace from apples at the earliest harvests and reflect the large amounts of ethanol and acetate available for ester synthesis. Hexyl acetate and hexyl hexanoate were the only other esters detected throughout the sampling period; 2-hexenyl acetate was detected sporadically during the

sampling period from both orchards. The presence of esters prior to the increase in ethylene production indicates the enzymes catalyzing ester synthesis are functional prior to attainment of physiological maturity of Bisbee Delicious apples. Ester synthesis by ripening apples after treatment with alcohol vapors has been previously observed (Knee and Hatfield, 1981). Ester synthesis was absent until ripening began in strawberries (Yamashita et al., 1977).

Many esters previously identified as odor active components from apple fruits (Cunningham et al., 1986) were detected near the end of the harvest period as fruit entered the climacteric stage of development. This phenomenon coincided with increased internal ethylene concentration, detected September 19 in orchard 2 fruits (Table IV) and September 25 in orchard 1 fruits (Table III). Similar results have been previously reported for McIntosh apples (Yahia et al., 1990; Sapers et al., 1977), where a large number of esters were detected coincident with increased fruit ethylene production. Because esters were present in samples collected prior to this sample date, the increase in number and concentration of esters may represent mobilization of substrate previously unavailable for ester

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Table IV. Headspace Volatiles (pL kg⁻¹ h⁻¹) of Bisbee Delicious Apples Harvested from Orchard 2 during Fruit Growth and Maturation in 1990[a]

| volatile | Aug | | | Sept | | | | Oct | |
|---|---|---|---|---|---|---|---|---|---|
| | 15 | 22 | 29 | 5 | 12 | 19[b] | 26 | 3 | 10 |
| **Aldehydes** | | | | | | | | | |
| propanal | 56.8 | 20.6 | 49.8 | 8.8 | 14.8 | 7.8 | 4.1 | 6.5 | 11.5 |
| butanal | 223.2 | 209.2 | 243.5 | 9.2 | 0 | 148.0 | 0 | 0 | 0 |
| pentanal | 387.3 | 230.7 | 321.0 | 12.1 | 40.5 | 209.1 | 0 | 0 | 0 |
| hexanal | 1324.2 | 966.4 | 954.1 | 73.2 | 169.3 | 730.0 | 215.6 | 139.6 | 80.6 |
| heptanal | 1308.6 | 1077.5 | 1124.6 | 33.9 | 58.3 | 516.8 | 71.1 | 0 | 0 |
| (E)-2-hexenal | 38.2 | 42.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| octanal | 1127.9 | 669.3 | 910.4 | 54.0 | 79.5 | 325.4 | 151.6 | 72.2 | 453.9 |
| nonanal | 2780.6 | 1906.4 | 2240.9 | 125.9 | 212.4 | 952.3 | 519.7 | 254.7 | 1180.3 |
| decanal | 966.7 | 267.6 | 709.2 | 119.1 | 128.3 | 575.8 | 263.4 | 274.5 | 1642.6 |
| benzaldehyde | 48.1 | 75.6 | 50.8 | 14.2 | 18.6 | 33.9 | 38.8 | 42.3 | 101.4 |
| **Alcohols** | | | | | | | | | |
| 2-propanol | 317.1 | 42.7 | 8.9 | 211.9 | 1102.0 | 1897.4 | 115.1 | 492.7 | 1006.5 |
| ethanol | 11500 | 228.1 | 4136.9 | 91.5 | 91.8 | 754.3 | 53.4 | 152.3 | 461.5 |
| 1-butanol | 41.5 | 35.8 | 25.4 | 11.8 | 24.1 | 14.4 | 93.9 | 105.4 | 235.4 |
| 2-methyl-1-butanol | 0 | 0 | 0 | 0 | 13.1 | 29.4 | 471.8 | 540.9 | 509.3 |
| 1-hexanol | 18.3 | 13.5 | 11.7 | 6.3 | 8.2 | 8.9 | 26.6 | 47.8 | 117.6 |
| 2-ethyl-1-hexanol | 28.3 | 37.9 | 32.8 | 11.8 | 16.8 | 36.5 | 73.9 | 48.3 | 31.7 |
| 2-methyl-2,4-pentanediol | 97.5 | 590.1 | 476.5 | 7.0 | 7.4 | 3.8 | 86.8 | 26.4 | 1463.0 |
| **Esters** | | | | | | | | | |
| ethyl acetate | 1372.5 | 27.4 | 57.2 | 159.7 | 5.9 | 911.3 | 6.8 | 12.8 | 12.4 |
| propyl acetate | 0 | 0 | 0 | 0 | 0 | 0 | 59.5 | 176.2 | 410.4 |
| propyl propanoate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24.8 | 87.4 |
| butyl acetate | 0 | 0 | 0 | 9.4 | 12.4 | 34.8 | 222.8 | 518.6 | 1961.0 |
| 2-methylbutyl acetate | 0 | 0 | 33.3 | 40.5 | 62.1 | 250.2 | 2921.1 | 7053.2 | 9883.6 |
| butyl propanoate | 0 | 0 | 0 | 0 | 0 | 0 | 54.9 | 69.0 | 266.6 |
| pentyl acetate | 0 | 0 | 0 | 0 | 0 | 2.6 | 4.8 | 101.2 | 191.8 |
| butyl butyrate | 0 | 0 | 0 | 0 | 0 | 11.2 | 97.8 | 303.1 | 1107.2 |
| butyl 2-methylbutyrate | 0 | 0 | 0 | 0 | 0 | 5.3 | 171.6 | 299.7 | 927.1 |
| 3-methyl-2-butenyl acetate | 0 | 0 | 0 | 0 | 0 | 9.0 | 72.6 | 90.8 | 83.5 |
| hexyl acetate | 11.3 | 30.7 | 9.8 | 6.4 | 21.8 | 40.2 | 248.1 | 367.0 | 529.8 |
| 2-methylbutyl 2-methylbutyrate | 0 | 0 | 0 | 0 | 0 | 0 | 278.2 | 557.4 | 651.3 |
| propyl hexanoate | 0 | 0 | 0 | 0 | 0 | 0 | 50.9 | 185.6 | 516.6 |
| 2-hexenyl acetate | 11.1 | 0 | 0 | 0 | 9.0 | 0 | 37.2 | 0 | 42.9 |
| hexyl propanoate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 283.8 |
| hexyl 2-methylbutyrate | 0 | 0 | 0 | 0 | 0 | 35.9 | 598.4 | 1179.3 | 2286.2 |
| hexyl hexanoate | 16.2 | 15.7 | 4.3 | 2.6 | 1.4 | 48.3 | 88.5 | 306.9 | 554.7 |
| **Ketones** | | | | | | | | | |
| 2-propanone | 473.6 | 269.3 | 409.1 | 93.0 | 168.4 | 393.0 | 135.0 | 343.2 | 912.3 |
| 6-methyl-5-hepten-2-one | 110.2 | 91.6 | 86.0 | 13.9 | 31.5 | 40.2 | 92.1 | 26.1 | 171.6 |
| **Acid** | | | | | | | | | |
| acetic acid | 349.6 | 1424.9 | 637.3 | 77.0 | 51.5 | 150.6 | 113.2 | 112.6 | 1785.3 |

[a] Values are means of three replicate 1-kg samples. [b] Optimum harvest date for long-term CA storage based on starch and ethylene values.

synthesis. Dates of initial detection of 2-methyl-1-butanol, presumably the alcohol moiety of 2-methylbutyl acetate, illustrate this possibility. Branched-chain alcohols are thought to arise from amino acid degradation in apple fruits (Myers et al., 1970). De novo synthesis or increased activity of enzymes necessary for ester formation may occur as ripening accelerates. Acetate, the other substrate for ester biosynthesis, was present in all headspace samples throughout the 8-week sampling period. The presence of acetate suggests alcohol substrate availability limits the extent of ester formation in developing fruits.

Several esters, specifically propyl acetate, propyl propanoate, and hexyl propanoate, were absent in samples collected from orchard 1 (Table III) but were present in fruits from orchard 2 (Table IV). The absence of these compounds may reflect lack of available substrate and could be due to differences in rootstock, cultural practices, nutrient availability (Somogyi et al., 1964), or other unknown factors. Studies with other cultivars of Delicious apples on two different rootstocks suggest rootstock differences may be responsible for differing volatile profiles

(Fellman and Mattheis, unpublished results).

2-Methylbutyl acetate was initially detected several weeks prior to the increase in ethylene synthesis, August 29 from orchard 2 fruits (Table IV) and a week later, September 4, from orchard 1 fruits (Table III). At the final sampling 2-methylbutyl acetate had the highest concentration of all esters detected, and due to its low olfactory threshold of 0.005 ppm (Flath et al., 1967) would be a major component of Bisbee aroma at this stage of development. Butyl acetate was also detected prior to the increase in internal ethylene in orchard 2 fruits (August 29; Table IV) but was missing until September 25 in orchard 1 fruits (Table III), coincident with the increase in internal ethylene. Internal ethylene concentration is used as an index of maturity for a number of apple varieties (Liu, 1978; Chu, 1988). Year to year variation in development of ethylene synthesis by different cultivars does, however, make the use of internal ethylene values for scheduling harvest for CA storage difficult (Chu, 1988). Analysis of additional compounds that appear prior to initiation of apple ethylene synthesis may provide another maturity index indicative of physiological development.

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Prediction of optimal harvest date using butyl acetate concentrations in headspace samples collected 2 days following harvest from Golden Delicious apples has previously been reported (Dirinck et al., 1989). For Bisbee Delicious apples, 2-methylbutyl acetate may be a more appropriate indicator compound to develop similar information.

Entry into the developmental stage where numerous esters are produced may be a prerequisite to development of characteristic flavor when apples are removed from storage (Yahia et al., 1990). Storage of apples in low-oxygen CA is known to adversely affect fruit flavor (Patterson et al., 1974); therefore, scheduling of harvest is even more critical. Harvesting too early may exacerbate the lack of flavor development, while late-harvested fruit experiences a rapid firmness loss in storage. For this reason, the disappearance of butanal and pentanal with the concomitant appearance of 2-methyl-1-butanol, butyl acetate, 2-methylbutyl acetate, butyl propanoate, pentyl acetate, butyl butyrate, butyl 2-methylbutyrate, and hexyl 2-methylbutyrate may provide an accurate assessment of the optimum harvest maturity of Delicious apples that will ensure a high flavor quality even after prolonged CA storage (Lidster et al., 1983).

In summary, qualitative and quantitative changes in headspace volatiles collected from Bisbee Delicious apples changed considerably over a developmental period during which fruit ripening began. A number of low molecular weight aldehydes detected while apples were still immature were absent as fruit development approached physiological maturity and entry into the ripening phase. Headspace samples collected from apples that had begun to ripen were composed largely of esters, with 2-methylbutyl acetate having the highest concentration. This compound was initially detected several weeks prior to increased ethylene synthesis and may be useful as a more accurate indicator of fruit maturity for scheduling commercial harvest. Accurate assessment of harvest maturity determines the postharvest utilization of Delicious apples. Harvest of climacteric fruit, such as the apple, at the proper prephysiological state ensures optimum quality after extended periods of CA storage. Previously developed indices involving measurements of fruit quality change gradually during fruit development and lack the precision necessary to predict absolute preclimacteric status. Measurements of ethylene production indicate apple development has progressed past the preclimacteric phase, whereas ester appearance seems to precede the onset of the ripening phase of development.

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