Quantification of α-Farnesene and Its Conjugated Trienol Oxidation Products from Apple Peel by C_{18}-HPLC with UV Detection

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Conjugated triene oxidation products of the sesquiterpene α-farnesene are thought to induce development of the storage disorder superficial scald. A C_{18}-HPLC method was devised which allows simultaneous quantification of α-farnesene and its major oxidation product, a conjugated trienol, in hexane extracts of apple peel using UV detection at 232 and 269 nm. Identification of the compounds was confirmed by UV spectrometry, HPLC with diode array detection, and GC−MS. Apples of high (Granny Smith, GS), moderate (Red Delicious, RD), and low (Gala) susceptibility to scald were stored for up to 6 months at 0 °C under 1.5, 3, or 100% oxygen or in air. Peel samples were analyzed for α-farnesene and conjugated trienol content, which correlated well with scald susceptibility and occurrence. Levels of both sesquiterpenoids were ≥20-fold higher in GS than in Gala. Storage under low (1.5 or 3%) oxygen decreased farnesene and trienol levels in each cultivar. Two lots of RD fruit harvested 1 week apart differed markedly in farnesene and trienol production; fruit from the earlier harvest had higher levels of both and developed scald, whereas fruit from the later harvest did not scald. These results support the proposal that scald susceptibility of apple cultivars is a function of farnesene production and its oxidation to conjugated trienols.

Keywords: *α-Farnesene; conjugated triene; C_{18}-HPLC; apple; storage; scald*

INTRODUCTION

The sesquiterpene α-farnesene accumulates in the peel of many apple and pear cultivars during low-temperature storage, and its oxidation products, conjugated trienes, have been linked with development of a serious physiological storage disorder known as superficial scald (Huelin and Murray, 1966; Anet, 1972; Ingle and D'Souza, 1989). Since the correlation between farnesene oxidation and scald was established 30 years ago (Huelin and Murray, 1966), many reports have been published concerning the influence of various pre- and postharvest factors on the accumulation of farnesene and conjugated trienes in the peel of stored apples and on the incidence and severity of superficial scald [e.g., Watkins *et al.* (1993) and Barden and Bramlage (1994)]. Generally these studies have relied on UV absorbance measurements of crude hexane or heptane dip extracts of whole fruit for quantification of α-farnesene (UV maximum at 232 nm) and conjugated trienes (UV maximum at 269 nm with secondary maxima at 259 and 281 nm) (Meir and Bramlage, 1988; Lurie *et al.*, 1989). This method is inherently subject to errors imposed by the presence of interfering, UV-absorbing compounds, including the antioxidant diphenylamine (DPA), which is used commercially to control scald (Huelin and Coggiola, 1970; Gallerani and Pratella, 1991).

Until quite recently, it was assumed that the conjugated trienes, which accumulate in apple peel after several months of storage, were identical with the hydro- and endoperoxides shown by Anet (1969) to be products of *in vitro* autoxidation of α-farnesene. However, Rowan *et al.* (1995) have now unequivocally demonstrated that the bulk of the conjugated trienes produced *in vivo* are the more stable 2,7,9,11-tetraen-6-ols, with the 7*E*,9*E* isomer generally composing 90% or more of the total and the 7*E*,9*Z* isomer constituting most of the remainder. Prior to publication of their work, we initiated an independent study with the aim of identifying and quantifying the conjugated trienes in the waxy coating of stored apples. We report here the development of a reversed phase (C_{18}, ODS-2) HPLC method with dual-wavelength UV detection that can be used to simultaneously quantify α-farnesene and conjugated trienes in hexane extracts of apple peel. We have corroborated the finding of Rowan *et al.* (1995) that the principle *in vivo* oxidation products of farnesene in apple are conjugated trienols. Furthermore, we have used the new HPLC method to examine the temporal relationship between farnesene and trienol accumulation during storage of three apple cultivars differing in scald susceptibility under various levels of oxygen and ventilation.

MATERIALS AND METHODS

Plant Material and Storage Conditions. The three apple cultivars used in the study were of high (Granny Smith, GS), moderate (Red Delicious, RD, or low (Gala) susceptibility to superficial scald. Two groups of RD fruit were harvested 1 week apart, on September 25 and October 2, 1995, at a commercial orchard in southern Pennsylvania. Gala apples were picked on August 26 and GS on October 10, 1995, at the University of Maryland orchard in western Maryland. RD fruit were stored for 2 months in tray-packed boxes in air, at which time a random sampling from both harvests was

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transferred to flow-through chambers (200 L volume) supplied with humidified air at 75 mL/min or a mixture of 1.5% O_2 + 98.5% N_2 at 95 mL/min. Gala and GS fruit were stored in boxes with plastic liners (air controls) or placed directly after harvest in 8-L desiccator jars supplied with a flow of air, 100% O_2, 1.5% O_2 + 98.5% N_2, or 3% O_2 + 97% N_2 at 60 mL/min (no Gala fruit were stored in 100% O_2 and no GS fruit were stored in 3% O_2). All fruit were stored at 0 ± 1 °C for a maximum duration of 5−6 months. Groups of 5−10 fruit were periodically removed from storage for analysis (usually at 4−5-week intervals). After 2−3 h at room temperature, the peel and outer 2−3 mm of cortical tissue were excised with a mechanical peeler and frozen in liquid N_2. Tissue from each apple was sealed in a separate small plastic bag. The total samples from each time × cultivar × storage atmosphere were then sealed in a large plastic bag and stored at −80 °C until used (not more than 3 weeks).

Isolation of α-Farnesene and Conjugated Trienes. Frozen peel tissue (10 g) from RD fruit from the 1994 season that had been stored for 6 months in air at 0 °C was lyophilized for 16 h, pulverized, and extracted 1 h with 20 mL of hexane at 5 °C under N_2 atmosphere. The extract was concentrated to 2 mL by N_2 evaporation and then held 2 h at −80 °C to precipitate much of the wax. After centrifugation for 2 min at 1000*g*, the extract was decanted and then fractionated on a small pipet column containing 3 g of 60−100-mesh Florisil. The column was washed with 2,2,4-trimethylpentane, the hexane extract was applied, and then the column was sequentially eluted with hexane (6 mL), hexane/ether (20:1, 8 mL), and hexane/ether (8:1, 9 mL). UV spectra of the Florisil column fractions indicated that all of the α-farnesene was in the hexane eluate (single absorbance maximum at 232 nm) and the bulk of the conjugated trienes was in the hexane/ether (8:1) eluate (absorbance maxima at 259, 269, and 281 nm). Aliquots of these farnesene- and conjugated triene-enriched fractions were dissolved in methanol after N_2 evaporation of the solvent and analyzed by HPLC on a 3.9 × 150 mm, 4-μm NovaPak C_{18} column (Waters Assoc.) using a Hewlett-Packard 79994A HPLC ChemStation and 1040A diode array detector. The mobile phase was methanol delivered at 0.5 mL/min by a Waters 510 pump, and 40 μL samples were injected by a Waters 712 WISP automatic injector. Under these conditions, prominent peaks eluting at 3.33 and 1.59 min were tentatively identified by their UV spectra as α-farnesene and a conjugated triene, respectively. HPLC-purified samples of these components were prepared for gas chromatographic and mass spectral analysis as follows: Peak eluates from a series of four injections were pooled and diluted 1:1 with 0.8% aqueous NaCl and then phase partitioned three times with 1 mL of hexane. The pooled hexane phases containing the pure farnesene or conjugated triene were N_2 evaporated, and the samples were redissolved in 200 μL of 2,2,4-trimethylpentane.

Capillary Gas Chromatography (GC). Capillary GC analysis of the HPLC-purified α-farnesene and conjugated triene samples was performed using a Hewlett-Packard 5890 Series II instrument equipped with a flame ionization detector and fitted with a 30 m × 0.25 mm i.d. (0.20 μm film thickness) SPB-1 column (Supelco). The column oven was isothermal at 150 °C, the injector was set at 200 °C and the detector at 250 °C, and the column head pressure was 10 psi of helium. Data were collected using an HP 3396 Series II integrator. The farnesene sample gave a single peak with a retention time of 13.1 min, whereas the conjugated triene sample gave a minor (≤10%) and a major (≥90%) peak with retention times of 22.4 and 23.7 min, respectively.

Capillary Gas Chromatography-Mass Spectrometry (GC−MS). Electron impact (EI) and chemical ionization (CI; methane and/or ammonia) capillary GC−MS analyses of the HPLC-purified α-farnesene and conjugated triene samples were carried out using a Finnigan-MAT 4510 mass spectrometer fitted with a 30 m × 0.32 mm i.d. (0.25-μm film thickness) DB-1 column (J&W Scientific, Folsom, CA). For both EI and CI analyses, the column head pressure was 13 psi of helium and the column temperature program was 2 min at 140 °C, heat to 200 °C at 5 °C/min, and then heat to 270 °C at 30 °C/min. The MS source temperature was set at 150 °C for the EI mode and at 60 °C for the CI mode.

The 70 eV EI spectrum of our farnesene sample matched that obtained by Murray (1969) and was confirmed as α-farnesene (MW = 204) by the NIST GC−MS library. The most intense ion was *m*/*z* 93, followed by *m*/*z* 69 (55), 55 (53), 79 (45), 107 (43), 123 (43), and 119 (42). Above *m*/*z* 140 the major ions, in decreasing order, were *m*/*z* 161, 147, 148, 189, and 204 (M^+), all ≤3 in relative intensity. In the methane CI spectrum of the farnesene sample, *m*/*z* 203 and 205, representing M^+ plus or minus a proton, were both prominent, with intensities of 75 and 89, respectively, relative to *m*/*z* 123 (100).

The major and minor conjugated triene peaks noted in the capillary GC chromatogram (with retention times of 23.7 and 22.3 min, respectively) had similar EI spectra. For the major peak the EI specturm showed *m*/*z* 220 (M^+, <1), 202 (11), 187 (3), 162 (42), 159 (31), 147 (12), 137 (24), 133 (21), 119 (43), 107 (37), 105 (61), 95 (65), 93 (86), 91 (67), 79 (51), 69 (80), and 55 (100), whereas for the minor peak the spectrum showed *m*/*z* 202 (13), 187 (2), 162 (23), 159 (33), 147 (7), 137 (15), 133 (27), 119 (36), 107 (34), 105 (63), 95 (47), 93 (83), 91 (72), 79 (61), 69 (100), and 55 (79). Both the methane and ammonia CI spectra of the two conjugated trienes showed *m*/*z* 203 as the predominant ion. The methane CI spectrum of the major component showed *m*/*z* 243 (1), 231 (4), 221 (9), 219 (6), 203 (100), 163 (30), 147 (18), 137 (8), 127 (13), 109 (29), and 95 (14) and that of the minor component showed *m*/*z* 243 (<1), 231 (2), 221 (1), 219 (3), 203 (100), 163 (20), 147 (10), 135 (5), 127 (13), 109 (31), 95 (12). The ammonia CI spectrum of the major triene showed *m*/*z* 255 (35), 238 (13), 220 (4), 203 (100), and 161 (3) and that of the minor triene showed *m*/*z* 255 (50), 238 (15), 220 (2), 203 (100), and 161 (3).

Preparation of Samples for HPLC Analysis. Initially samples of peel tissue were lyophilized overnight, pulverized, and extracted with hexane, but this procedure was found to result in ≥50% loss of α-farnesene because of its volatility. Subsequently, 10 g frozen peel samples from individual fruit were ground to a powder in liquid N_2 with a mortar and pestle and transferred to 50-mL Teflon-lined screw-cap culture tubes containing 20 mL of HPLC-grade hexane. The tubes were flushed with N_2, sealed, and agitated in the dark at 5 °C for 1 h. Extracts were vacuum filtered through glass fiber disks and restored to a 20-mL total volume. Aliquots (1 mL) of the extracts were transferred to 2-mL vials, and the hexane was evaporated under a gentle stream of N_2 without heating. The residue was dissolved in 400 μL of HPLC-grade methanol and filtered through a 0.45-μm PTFE membrane prior to HPLC analysis. Comparison (by HPLC) of samples prepared in this way with samples containing an equivalent amount of crude hexane extract (after dilution with one volume of 2-propanol and two volumes of methanol) showed that this procedure gave 86 ± 2% recovery of α-farnesene and 97 ± 2% recovery of conjugated trienes in a matrix suitable for C_{18}-HPLC while removing a substantial amount of methanol-insoluble wax.

HPLC Analytical Conditions. Samples (80 μL) were injected manually by a Rheodyne injector with a 200-μL loop into a Waters 600MS HPLC system fitted with a 4.6 × 250 mm, 5-μm Spherisorb C_{18} column (Alltech). The mobile phase was methanol/acetonitrile/water (90:5:5) pumped at 0.8 mL/min. Absorbance at 232 (α-farnesene) and 269 nm (conjugated trienes) was monitored simultaneously by a Waters 490 programmable wavelength detector, and data were gathered and processed using the Waters Maxima 820 program operating in a 286 PC. With this HPLC system, α-farnesene gave a single peak that eluted at 10.7 min, whereas the conjugated trienes gave a prominent peak at 5.0 min with a small shoulder discernable at 5.2 min. Calculation of α-farnesene and conjugated triene concentrations was based on their respective published molar extinction coefficients, $\epsilon_{232\ nm} = 27\ 740$ (Huelin and Coggiola, 1968) and $\epsilon_{269\ nm} = 42\ 500$ (Anet, 1969). HPLC-purified farnesene and conjugated triene samples of known concentration (determined by UV spectrometry) were used to establish conversion factors for calculation of concentrations of the compounds in apple peel extracts from their HPLC peak areas.

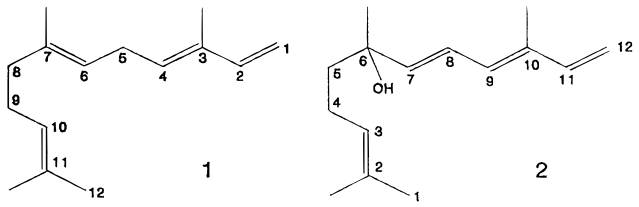

Figure 1. Structures of α-farnesene [3,7,11-trimethyldodeca-1,3(*E*),6(*E*),10-tetraene] (**1**) and a conjugated trienol [2,6,10-trimethyldodeca-2,7(*E*),9(*E*),11-tetraen-6-ol] (**2**) identified by Rowan *et al.* (1995) as the major oxidation product of α-farnesene in the natural coating of apple peel.

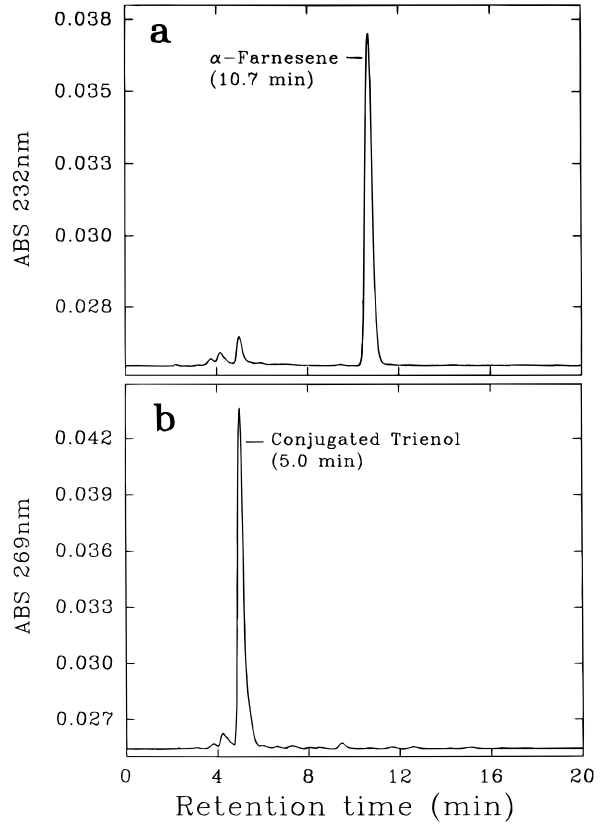

Figure 2. C_{18}-HPLC chromatograms of (a) α-farnesene and (b) conjugated trienols in hexane extracts of peel samples from Granny Smith apples with moderately high levels of the compounds. The trienol peak was shown to include two components, major and minor isomers, by capillary GC and GC−MS.

RESULTS

α-Farnesene and Conjugated Trienols in Hexane Extracts of Apple Peel. Figure 1 shows the structures of α-farnesene [3,7,11-trimethyldodeca-1,3(*E*),6(*E*),10-tetraene] and its major conjugated trienol oxidation product in the natural coating of apple peel [2,6,10-trimethyldodeca-2,7(*E*),9(*E*),11-tetraen-6-ol] identified by Rowan *et al.* (1995). Typical C_{18}-HPLC chromatograms of α-farnesene and conjugated trienes in extracts of peel samples from GS apples with moderately high levels of the compounds are shown in Figure 2. The 232-nm-absorbing component eluting at 10.7 min was identified as α-farnesene by EI and methane CI GC−MS. The molecular weight of the major and minor 269-nm-absorbing components eluting at 5.0 min was deduced to be 220 from their EI and CI mass spectra, and it was also apparent that these compounds readily lose a molecule of water and are then protonated in the CI mode ($M^+ - 18 + H^+ = m/z$ 203). These data, along with the characteristic conjugated triene UV spectra showing maxima at 259, 269, and 281 nm, support the conclusion that the two compounds are isomers of the conjugated trien-6-ols reported by Anet (1969) and Rowan *et al.* (1995). The slight shoulder on the right side of the 5.0-min peak in Figure 2b is probably the minor trienol isomer that was baseline-resolved by capillary GC and GC−MS. The small peaks present at 3.8 and 4.2 min are not likely to be conjugated trienes because their absorbance at 232 nm is almost equal to that at 269 nm. The ratio of the major to minor trienol, determined by GC, was ≥9:1, and based on the findings of Rowan *et al.* (1995), they are tentatively identified as the 7(*E*),9(*E*) (Figure 1) and 7(*E*),9(*Z*) isomers, respectively.

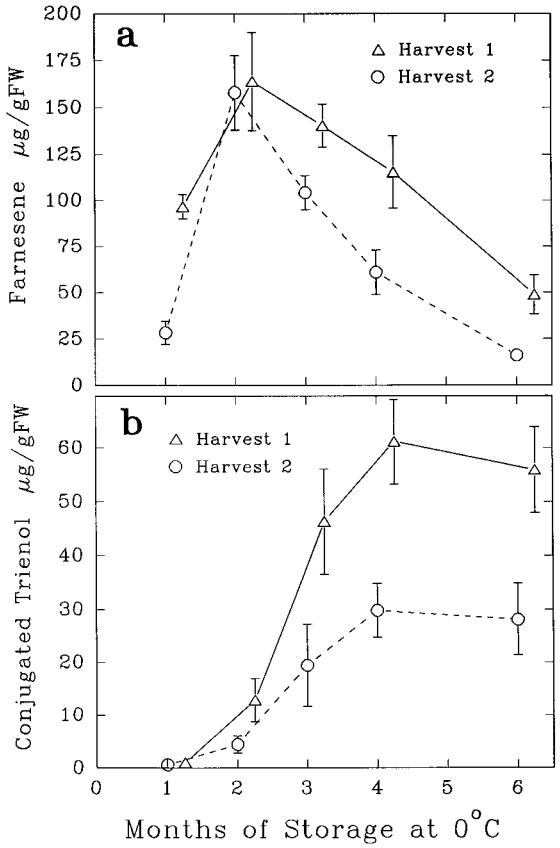

Figure 3. Time course of changes in (a) α-farnesene and (b) conjugated trienol levels in two groups of Red Delicious apples harvested 1 week apart during storage at 0 °C in air. Vertical bars indicate ±SD (n = 6).

Influence of Maturity and Storage Atmosphere on Farnesene and Trienol Levels in Red Delicious Apples. Two groups of RD apples harvested 1 week apart showed the same time course of changes in farnesene and trienol levels during storage at 0 °C in air, but fruit from the first harvest produced greater amounts of both sesquiterpenoids (Figure 3). Although the peak of farnesene accumulation, which occurred at 2 months, was about the same in the two groups of fruit (∼160 μg/g FW), the level increased sooner and declined more gradually in apples from the earlier harvest (Figure 3a). In contrast with farnesene, there was a lag of 1 month before conjugated trienols began to increase, and the peak accumulation was reached at 4 months, with little decline from 4 to 6 months (Figure 3b). From 2 months through the end of storage, the level of trienols was consistently ∼2-fold higher in RD fruit from the first harvest, reaching a maximum of ∼60 μg/g FW.

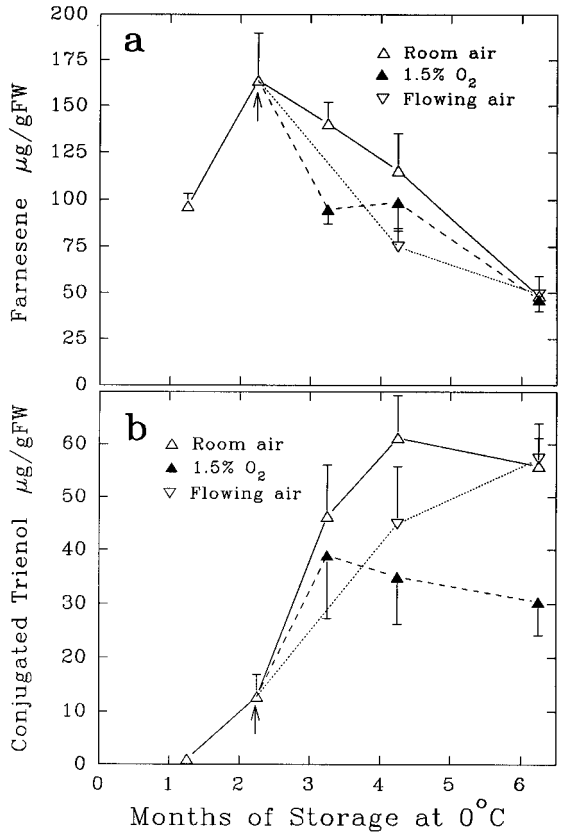

Figure 4. Changes in levels of (a) α-farnesene and (b) conjugated trienols after transfer of first-harvest Red Delicious fruit from boxes ("room air") to flow-through chambers supplied with air or 1.5% O_2 after 2 months of storage. Vertical bars indicate ±SD ($n = 6$).

Transfer of RD fruit after 2 months of storage from boxes to flow-through chambers supplied with humidified air or 1.5% O_2 caused a subsequent decrease in the levels of farnesene and trienols. Data for RD apples from the first harvest show that flowing air and 1.5% O_2 were equally effective in reducing farnesene over the 2-month interval following transfer (Figure 4a), whereas 1.5% O_2 was much more effective than flowing air in decreasing accumulation of trienols, reducing the level to about half that in the room air (boxed) controls over the last 2 months of storage (Figure 4b). Similar results were obtained with RD fruit from the second harvest (data not shown), although the reductions in farnesene relative to the room air controls were not as great, and the long-term (through the end of storage) reductions in trienols were more substantial with both flowing air and 1.5% O_2. At the end of the 6-month storage period, all remaining RD fruit were transferred to 20 °C in air for 8 days to monitor development of superficial scald. Apples from the first harvest stored continuously in boxes in air were the only group to exhibit scald symptoms. Of these, ~60% (14 of 23) showed brown discoloration on ≥25% of the fruit surface.

Influence of Storage Atmosphere on Farnesene and Trienol Levels in Granny Smith Apples. The time course of changes in α-farnesene and conjugated trienol levels in the peel of GS apples stored in boxes in air or in flow-through jars supplied with low (1.5%) or high (100%) O_2 is depicted in Figure 5. After 3 weeks at 0 °C, farnesene had accumulated to nearly 60 μg/g FW in the room air (boxed) controls but the level of trienols was still negligible. Farnesene peaked at 7 weeks at ~265 μg/g FW in both air control and 100% O_2 fruit, whereas for fruit in 1.5% O_2, the maximum

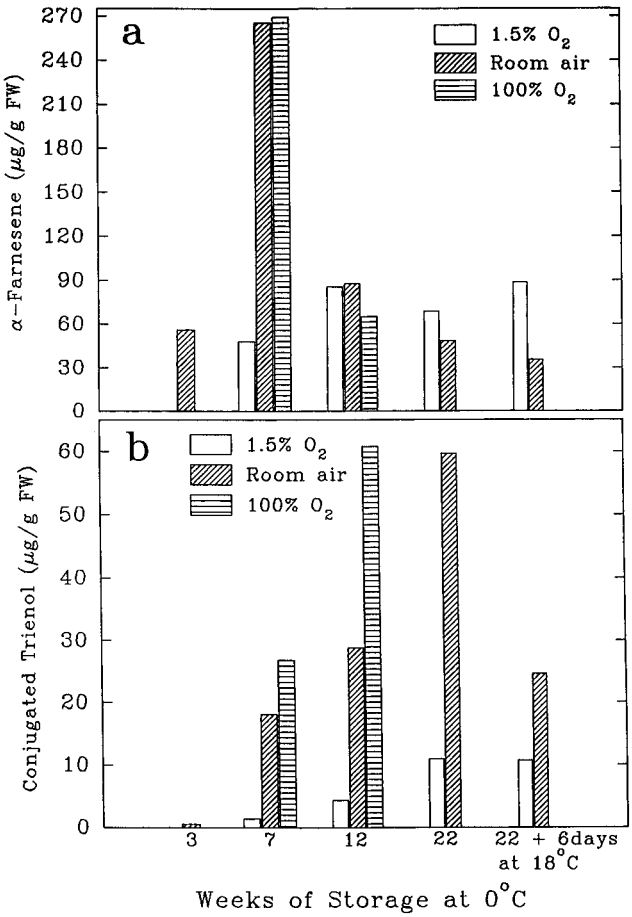

Figure 5. Changes in (a) α-farnesene and (b) conjugated trienol levels in the peel of Granny Smith apples stored at 0 °C in boxes in air ("room air") or in flow-through jars supplied with low (1.5%) or high (100%) O_2. Values are the mean of analyses of peel samples from five fruit. Apples in 100% O_2 were severely injured at 12 weeks and were discarded. After 22 weeks, air control and 1.5% O_2 fruit were transferred to 18 °C for 6 days prior to a final sampling and assessment of scald.

level was reached at 12 weeks and was ~3-fold lower than the peak in the other two groups. Conjugated trienols accumulated more rapidly in fruit under 100% O_2 than in those stored in air. Peak accumulation of trienols, ~60 μg/g FW, was reached at 12 weeks in 100% O_2 and at 22 weeks in air. The increase in trienols during storage followed a similar time course in 1.5% O_2 compared with air control fruit, but trienol levels were at all times ≥5-fold lower in apples stored under the low-O_2 atmosphere. A small group of GS fruit was also stored in jars supplied with humidified air and sampled after 22 weeks at 0 °C (data not shown). The level of farnesene in the peel of these fruit was equal to that in the 22-week room air samples, whereas the level of trienols was ~20% lower.

GS fruit stored in 100% O_2 showed symptoms of extensive injury after 12 weeks; the entire peel was "bronzed" and a high level of ethanol had accumulated in the cortex. Hence, these fruit were discarded after the 12-week time point, with no further analyses of farnesene and trienols. After 22 weeks, all remaining GS apples stored in boxes in air, in flowing air, or in 1.5% O_2 were transferred to 18 °C (in air) for 6 days. At this time, scald development was evaluated and final peel samples were taken for sesquiterpenoid analysis. Over 90% of the boxed fruit (32 of 35) and 88% of the flowing air fruit (22 of 25) showed moderate to severe scald, with blackened patches covering ≥25% of the

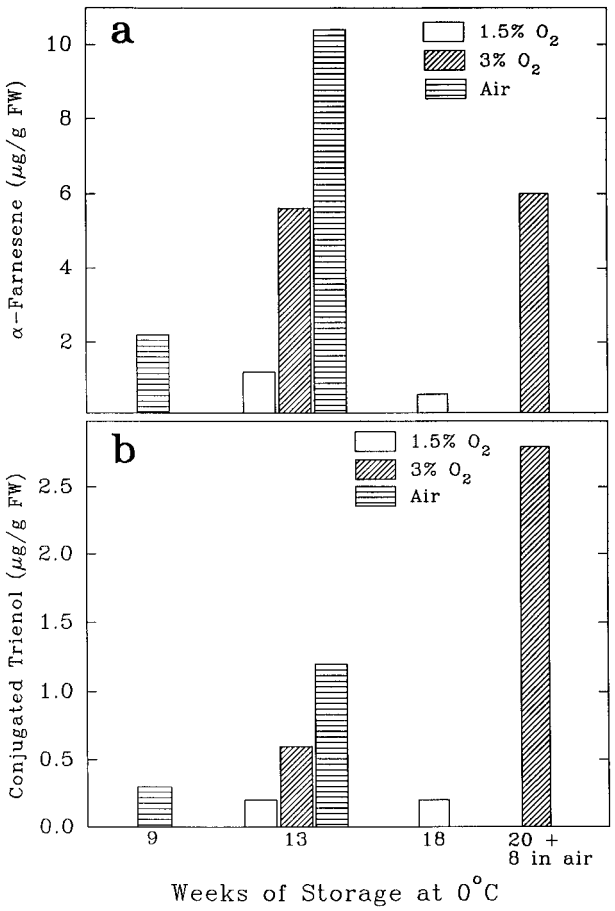

Figure 6. Effect of low O_2 (1.5 or 3%) atmosphere on levels of (a) α-farnesene and (b) conjugated trienols in Gala apples stored at 0 °C. Values are the mean of analyses of samples from five fruit. Air control fruit were discarded after 13 weeks because of extensive softening. After 20 weeks, a group of fruit was transferred from 3% O_2 to air for 8 weeks prior to a final sampling.

surface. In sharp contrast, fruit stored in the flow-through system under 1.5% O_2 ($n = 22$) were completely free of scald. During the 6 days after transfer to 18 °C, the levels of farnesene and trienols in the peel of air control apples decreased by ~30 and 60%, respectively, whereas in low-O_2 fruit farnesene increased ~25% and trienols remained unchanged. In apples from the flow-through air jars, farnesene did not change and trienols decreased by ~40%.

Influence of Storage Atmosphere on Farnesene and Trienol Levels in Gala Apples. Small groups of Gala apples were stored in flow-through jars supplied with humidified air or 1.5 or 3% O_2. Peel samples were taken from each group at two time points for farnesene and trienol analysis. Regardless of the storage atmosphere, levels of the sesquiterpenoids were very low; farnesene never exceeded 11 μg/g FW and trienols were always ≤3 μg/g FW (Figure 6). Both farnesene and trienols increased ~5-fold in air-stored fruit over the span of 9−13 weeks postharvest. At the 13-week sampling, storage under low O_2 had clearly reduced accumulation of the sesquiterpenoids. Relative to air, 1.5% O_2 decreased the levels of farnesene and trienols about 10- and 6-fold, respectively. Storage in 3% O_2 was less effective than 1.5% O_2. Beyond 13 weeks, the air-stored fruit had ripened and softened extensively and were discarded without further sampling. Between 13 and 18 weeks of storage, farnesene decreased slightly and trienols did not change in the peel of fruit in 1.5% O_2. A final set of samples was taken from apples stored in 3% O_2 for 20 weeks and then transferred to boxes in air for a subsequent 8 weeks. Relative to the 3% O_2 fruit sampled at 13 weeks, farnesene was at the same level, whereas trienols had increased nearly 5-fold.

DISCUSSION

The findings of this study are thoroughly consistent with those of Rowan *et al.* (1995) with regard to the oxidation products of α-farnesene in apple peel; i.e., the conjugated trienes that accumulate are two isomers of 2,6,10-trimethyldodeca-2,7,9,11-tetraen-6-ol. On the basis of the proportions of the major and minor trienols, ≥9:1, they are presumed to be the 7(*E*),9(*E*) and 7(*E*),9(*Z*) isomers, respectively, as determined by ^{1}H NMR in the earlier investigation (Rowan *et al.*, 1995). The isocratic C_{18}-HPLC method we have developed allows simultaneous quantification of α-farnesene and conjugated trienols with a run time of ≤15 min and no need for re-equilibration. The normal phase (silica) HPLC method used by Rowan *et al.* gave slightly better separation of the trienol isomers but required a prior cleanup step on a silica SPE column with inclusion of an internal standard for quantitative analysis (D. Rowan, personal communication). In addition, because of the SPE column elution procedure, their silica HPLC method could not be used for simultaneous analysis of α-farnesene and trienols. A normal phase HPLC method was also devised by Gallerani and Pratella (1991) and used to quantify conjugated trienes in hexane extracts of GS apples that were or were not treated with DPA prior to storage. Curiously, the major HPLC peak that gave a typical conjugated triene UV spectrum was tentatively identified as a hydroperoxide which upon reduction with sodium borohydride was converted to a conjugated trienol [as in Anet (1969)]. At present we can offer no explanation for this apparent discrepancy.

The time course of α-farnesene and conjugated triene accumulation in the peel of GS and RD apples determined using our C_{18}-HPLC method is in accord with a number of earlier reports [e.g., Huelin and Coggiola (1968, 1970), Lurie *et al.* (1989), and Barden and Bramlage (1994)]. Farnesene peaked and then declined well before the trienols, which remained elevated through the end of storage. High levels of trienols were correlated with the incidence of scald, but the fact that conjugated trienes decline with prolonged storage or after rewarming of stored fruit [Huelin and Coggiola (1970); this study] suggests that a degradation product of trienols may trigger scald development or that other factors such as depletion of endogenous antioxidants are involved in the deteriorative chain of events (Anet, 1972; Meir and Bramlage, 1988).

Many reports have indicated that the apple cultivar, harvest maturity, and storage atmosphere are three factors important in determining the susceptibility to and incidence of superficial scald (Ingle and D'Souza, 1989). Some have shown a close correlation between the influence of these factors on production of farnesene and/or trienes and development of scald (Huelin and Coggiola, 1968, 1970; Barden and Bramlage, 1994), whereas others (Meigh and Filmer, 1969; Anet, 1972) have not. Using the new HPLC method for quantification, we found the following: (1) Highly scald-susceptible GS accumulated ≥20-fold more farnesene and trienols than scald-resistant Gala and ≥60% more farnesene than moderately scald-susceptible RD. (2) Early-harvest RD produced more farnesene and about

twice as much trienol as fruit harvested just 1 week later. (3) The only fruit with a high incidence of scald, GS and first-harvest RD stored in air, also had the highest accumulation of trienols (peak ~60 μg/g FW). (4) Production of both farnesene and trienols was reduced considerably in all three cultivars by storage under 1.5% O_2 in a flow-through system. Concerning the last observation, experiments over the last three seasons have shown good control of scald in GS apples by storage in 1.5% O_2 (T. Solomos, unpublished). Other studies with RD apples have determined that, for storage durations of 7−9 months, levels of O_2 below 1% may be required to control scald, particularly in early-harvest fruit (Chen *et al.*, 1985; Lau, 1990).

Overall our results support the 30-year-old hypothesis of Huelin and Murray (1966) that farnesene oxidation and the consequent accumulation of conjugated trienes is a primary factor leading to the development of scald. The C_{18}-HPLC method devised for this study will be useful to further test the hypothesis in a wider selection of cultivars under various storage regimes.

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