# Ester Variability in Apple Varieties As Determined by Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry

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Solid-phase microextraction (SPME) with a polydimethylsiloxane fiber coupled with gas chromatography–mass spectrometry (GC-MS) was applied to the study of variability in volatiles released by 13 apple varieties. The relative amounts of 40 esters and $\alpha$-farnesene were determined. Principal component analyses of these results clustered the apples into three groups according to skin color: red, green, and red-green. Total ester contents were highest with the red cluster apples, and the green cluster apples had the highest $\alpha$-farnesene levels. This technology was also applied to the monitoring of changes in volatiles for apples removed from controlled-atmosphere storage with subsequent storage at 4 °C and room temperature. Total ester contents increased 25-fold, with the greater increases coming at room temperature, whereas $\alpha$-farnesene levels increased only 5-fold. For apples stored at room temperature, after 11 days, the amount of increase was inversely proportional to the size of the ester: levels of smallest esters (molecular weight 116) increased 12.5-fold, and the largest esters (molecular weight 228) increased ~1.3-fold.

**KEYWORDS: Apple;** *Malus domestica***; SPME; volatile esters; GC-MS;** $\alpha$**-farnesene; skin color**

## INTRODUCTION

The majority of aroma compounds from apples (*Malus domestica*) are volatile esters; however, apples also produce a relatively large amount of $\alpha$-farnesene (*1*–*4*). The products of $\alpha$-farnesene oxidation could induce the scald disorder (*5*–*7*). Scald susceptibility of apple cultivars is a function of $\alpha$-farnesene production and its oxidation to conjugated trienols (*8*). The inhibition of ester production may occur as a result of the physiological changes associated with susceptibility to, rather than expression of, scald symptoms (*9*).

Increases in the concentration of volatile compounds have been detected several weeks before the respiratory climacteric. Following the climacteric, the volatile concentrations decline (*10*). The large increase in $\alpha$-farnesene concentration also coincides with the rise in the respiratory (*11*) and ethylene (*12*) climacterics.

Apples are often held for several months at low temperature either in air or in controlled-atmosphere (CA) storage. During CA storage, the production of volatiles decreases, and the capacity for their production after storage declines (*13*). Reduced emission of aroma volatiles has been reported as the factor most likely responsible for diminished flavor (*14*).

Most reports on the ester content of apples (*1*, *3*, *4*, *9*, *10*, *15*–*42*) have been restricted to one or two varieties of apples and different sampling techniques were used. Kakiuchi et al. (*43*) noted variability in the volatile composition in five apple cultivars and that the ranking depended upon the sampling method used. Our objectives for this paper were to use a single technique, solid-phase microextraction (SPME) (*44*, *45*), to (1) examine the relationships between total esters and $\alpha$-farnesene of 13 apple varieties and (2) study the effect of temperature and time after CA storage on the capability of total ester and $\alpha$-farnesene production of cv. Royal Gala apple. SPME has been applied in several studies of apple volatiles and $\alpha$-farnesene (*17*, *18*, *20*, *41*, *42*).

## MATERIALS AND METHODS

**Apple Samples.** Thirteen varieties (listed in **Table 1**) of apples were purchased from local supermarkets in December 2001. These apples had come from storage at 4 °C in the produce coolers earlier in the day. The produce managers were unable to provide storage details for the apples prior to arrival in their stores; however, it is usual commercial practice for apples to be stored and shipped in cold temperature until they reach the retail stores. Care was taken to obtain four apples of approximately the same size for each variety. The masses and volumes of each apple were determined. The apples were analyzed in pairs the day immediately following purchase.

In addition, for the total ester and $\alpha$-farnesene study, 50 Royal Gala apples that had been stored in CA conditions were used. These apples

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**Table 1.** Varieties and Sources of Apples Used in This Study

| variety | source[a] | wt[b] (g) | vol[b] (mL) | density (g/mL) |
|---|---|---|---|---|
| Braeburn (Br) | WA | 200.8 ± 1.9[c] | 247.8 ± 2.0 | 0.810 ± 1.2 |
| Crispin (Cr) | ? | 292.6 ± 8.8 | 366.5 ± 9.6 | 0.798 ± 1.2 |
| Empire (Em) | ON | 197.3 ± 1.6 | 258.0 ± 3.0 | 0.765 ± 1.8 |
| Fuji (Fu) | BC | 265.1 ± 2.3 | 350.5 ± 3.2 | 0.756 ± 1.1 |
| Golden Delicious (GD) | WA | 199.7 ± 2.4 | 265.8 ± 3.6 | 0.752 ± 1.2 |
| Granny Smith (GS) | WA | 180.4 ± 1.8 | 233.8 ± 2.2 | 0.772 ± 1.0 |
| Ida Red (IR) | ON | 236.9 ± 3.2 | 320.8 ± 3.9 | 0.720 ± 0.8 |
| Jonagold (Jg) | ON | 196.6 ± 1.3 | 251.3 ± 0.5 | 0.782 ± 0.9 |
| McIntosh (Mc) | ON | 178.0 ± 5.5 | 251.3 ± 6.0 | 0.708 ± 1.9 |
| Pink Lady (PL) | WA | 198.2 ± 2.7 | 269.3 ± 2.9 | 0.736 ± 0.3 |
| Red Delicious (RD) | WA | 232.5 ± 2.0 | 299.0 ± 2.8 | 0.778 ± 1.1 |
| Royal Gala (RG) | BC | 215.3 ± 1.3 | 274.5 ± 0.2 | 0.784 ± 1.1 |
| Spartan (Sp) | BC | 200.0 ± 1.4 | 279.5 ± 2.2 | 0.715 ± 1.1 |

[a] BC, British Columbia, Canada; ON, Ontario, Canada; WA, Washington, USA; ?, unknown. [b] Values are the averages for four apples. [c] Relative standard deviation in percent.

were harvested from the University of Guelph's research orchard in Simcoe, ON, and stored in CA (2.5% oxygen + 2.5% carbon dioxide + 95% nitrogen) at 1 °C for ~6.5 months. Apples were then stored in cold storage at 1 °C for 10 days under ambient atmosphere before the start of this study, which had two holding periods. During the first holding period, apples were placed in a refrigerator at 4 °C for 1, 4, 11, 18, or 25 days. At the end of each of the various first holding periods, the same apples were further held at 23 °C in the dark for an additional 4, 11, and 18 days.

**Synthesis of Esters.** Because only a few of the many potential esters were commercially available, >100 esters were synthesized according to the method of Tsao et al. (*46*) to obtain standards for gas chromatographic (GC) retention times and mass spectra (MS) determinations. Typically, 8 mmol of an organic acid was added to a mixture of 1 mmol each of eight alcohols in 12 mL of dichloromethane. Then 8 mmol of 1,3-dicyclohexylcarbodiimide was added followed by a crystal or two of 4-(dimethylamino)pyridine. This reaction mixture was stirred at room temperature for several hours and filtered, and ~1 mL of filtrate was taken to dryness under a stream of nitrogen in a 1.8 mL vial.

The reagents methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, 1-pentanol, 2-methyl-1-butanol, 1-hexanol, 1-heptanol, 1-octanol, 1 nonanol, acetic acid, propionic acid, butyric acid, pentanoic acid, 2-methylbutyric acid, hexanoic acid, 4-methylpentanoic acid, heptanoic acid, and octanoic acid were obtained from Sigma-Aldrich (Sigma-Aldrich Canada, Oakville, ON). Esters were synthesized from every possible alcohol–acid combination.

**Solid-Phase Microextraction of Apple Volatiles.** A SPME fiber (Supelco, Bellefonte, PA) coated with a 100 $\mu$m layer of polydimethylsiloxane (PDMS) was used in all of the experiments. For standards, the fiber was exposed to the contents of the vial for only 10–15 s to avoid overloading the fiber. For apples, the fibers were exposed for 25 min. All extractions were carried out at room temperature (23 °C) in a climate-controlled laboratory. In preliminary studies, the utility of polydimethylsiloxane/divinylbenzene (PDMS/DVB), Carboxen/poly-dimethylsiloxane (CAR/PDMS), and polyacrylate fibers was also investigated.

Late in the afternoon on the day of purchase, a pair of apples was placed onto a fresh layer of aluminum foil in a clear plexiglass box of dimensions 18 × 12 × 21.5 cm (4.6 L), which was then sealed shut. One end had a circular 3 mm i.d. hole that was covered with a PTFE/silicone septum. For sampling beginning the following morning, the SPME fiber assembly was placed through the septum.

In the store-purchased apple study, two apples were placed in one sampling chamber and the other two apples placed in a separate chamber. Each chamber was analyzed twice, and the results were averaged. For the Royal Gala CA storage study, apples were paired such that the mass of each pair was as equal as possible. Five pairs (replicates) of apples were used for each treatment period, and each pair was sampled twice and the results were averaged. One separate

set of apples was used for each of the holding periods at 4 °C in a refrigerator, and those same sets of apples were analyzed after 1, 4, 11, and 18 days at 23 °C in the dark. Between analyses, apples were placed in paper bags and stored in the dark. Samples of given varieties or times were analyzed on the same day.

After being used for analyses, the boxes were washed in hot soapy water, rinsed with distilled water and methanol, and air-dried before being used for the next pair of apples.

**Gas Chromatography–Mass Spectrometric (GC-MS) Analysis.** The SPME fiber was immediately placed into a splitless mode injection port of a Thermo-Finnigan GCQ GC-MS (Thermo-Finnigan, San Jose, CA) for 3 min. The injection port temperature was set at 250 °C. Desorbed volatiles were swept by a 1 mL/min flow of helium onto a 30 m × 0.25 mm capillary column with a 0.25 $\mu$m film of Stabilwax (Restek, Bellefonte, PA). The column was heated at 50 °C for 5 min and then temperature programmed to 220 °C at 10 °C/min and held for 8 min. Effluents were detected by an ion trap MS operating in the electron ionization mode at 70 eV. The transfer line and ion source temperatures were set at 230 and 200 °C, respectively, and masses $m/z$ 35–650 were scanned.

Esters were identified by the congruence of retention times and MS spectra with those of the synthesized standards. Peak areas in total ion counts were used to compare the amounts of analytes.

**Statistical Analysis.** The statistics program S-Plus 2000 (MathSoft, Seattle, WA) was used to perform the analysis. This program plots the data in a two-dimensional space, using principal components or multidimensional scaling. The data points are then clustered in such a way that objects belonging to the same cluster resemble each other, whereas objects in different clusters are dissimilar. The variables used in the analysis were the peak areas from the GC-MS data.

## RESULTS AND DISCUSSION

**Determination of Ester and α-Farnesene Levels in Apple Varieties.** Extraction of esters and α-farnesene by SPME was first optimized by choice of fiber. At the outset of a related study, α-farnesene was the target molecule, and of the four fibers evaluated (PDMS, PDMS/DVB, CAR/PDMS, and polyacrylate), PDMS gave the highest levels of this molecule. It was also the best for ester recovery and was thus chosen for this study.

To maximize extractions, the volatiles in the headspaces needed to reach equilibrium concentrations. This was achieved after 16 h, so samples were kept in the headspace containers overnight. Then sufficient time to achieve equilibrium concentrations on the fiber was needed. Although this occurred after ~20 min, extractions were continued for 25 min because this fitted conveniently into the cycle of extracting a sample while the previous sample was being analyzed by GC-MS.

In the initial methodology development stages, we also noted some carry-over of α-farnesene from one analysis to the next, presumably due to adsorption on the walls of the container. Matich et al. (*9*) also noted that α-farnesene adsorbed onto glass. For this reason, containers were washed well between analyses. Carry-over on the fiber was ruled out when reanalysis of fibers resulted in blank chromatograms.

It quickly became apparent that the α-farnesene and ester profiles of different varieties were not the same, for example, for cvs. Ida Red and McIntosh illustrated in **Figure 1**. Thus, as many varieties as were available in local supermarkets at the time were purchased and analyzed. **Table 1** summarizes the varieties analyzed, locale grown, weight, and volume. The calculated densities ranged from about 0.71 to 0.81 g/mL.

**Table 2** summarizes the MS ion count abundances for α-farnesene and the 40 esters observed in the 13 apple cultivars studied. Nineteen compounds in this list showed reasonable quantities (with an ion count of >200 000) and existed in at least one apple cultivar. The most significant compound (peak) was α-farnesene. The esters labeled as peaks 30 and 31 were
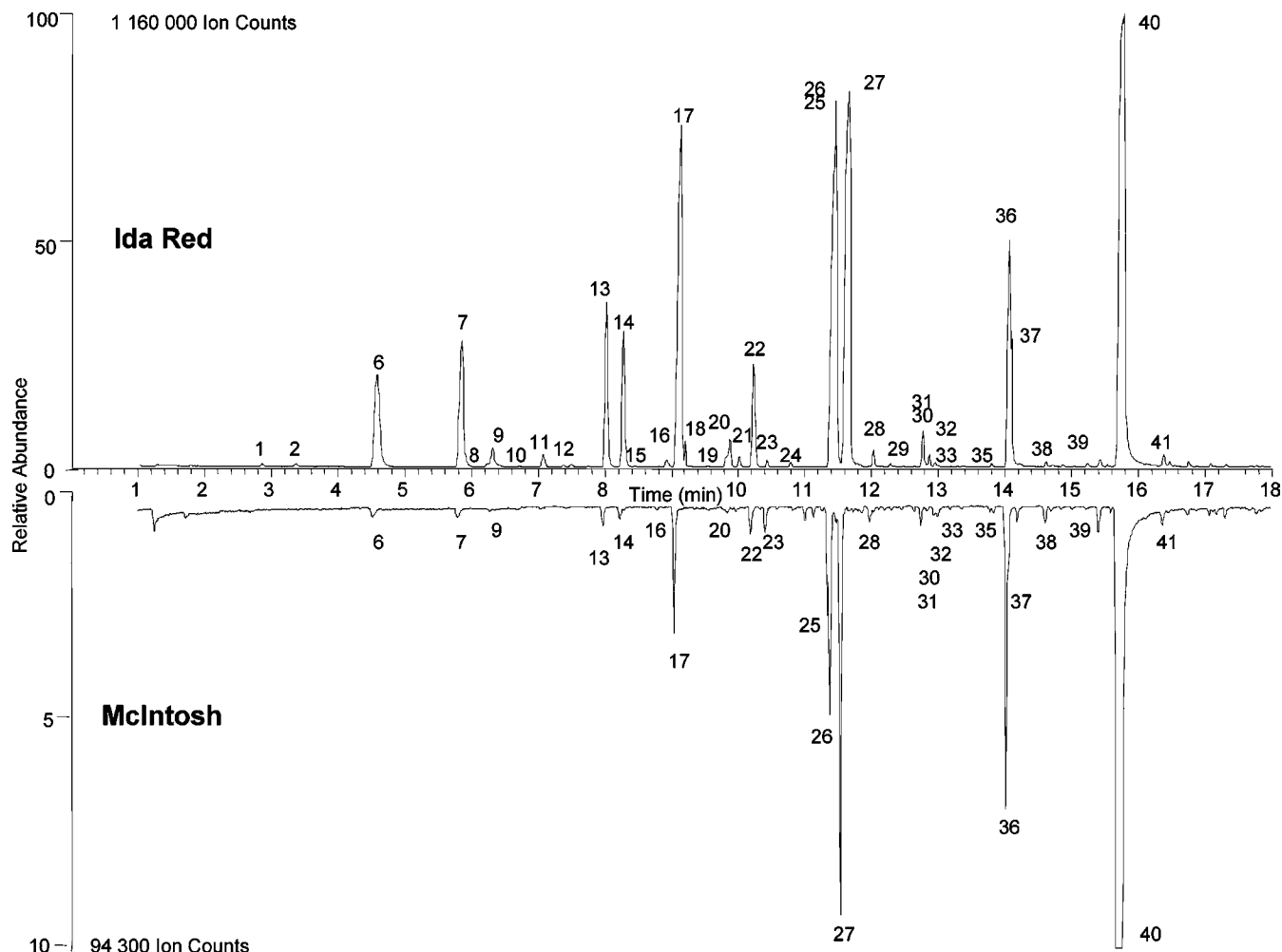
**Figure 1.** Gas chromatography–mass spectrometric chromatograms of headspace volatiles for Ida Red and McIntosh apples sampled with polydimethylsiloxane solid-phase microextraction fibers. See **Table 2** for identity of the various peaks.

not resolved enough to permit separate quantification. Three standards, pentyl octanoate, hexyl heptanoate, and heptyl hexanoate, all had similar retention times very close to that of peak 39. Being a minor component, it was not possible to confirm if this peak was a single component or a mixture of these three possible 13-carbon esters.

Others over the past two decades have reported on ester profiles (*1, 3, 4, 9, 10, 15−42*), and the compounds reported in those studies are also listed in **Table 3**. In each of those studies, the number of esters reported ranged from 7 to 27 (median = 16). In this study we observed 40 esters and, when combined with the other studies, ∼70 esters in total have been noted. All told, nearly 300 volatile compounds have been isolated from apple (*47*).

A review of the 33 other studies cited in **Table 3** revealed that hexyl acetate was the only ester reported in every study. If one includes all of the esters that are reported in at least half of those studies, the 15 such esters have a common feature: they each have only two, four, or six carbons in the alcohol and/or acid moiety. This is not surprising, considering that these esters are likely derived from the acetate biosynthetic pathway.

To compare similarities or differences between the complex mixtures of individual esters and α-farnesene, the raw ion count data in **Table 2** were subjected to principal component analysis (PCA). **Figure 2** illustrates how the data grouped into three clusters that coincided with peel color. The first cluster contained apples (cvs. Red Delicious, Fuji, Royal Gala, Pink Lady,

Jonagold, Ida Red, and Spartan) that had dominant red peel color. The second cluster contained apples (cvs. Golden Delicious, Crispen, and Granny Smith) that had dominant green peel color. The third cluster contained apples (cvs. McIntosh, Empire, and Braeburn) that had both red and green peel colors. This clustering relationship may be genetically linked. For example, in the first cluster, Fuji is a cross of Red Delicious and cv. Ralls, and clustered with Red Delicious. Jonagold and Ida Red both had the same parent, Jonathan, and appear in the same cluster. Although Royal Gala, Pink Lady, and Jonagold varieties all had Golden Delicious as one of their parents, because of their dominant red peel color, they are not clustered with the non-red peel parent Golden Delicious. Spartan is a cross of cv. Newton and McIntosh, but because of its dominant red peel color, it is not clustered with McIntosh. In the second cluster, Crispen is a cross of Golden Delicious and cv. Indo; therefore, it is clustered with Golden Delicious. In the third cluster, Empire is a cross between McIntosh and Red Delicious; it is clustered with McIntosh and not with Red Delicious because its appearance is more like that of a McIntosh than a Red Delicious. Karlsen et al. (*21*) also applied PCA to volatiles from about a dozen apple varieties, although they used apple slices, which would have created profiles different from whole apples.

A re-examination of the data in **Table 2** then revealed that those apples clustering as red peeled showed the highest total ester values, with the green peeled having lower values. Fellman and co-workers (*48, 49*) have suggested that the intensity of

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**Table 2.** Levels of α-Farnesene and Esters Observed in the Headspace Volatiles Released by Different Varieties of Whole Apples As Determined by Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry

| peak | compound | retention time (min) | Br[a] | Cr | Em | Fu | GD | GS | IR | JG | Mc | PL | RD | RG | Sp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | propyl acetate | 2.86 | —[b] | – | 3[c] | – | 6 | – | 17 | 6 | – | 7 | 16 | 4 | – |
| 2 | methyl 2-methyl-1-butanoate | 3.38 | 2 | – | – | – | 9 | – | 18 | 7 | – | 6 | 10 | 6 | 5 |
| 3 | ethyl butanoate | 3.78 | – | 60 | 38 | 9 | – | 2 | – | – | – | 7 | 3 | – | 17 |
| 4 | propyl propanoate | 3.93 | – | – | 1 | 10 | – | 4 | – | 4 | – | 10 | – | – | 6 |
| 5 | ethyl 2-methyl-1-butanoate | 4.06 | – | – | 13 | 16 | – | 2 | – | – | – | 12 | 8 | – | 7 |
| 6 | butyl acetate | 4.58 | 29 | 143 | 388 | 70 | 681 | 5 | 1015 | 598 | 8 | 93 | 255 | 445 | 208 |
| 7 | propyl butanoate | 5.86 | 244 | 94 | 383 | 323 | 287 | 17 | 1110 | 371 | 7 | 401 | 827 | 288 | 459 |
| 8 | 2-propyl pentanoate | 6.25 | 9 | – | 14 | 154 | 6 | 21 | 23 | 15 | – | 228 | 194 | – | 29 |
| 9 | butyl propanoate | 6.31 | 21 | 37 | 85 | 60 | 111 | – | 210 | 192 | 2 | 32 | 37 | 43 | 104 |
| 10 | 2-methyl-1-propyl butanoate | 6.72 | – | 7 | 2 | 4 | 5 | 4 | 8 | – | – | 11 | – | – | 18 |
| 11 | pentyl acetate | 7.08 | 10 | 23 | 56 | 28 | 61 | 4 | 85 | 48 | 2 | 39 | 50 | 42 | 51 |
| 12 | 2-methyl-1-butyl propanoate | 7.38 | 12 | 3 | 2 | 20 | 2 | 2 | 19 | 7 | – | 84 | 14 | 1 | 10 |
| 13 | butyl butanoate | 8.04 | 52 | 358 | 426 | 287 | 570 | 5 | 1018 | 429 | 11 | 155 | 195 | 190 | 691 |
| 14 | butyl 2-methyl-1-butanoate | 8.29 | 167 | 245 | 279 | 948 | 499 | 9 | 853 | 783 | 6 | 792 | 833 | 148 | 447 |
| 15 | pentyl propanoate | 8.46 | 3 | 2 | 3 | 8 | 4 | – | 9 | 9 | – | 7 | 5 | 3 | 7 |
| 16 | 2-methylbutyl butanoate | 8.94 | 21 | 32 | 14 | 83 | 9 | 2 | 62 | 15 | – | 30 | 30 | 7 | 54 |
| 17 | hexyl acetate | 9.08 | 186 | 798 | 1651 | 253 | 2584 | 156 | 2807 | 2884 | 57 | 824 | 849 | 2188 | 1689 |
| 18 | 2-methyl-1-butyl 2-methyl-1-butanoate | 9.15 | 86 | 17 | 25 | 247 | 21 | 6 | 67 | 44 | – | 158 | 153 | 16 | 143 |
| 19 | butyl pentanoate | 9.85 | 20 | 83 | 36 | 0 | 42 | – | 59 | 41 | – | 31 | 24 | 20 | 94 |
| 20 | propyl hexanoate | 9.88 | 74 | – | 67 | 284 | 57 | 15 | 139 | 75 | 4 | 231 | 584 | 32 | 80 |
| 21 | pentyl 2-methyl-1-butanoate | 10.01 | 46 | 29 | 31 | 165 | 23 | 3 | 62 | 41 | – | 138 | 123 | 20 | 81 |
| 22 | hexyl propanoate | 10.24 | 138 | 226 | 383 | 276 | 409 | 67 | 609 | 1055 | 13 | 563 | 232 | 412 | 683 |
| 23 | 2-methyl-1-propyl hexanoate | 10.43 | 16 | 20 | 34 | 21 | 29 | 13 | 43 | 30 | 12 | 23 | 30 | 31 | 39 |
| 24 | heptyl acetate | 10.79 | 2 | 8 | 7 | 5 | 14 | 4 | 15 | 20 | 2 | 14 | 11 | 27 | 15 |
| 25 | butyl hexanoate | 11.45 | 618 | 2664 | 1355 | 1828 | 2444 | 9 | 2156 | 2223 | 39 | 1026 | 2427 | 2171 | 3014 |
| 26 | hexyl butanoate | 11.48 | 190 | 1239 | 1043 | 831 | 645 | 14 | 1700 | 1450 | 88 | 798 | 529 | 1247 | 2207 |
| 27 | hexyl 2-methyl-1-butanoate | 11.61 | 1095 | 1287 | 1219 | 2909 | 1948 | 105 | 4592 | 3726 | 169 | 4652 | 3044 | 1130 | 3493 |
| 28 | 2-methyl-1-butyl hexanoate | 12.03 | 190 | 38 | 39 | 265 | 25 | 10 | 94 | 51 | 9 | 122 | 278 | 34 | 198 |
| 29 | octyl acetate | 12.29 | – | 22 | 10 | – | 14 | – | 12 | 34 | – | 11 | 10 | 61 | 21 |
| 30, 31 | pentyl hexanoate and butyl heptanoate | 12.78 | 122 | 254 | 136 | 336 | 146 | 10 | 166 | 229 | 9 | 285 | 323 | 232 | 392 |
| 32 | propyl octanoate | 12.88 | 24 | 23 | 37 | 202 | 11 | 4 | 52 | 48 | 3 | 117 | 219 | 45 | 64 |
| 33 | heptyl 2-methyl-1-butanoate | 12.96 | 7 | 5 | – | 34 | 11 | 5 | 24 | 12 | 5 | 54 | 41 | 6 | 17 |
| 34 | 2-methyl-1-propyl octanoate | 13.29 | 2 | – | – | 9 | – | – | 4 | 3 | – | 8 | 6 | 6 | 9 |
| 35 | 2-methyl-1-butyl heptanoate ? | 13.38 | 9 | 3 | 9 | 22 | 12 | – | 9 | 10 | 2 | 14 | 16 | 19 | 32 |
| 36 | hexyl hexanoate | 14.08 | 466 | 1708 | 1034 | 976 | 1139 | 48 | 1381 | 1655 | 134 | 1470 | 1199 | 2539 | 3390 |
| 37 | butyl octanoate | 14.11 | 102 | 465 | 688 | 535 | 400 | – | 451 | 809 | – | 279 | 433 | 1114 | 1181 |
| 38 | nonyl propanoate | 14.63 | 44 | 49 | 45 | 105 | 7 | – | 28 | 24 | 8 | 33 | 88 | 77 | 169 |
| 39 | C13 ester[d] | 15.25 | – | 19 | 25 | 23 | 16 | – | 15 | 23 | 2 | 30 | 23 | 60 | 51 |
| 41 | hexyl octanoate | 16.39 | 19 | 94 | 95 | 62 | 69 | 30 | 70 | 79 | 13 | 70 | 65 | 246 | 155 |
| | total ester | | 4025 | 10055 | 9680 | 11408 | 12314 | 578 | 19001 | 17049 | 602 | 12869 | 13185 | 12911 | 19329 |
| | RSD% | | 39 | 19 | 14 | 4 | 19 | 26 | 17 | 8 | 7 | 19 | 3 | 7 | 6 |
| 40 | α-farnesene | 15.80 | 4256 | 8767 | 2644 | 6640 | 9345 | 8786 | 6547 | 6322 | 4117 | 6005 | 6611 | 5433 | 7718 |
| | RSD% | | 27 | 14 | 8 | 3 | 13 | 27 | 8 | 7 | 5 | 9 | 6 | 23 | 8 |

[a] See **Table 1** for meaning of apple variety abbreviations. [b] Not detected. [c] Values are integrated responses in thousand ion counts and are the averages of duplicate analyses of two replicates. [d] Hexyl heptanoate, heptyl hexanoate, and/or pentyl octanoate.

peel color, as determined by anthocyanin content, may be correlated with total ester content, at least for Red Delicious apples. The highest α-farnesene values were observed for the green-peeled apples, with lower levels for those apples in the red peel cluster. The PCA revealed that nearly 80% of the point variability could be explained by two components. A simple plot of total ester content versus α-farnesene content (not illustrated) showed that on this basis, the apple varieties studied clustered as in the PCA (**Figure 2**), which suggests that the two major components were total ester and α-farnesene.

Saevels et al. (*42*) have just reported on the use of a mass spectrometer-based electronic nose for assessing apple quality during shelf life. In that study, headspace volatiles were transported simultaneously and in total into a mass spectrometer, and a composite mass spectrum was generated for each sample. These spectra were used for statistical analysis. PCAs were able to distinguish between various storage conditions and times of storage. This study prompted us to re-evaluate our MS data. The Xcalibur software enabled summation of all spectra from all peaks in a chromatogram into a single composite spectrum analogous to that obtained by Saevels et al. (*42*). Spectra

achieved by summation of all ester peaks and separately of the α-farnesene peak revealed that the ions at $m/z$ 43, 55, 56, 57, 89, 99, 145, 159, 173, 187, and 201 were characteristic of esters and that ions at $m/z$ 77, 79, 81, 91, 92, 93, 105, 106, 107, 119, and 123 were characteristic of α-farnesene. When the total ion counts for the two sets of ions were summed and plotted against one another for the different apple varieties (not illustrated), a clustering very similar to that in **Figure 2** was observed. A further simplification by plotting only the $m/z$ 43 ion to represent esters and $m/z$ 91 to represent α-farnesene also gave a similar clustering (**Figure 3**). In this study, the raw ion count data were used, whereas Saevels et al. (*42*) normalized their data and used relative intensities. Here, normalized spectra did not result in clusters that could be explained as above.

**Variation in Ester and α-Farnesene Levels in Royal Gala Apples Removed from Controlled-Atmosphere Storage.** When apples go into CA storage, there is a decrease in volatiles (*1*, *3*, *15*, *16*, *29*, *31*, *34*, *37*, *48*, *50*), and there can be some increase in volatiles when they are subsequently placed into a regular atmosphere (*1*, *22*, *31*, *34*, *37*). **Figure 4** illustrates changes in α-farnesene and total ester composition when apples

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**Table 3.** Literature Citations to Esters Reported in the Headspace Volatiles Released by Different Varieties of Whole Apples

| compound | mol wt | literature observances |
|---|---|---|
| ethyl acetate | 88 | *10, 15, 16, 21, 22, 29–31, 33, 38, 40* |
| ethyl propanoate | 102 | *15, 22, 24, 28, 31, 34* |
| methyl butanoate | 102 | *9, 31* |
| methyl methylpropanoate | 102 | *40* |
| propyl acetate | 102 | *ts,[a] 1, 3, 15, 16, 20–23, 26, 28, 31, 32–35, 37, 38, 42* |
| ethyl butenoate | 114 | *31* |
| 2-methyl-1-propyl acetate | 116 | *16, 22, 23, 26, 31, 32–35, 37, 38, 42* |
| butyl acetate | 116 | *ts, 1, 3, 4, 10, 15, 16, 19–24, 26–38, 40–42* |
| ethyl butanoate | 116 | *ts, 1, 9, 15–22, 24, 28–31, 33, 34, 40* |
| ethyl methylpropanoate | 116 | *40* |
| methyl 2-methyl-1-butanoate | 116 | *ts, 17, 20, 31, 40* |
| propyl propanoate | 116 | *ts, 16, 18–20, 23, 25, 26, 31, 32, 34, 37, 38, 42* |
| 2-methylbut-3-enyl acetate | 128 | *35* |
| 3-methylbut-2-enyl acetate | 128 | *35, 37* |
| 3-methylbut-3-enyl acetate | 128 | *35* |
| 2-methyl-1-butyl acetate | 130 | *1, 4, 10, 15, 16, 21–23, 25–27, 31, 33–38, 40–42* |
| 3-methyl-1-butyl acetate | 130 | *30, 32, 40* |
| butyl propanoate | 130 | *ts, 4, 10, 16, 18–20, 23, 25–29, 31–35, 37, 38, 42* |
| ethyl 2-methyl-1-butanoate | 130 | *ts, 9, 15–17, 19, 20, 22, 28–31, 33, 34, 37, 39, 40* |
| ethyl 3-methyl-1-butanoate | 130 | *22* |
| ethyl pentanoate | 130 | *23, 24, 30* |
| methyl hexanoate | 130 | *9, 24, 31, 40* |
| pentyl acetate | 130 | *ts, 1, 3, 4, 10, 15, 16, 18–20, 22–24, 26, 30, 32–35, 37, 38, 42* |
| propyl butanoate | 130 | *ts, 1, 3, 4, 10, 15, 16, 19–24, 26–32, 34–38, 40–42* |
| *tert*-butyl propanoate | 130 | *22* |
| 2-hexenyl acetate | 142 | *10, 24, 25* |
| hexenyl acetate | 142 | *19, 35, 40* |
| 1-butanol 2-methylpropanoate | 144 | *23* |
| 2-methyl-1-butyl propanoate | 144 | ts, *3, 25, 28, 33, 34* |
| 2-methyl-1-propyl butanoate | 144 | ts, *17, 24, 25, 34, 42* |
| 2-propyl pentanoate | 144 | ts |
| 3-methyl-1-butyl propanoate | 144 | *37* |
| butyl 2-methylpropanoate | 144 | *37* |
| butyl butanoate | 144 | *ts, 3, 4, 9, 10, 15, 16, 18, 20, 22–29, 32–34, 37, 38, 42* |
| ethyl hexanoate | 144 | *9, 15, 16, 22, 28–30* |
| hexyl acetate | 144 | *ts, 1, 3, 4, 9, 10, 15–42* |
| pentyl propanoate | 144 | *ts, 3, 34, 37* |
| propyl 2-methylbutanoate | 144 | *23, 37, 40* |
| hexenyl proprionate | 156 | *23* |
| 2-methylbutyl butanoate | 158 | *ts, 3, 9, 19, 25, 28, 34, 37* |
| 3-methylbutyl butanoate | 158 | *ts, 37* |
| butyl 2-methyl-1-butanoate | 158 | *ts, 3, 9, 10, 15–17, 20, 23, 25, 26, 29, 32–34, 37, 38, 40, 42* |
| butyl pentanoate | 158 | *ts, 18, 20, 25, 32, 34, 42* |
| heptyl acetate | 158 | *ts, 18, 33, 34, 37* |
| hexyl propanoate | 158 | *ts, 3, 4, 9, 10, 15–18, 20, 23–29, 31–34, 37, 38* |
| methyl 2-ethylhexanoate | 158 | *42* |
| pentyl butanoate | 158 | *1, 4, 9, 25, 26, 28, 32, 33, 37* |
| propyl hexanoate | 158 | *ts, 3, 10, 16, 17, 23–27, 31, 32, 34, 37, 38, 41, 42* |
| hexenyl butanoate | 170 | *23, 25* |
| hexyl 2-methyl-2-propenoate | 170 | *9* |
| hexyl butenoate | 170 | *29* |
| 2-methyl-1-butyl 2-methyl-1-butanoate | 172 | *ts, 10, 25, 26, 33, 34, 42* |
| 2-methyl-1-propyl hexanoate | 172 | *ts, 17, 34* |
| 2-methylhexyl propanoate | 172 | *25* |
| butyl hexanoate | 172 | *ts, 3, 4, 9, 16, 17, 19, 20, 23–27, 32–34, 37, 38, 41, 42* |
| ethyl octanoate | 172 | *15, 28* |
| hexyl 2-methylpropanoate | 172 | *37* |
| hexyl butanoate | 172 | *ts, 1, 3, 4, 9, 15–20, 22–29, 31–34, 37, 38, 42* |
| octyl acetate | 172 | *ts, 20* |
| pentyl 2-methyl-1-butanoate | 172 | *ts, 4, 9, 23, 25, 33, 34* |
| hexenyl 2-methyl-1-butanoate | 184 | *23, 25* |
| 2-methyl-1-butyl hexanoate | 186 | *ts, 17, 25, 28, 34, 42* |
| hexyl 2-methyl-1-butanoate | 186 | *ts, 1, 3, 4, 9, 10, 15–17, 19–26, 28, 29, 31–35, 37, 38, 42* |
| hexyl pentanoate | 186 | *32–34* |
| pentyl hexanoate and butyl heptanoate | 186 | *ts, 23, 28, 34, 37, 42* |
| propyl octanoate | 186 | *ts, 18, 20, 34, 41, 42* |
| hexenyl hexanoate | 198 | *4, 23, 25* |
| 2-methyl-1-butyl heptanoate ? | 200 | *ts, 18, 20, 34, 41, 42* |
| 2-methyl-1-propyl octanoate | 200 | *ts, 18, 20, 34, 41, 42* |
| butyl octanoate | 200 | *ts, 25, 34, 41, 42* |
| heptyl 2-methyl-1-butanoate | 200 | *ts, 18, 20, 34, 41, 42* |
| hexyl hexanoate | 200 | *ts, 3, 9, 10, 15–18, 20, 23–25, 27, 29, 32–34, 37, 38, 41, 42* |
| nonyl propanoate | 200 | *ts, 28* |
| octyl butanoate | 200 | *28* |
| 2-methyl-butyl octanoate | 214 | *25* |
| C13 ester | 214 | ts |
| hexyl octanoate | 228 | *ts, 37* |

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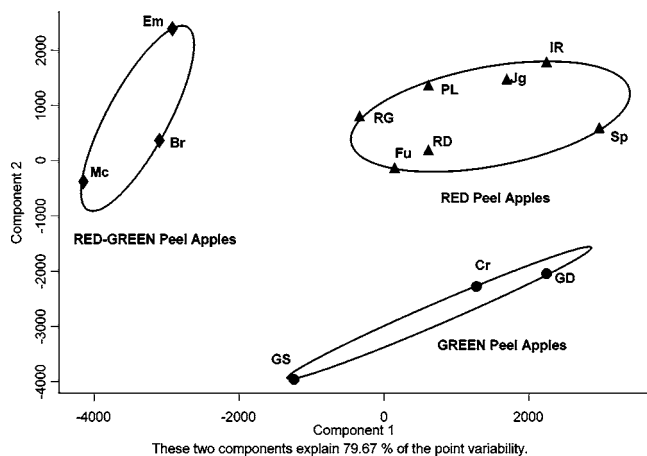*J. Agric. Food Chem.,* Vol. 52, No. 26, 2004  **8091**



**Figure 2.** Principal component analysis clustering of headspace levels of α-farnesene and esters in 13 varieties of apples. See **Table 1** for meaning of apple variety abbreviations. See **Table 2** for raw data used for this analysis.
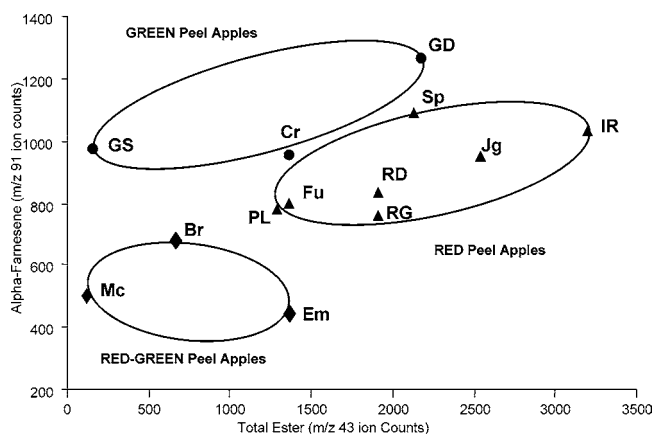


**Figure 3.** Clustering of headspace levels of α-farnesene and total esters in 13 varieties of apples. α-Farnesene levels were based on summation of the ion counts for *m/z* 91, and total ester levels were based on summation of the ion counts for *m/z* 43. See **Table 1** for meaning of apple variety abbreviations.



**Figure 4.** Relative changes in headspace levels of α-farnesene and total esters for Royal Gala apples under different storage times and conditions. Levels were determined by sampling with polydimethylsiloxane solid-phase microextraction fibers followed by gas chromatography–mass spectrometry. Initial levels were normalized to equal 1, and α-farnesene and esters are shown on same scale. Apples had been held under controlled atmosphere conditions for about 6.5 months at 1 °C and then stored in cold storage at 1 °C for 10 days under ambient atmosphere before this study. Apples were placed in a refrigerator at 4 °C and sampled periodically. At the end of each sampling period, the same apples were further held at 23 °C for additional sampling periods.

taken out of CA were held under the following conditions: Apples were stored in cold storage at 1 °C for 10 days before the start of the two holding periods. During the first holding period, apples were placed in a refrigerator at 4 °C for 1, 4, 11, 18, or 25 days. At the end of each of the various first holding periods, the same apples were further held at 23 °C for an additional 4, 11, and 18 days. At 4 °C, the total ester content slowly increased and reached a maximum at 18 days. However, when apples were then stored at room temperature, there was a more rapid increase in the esters released, and the total contents reached a maximum after 11 days. Overall, from the initial day, there was a maximum 25-fold increase in total ester content. On the other hand, levels of α-farnesene increased slowly in the refrigerator or at room temperature after coming from CA storage, and the overall increase was only 5-fold.

As noted above, the total ester contents released by apples under CA conditions increased when stored at higher temperatures and normal atmosphere. This prompted us to determine if there were any changes in the proportions of the individual constituents. **Figure 5** shows that for Royal Gala apples coming out of (a) 6.5 months of CA storage at 0 °C, (b) 10 days of cold storage at 1 °C, (c) 4 °C for 18 days, and (d) finally at room temperature for 11 days, for the final stage, the increase in levels of lower molecular weight esters in the volatiles was
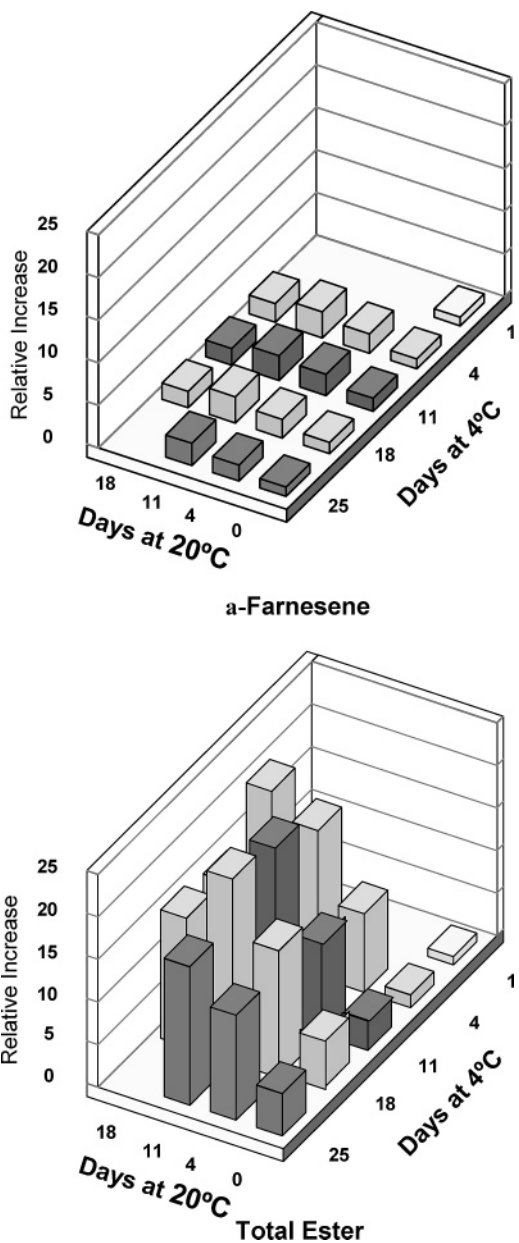
greater than for higher molecular weight esters. For example, the increases over 11 days at room temperature were 12.5-fold for the isomeric esters of molecular weight 116 but only 1.3-fold for the isomeric esters of molecular weight 214. When data reported by Fellman et al. (Table 3 of ref *31*) for esters in Red Delicious apples 8 days after removal from CA or refrigerated air were plotted the same way, a similar trend was observed.

In summary, SPME coupled with GC-MS has been demonstrated to be a useful technique for determining the identity and content of volatiles released by different apple varieties. Statistical analysis of the ester and α-farnesene levels thus
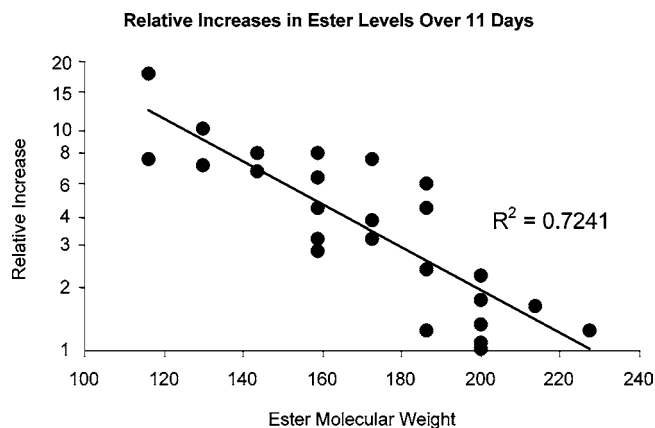
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**Figure 5.** Relative increases in individual ester levels in headspaces of Royal Gala apples coming from controlled atmosphere storage at 1 °C, then storage at 1 °C for 10 days under ambient atmosphere and at 4 °C for 18 days. Changes were measured from then until after storage at 23 °C for 11 days. Levels were determined by sampling with polydimethylsiloxane solid-phase microextraction fibers followed by gas chromatography–mass spectrometry.

generated correlated with clustering of apple varieties on the basis of peel color. In a study of Golden Delicious and Granny Smith apples, Lopez et al. (*51*) reported that although ∼80% of total volatile components in the two varieties were esters, the aroma components varied in class and quantity and thus provided characteristic sensory properties for each variety. Our results also showed variation in the quantity of individual and total esters measured: McIntosh and Granny Smith apples had low levels of total esters, and Spartan, Ida Red, and Jonagold apples had high levels of total esters. It is well established that individual esters have unique odors. The combination of up to 40 individual esters and their relative quantities present in the mixture of volatiles released by apple fruit would explain the characteristic aroma for each apple variety.

The technique also showed that monitoring of changes in ester content under different storage conditions was facile. The data revealed that low molecular weight ester levels increase more quickly after coming out of CA conditions than do high molecular weight esters. This may have biosynthetic implications.

**ABBREVIATIONS USED**

Br, Braeburn apples; CA, controlled atmosphere; CAR, Carboxen; Cr, Crispin apples; DVB, divinylbenzene; Em, Empire apples; Fu, Fuji apples; GC, gas chromatography; GD, Golden Delicious apples; GS, Granny Smith apples; IR, Ida Red apples; Jg, Jonagold apples; Mc, McIntosh apples; MS, mass spectrometry; PCA, principal component analysis; PDMS, polydimethysiloxane; PL, Pink Lady apples; RD, Red Delicious apples; RG, Royal Gala apples; Sp, Spartan apples; SPME, solid-phase microextraction.

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