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Effect of maturity and storage on quality and volatile production of 'Jonagold' apples

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'Jonagold' apples (*Malus domestica* Borkh.) harvested 3 times at bi-weekly intervals starting approximately two weeks prior to commercial harvest were stored at 0°C in air and controlled atmosphere (CA, 1·5% O_2 + 1·5% CO_2) for six months. Starch index (SI) increased from 2·1 to 8·4 during the harvest period. Early harvests and CA significantly ($P \leq 0{\cdot}05$) reduced the loss of acidity and firmness in stored apples but did not influence total soluble solids content. CA decreased the volatile production (esters, alcohols, and hydrocarbons) by half. The last harvest (SI = 8·4) provided an increase of 14% in volatiles compared to the first date (SI = 2·1). These increases were mostly due to straight C3 C6 chain acetates and alcohols, and the hydrocarbon α-farnesene. Variation in background skin colour within the same harvest period had a negligible effect on volatile patterns. Harvest at starch index values of 6·5–8·5 allowed higher concentration of odour-active compounds while retaining acceptable levels of acidity and firmness.

Keywords: Malus domestica, harvest date, storage regime, volatile production, starch index, titratable acidity, soluble solids concentration, colour.

INTRODUCTION

'Jonagold' apple was derived from a cross of 'Jonathan' by 'Golden Delicious' and has gained popularity in North America and Northern Europe for its high yield and dessert quality (Lovelidge, 1986). It is a late-keeping cultivar that can be stored for 9–10 months under controlled atmosphere (Stow, 1987; Lau, 1988).

Several factors influence post-storage apple quality including harvest maturity and storage conditions. Fruit harvested before or during the climacteric period achieved better quality retention during long-term storage than those harvested at the postclimacteric stage (Lau, 1985). Harvest indices have been developed to assess 'Jonagold' maturity and predict the harvest window. They include starch index (SI), internal ethylene concentration (IEC), soluble solids concentration (SSC), flesh firmness (FF) and titratable acidity (TA) (Lau, 1988). IEC is used as an index of maturity for other apple varieties (Liu, 1978; Chu, 1988). Increases in the concentration of esters have been detected several weeks prior to ethylene synthesis and ester appearance was suggested as an additional indicator of fruit maturity (Mattheis *et al.*, 1991).

Effect of refrigerated storage on apple quality depends on both the composition of the atmosphere and the length of storage. In comparison to conventional air storage, low O_2 and elevated CO_2 environments attenuate the loss of acidity, firmness and chlorophyll, and decrease the incidence of physiological disorders such as watercore, flesh breakdown, coreflush, and scald (Streif, 1985; Lau, 1988). CA conditions however suppress flavour development as observed in 'McIntosh' (Lidster *et al.*, 1981), 'Cox Orange' (Knee & Hatfield, 1981), and 'Golden Delicious' (Streif & Bangerth, 1988). Harvesting fruit at a proper stage of maturity may improve ester synthesis and thus the development of characteristic flavour after storage (Yahia *et al.*, 1990; Mattheis *et al.*, 1991).

Harvesting practices can also be designed to obtain desirable colour and appearance necessary for optimum grade classification. 'Jonagold' apple requires multiple picking for a higher packout based on the break of ground colour in the skin (from green to pale green or white–yellow). Whether background colour has any relationship to the volatile profile after storage is not known. The objective of this study was to investigate the effect of harvest maturity and type of storage on the quality and volatile profile of stored 'Jonagold' apples.

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MATERIALS AND METHODS

Plant source

Apples from 'Jonagold' trees grown on MM.111 rootstock planted at the Summerland Research Station in 1974 were harvested on 13 September, 27 September and 11 October 1991.

Harvest maturity and quality evaluation

Maturity and quality of fruit were determined on a 10-fruit sample immediately after each harvest according to methods of Lau (1988). Ethylene in a 1-ml gas sample from the core cavity of each fruit was determined on a Hewlett-Packard 5980 gas chromatograph equipped with a flame ionization detector (FID) and a stainless steel column (1·5 m × 3·2 mm — Supelco Inc., Toronto, ON) packed with Alumina F1 (60–80 mesh). The column was held isothermally at 130°C. Gas flows for He, H_2 and air were 30, 30, and 300 ml min^{-1}, respectively. Starch index (SI) at harvest was determined by dipping a thin, transversely cut slice taken from the equator of each fruit in a I_2–KI solution (7·5 g I_2 and 30 g KI in 3·4 l) for approximately 30 s and comparing the stained starch pattern to a 'Jonagold' starch chart developed by Lau (1988); higher values on a 0–9 scale are indicative of lower starch content and more advanced maturity. Yellow ground color was measured with the 'Golden Delicious Color Meter' (Techwest Enterprises Ltd, Vancouver, BC); higher values on a 0–10 scale indicated yellower fruit. Apple firmness was measured by a Magness–Taylor penetrometer equipped with a 11·1 mm diameter tip on opposite sides of each fruit (skin removed). Juice prepared with a Champion juicer was used in determining titratable acidity (TA, titration with 0·1 N NaOH to pH 8·1 and expressed as malic acid) and soluble solids concentration (SSC, refractometry).

Storage mode and post-storage fruit evaluation

Samples of 27 apples were assigned to each combination of harvest date (13 September, 27 September or 11 October) and storage modes (air or 1·5% O_2 + 1·5% CO_2 at 0°C and 90–92% RH). Apples within each sample were ranked according to their yellow ground colour intensity, and divided into three groups of nine fruits. All samples were removed after 6 months of storage and held for an additional 7 days in 20°C air before evaluation of fruit quality (described above) and analysis of volatile production (described below).

Solvent extraction of volatile compounds

Two slices of tissue (8 g each) were taken along the median between the blushed and the non-blushed sides of each of the three fruits within each treatment to make a composite sample of 48 g per treatment. Each composite was immediately homogenized in a two-speed Waring-blender with 100 ml methanol and 100 ml dichloromethane for 2 min; 1 min at low speed and 1 min at high speed. Dichloromethane contained 0·5 mg/l cyclohexanone as the internal standard. Homogenates were filtered through Whatman No. 1 filter paper. Deionized distilled water (50 ml) was added to the filtered samples which were then thoroughly mixed and stored at –20°C for 8 h. Dichloromethane fractions were carefully decanted and kept at –20°C. Dichloromethane fractions were concentrated to 5 ml in a Kuderna-Danish concentrator (Kontes Scientific Glassware/Instruments, Vineland, NJ), and reduced further to 0·2 ml with a gentle stream of purified N_2.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

A 2 μl aliquot of each concentrated extract was injected into a HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector (FID). The column headpressure was maintained at 220 kPa and the injector split ratio was adjusted to 10 : 1. Volatiles were separated on a Supelcowax 10 fused silica capillary column (90 m length × 0·25 mm i.d × 0·25 μm film thickness) with helium as the carrier gas. Chromatographic conditions were 35°C for 20 min, then temperature programmed to 220°C at 2°C min^{-1}, followed by 30 min at 220°C. Data were processed with a GC Star Workstation (Varian Associates Inc., Walnut Creek, CA).

A HP 5890-5970 GC-MSD system (Hewlett-Packard, Avondale, PA) recorded the mass spectral data. The MS operated with an ion source temperature of 250°C, ionizing energy of 70 eV, and scan range of 25–300 amu. Column and temperature programming for volatile separation were similar to that for the analytical GC-FID described above. Initial identification of volatile compounds was made with the NBS (Rev. E) library. Spectra and Kovats retention indices were compared with those of authentic compounds to confirm volatile identification.

Analysis of data

Statistical Analysis System ver. 6.07 (SAS Inc., Cary, NC) was employed for data analysis. ANOVA was used to determine significance of variations in fruit quality parameters and volatile compounds. Fruit quality results were based on a 27-fruit sample. Volatile analysis included two storage regimes (SR), three harvest dates (HD) and three skin colour groups (SC) nested within harvest date [SR × HD(SC)] in three replicates.

Table 1. Changes in quality characteristics of 'Jonagold' apples during maturation in 1991[a]

Harvest date	Fruit weight (g)	Flesh firmness (N)	IEC (μl/l)	Starch index	Yellow colour[b,c] Gr1	Gr2	Gr3
13 September	222 ± 43	87·6 ± 9·0	0·25 ± 0·18	2·1 ± 1·1	2·2[a]	2·9[b]	3·5[c]
27 September	260 ± 51	79·2 ± 5·8	0·89 ± 0·29	5·6 ± 1·3	2·6[a]	3·4[b]	4·1[c]
11 October	240 ± 53	79·6 ± 5·8	0·86 ± 0·35	8·4 ± 1·6	2·5[a]	3·0[b]	4·4[c]

[a]Values are means and SD for analysis of 27 fruits.
[b]Gr, group.
[c]Means within a row for each harvest followed by different letters are significantly different at $P \leq 0{\cdot}05$.

RESULTS AND DISCUSSION

'Jonagold' are considered relatively large apples and averaged between 220 and 260g in weight during the harvest period (Table 1). Flesh firmness decreased during the first two weeks of harvest and did not vary subsequently. Internal ethylene concentration remained mostly below or close to 1 μl/l. Starch index values increased on each successive harvest date averaging approximately 1·6 index number per week. The steady and gradual increase in the SI values confirmed the reliability of this harvest predictor for 'Jonagold'. Although the yellowness values for 'Jonagold' were found at the low end (2–4 = green range) of the 0–10 scale, every group of apples within each harvest date destined for storage were different. Apples harvested at the last two dates (27 September and 11 October) met the suggested levels of fruit parameters for harvesting 'Jonagold' (Lau, 1988).

Harvest date and storage regime had notable impacts on the FF and TA of 'Jonagold' (Table 2). SSC however remained at 13.4 (± 2·4) for all treatments. Early-picked fruits had higher acidity and firmness values. Controlled atmosphere was more effective than air storage in maintaining FF and TA. Reduction of acid loss in CA-stored apples has been associated with the reduced loss of malic acid, and linked to an increase in

Table 2. Quality of 'Jonagold' apples after 6 months of storage[a]

Harvest date	Flesh firmness (N) Air	CA	Titratable acidity (mg/100 ml juice) Air	CA
13 September	56·9	81·0	360	510
27 September	49·4	67·6	336	495
11 October	51·6	64·0	301	459
Std err	4·8		36·3	
Significance[b,c]				
HD	***		***	
SR	***		***	
HD × SR	***		***	

[a]Air, air storage at 0°C; CA, 1·2% O_2 + 1·5% CO_2 storage at 0°C.
[b]HD, harvest date, SR, storage regime
[c]***Significant at 0·1% level.

Table 3. Volatile constituents in 'Jonagold' apples after 6 months of storage

Peak no.[a]	Constituent	Retention index	Average area count[b] Air	CA
	Esters			
1	Ethyl propanoate	961	1396	2537
2	Propyl acetate	970	6514	2763
3	Methyl butyrate	985	18548	21494
7	Butyl acetate	1070	127254	30269
11	2-Methylbutyl acetate	1126	21450	22576
14	Pentyl acetate	1180	4068	1246
15	2-Methylbutyl propionate	1197	897	1293
17	Butyl butyrate	1216	1640	789
18	Butyl 2-methylbutyrate	1231	5742	5304
20	2-Methylbutyl butyrate	1255	1244	919
21	Hexyl acetate	1270	73380	18749
23	Propyl hexanoate	1298	26380	26936
24	Hexyl propionate	1316	2309	2149
29	Butyl hexanoate	1402	817	751
30	Hexyl butanoate	1398	3221	1959
31	Hexyl 2-methytbutyrate	1418	3792	3572
35	Hexyl hexanoate	1619	1413	1137
38	Butyl 3-hydoxybutyrate	1688	2268	2953
	Alcohols			
5	1-Propanol	1002	2401	1005
12	1-Butanol	1140	73649	18330
16	2-Methylbutanol	1203	26762	20070
19	Pentanol	1244	2274	1502
25	1-Hexanol	1320	42959	22749
27	(Z)-3-Hexenol	1369	5753	2536
28	(Z)-2-Hexenol	1397	765	752
32	6-Methyl-5-hepten-2-ol	1452	4309	1429
33	Octanol	1558	1961	1423
34	(Z)-5-octenol	1615	1716	1491
	Aldehydes			
8	Hexanal	1084	10434	11645
10	2-Methyl-2-butenal	1114	5545	5717
22	Octanal	1284	1701	1582
26	Nonanal	1338	3498	3136
	Hydrocarbons			
6	Methylbenzene	1060	7374	7410
9	Undecane	1100	5910	4614
41	alpha-Farnesene	1775	62039	25392
43	Nonadecane	1900	6292	6959
	Ketones			
4	2-Methyl-3-pentanone	989	19456	19841
13	4-Heptanone	1170	4216	4709
37	gamma-Hexalactone	1677	2650	3177
	Phenol			
36	Methyl chavicol	1670	4785	6038
39	Anethole	1730	8575	5923
	Acid			
42	Hexanoic acid	1871	7521	9711
	Sulfur			
40	3-Methylthio-1-propanol	1763	8533	6744

[a]The peak numbers correspond to the numbers in Figure 1.
[b]Areas counts were standardized with that of cyclohexanone as described in Materials and Methods.

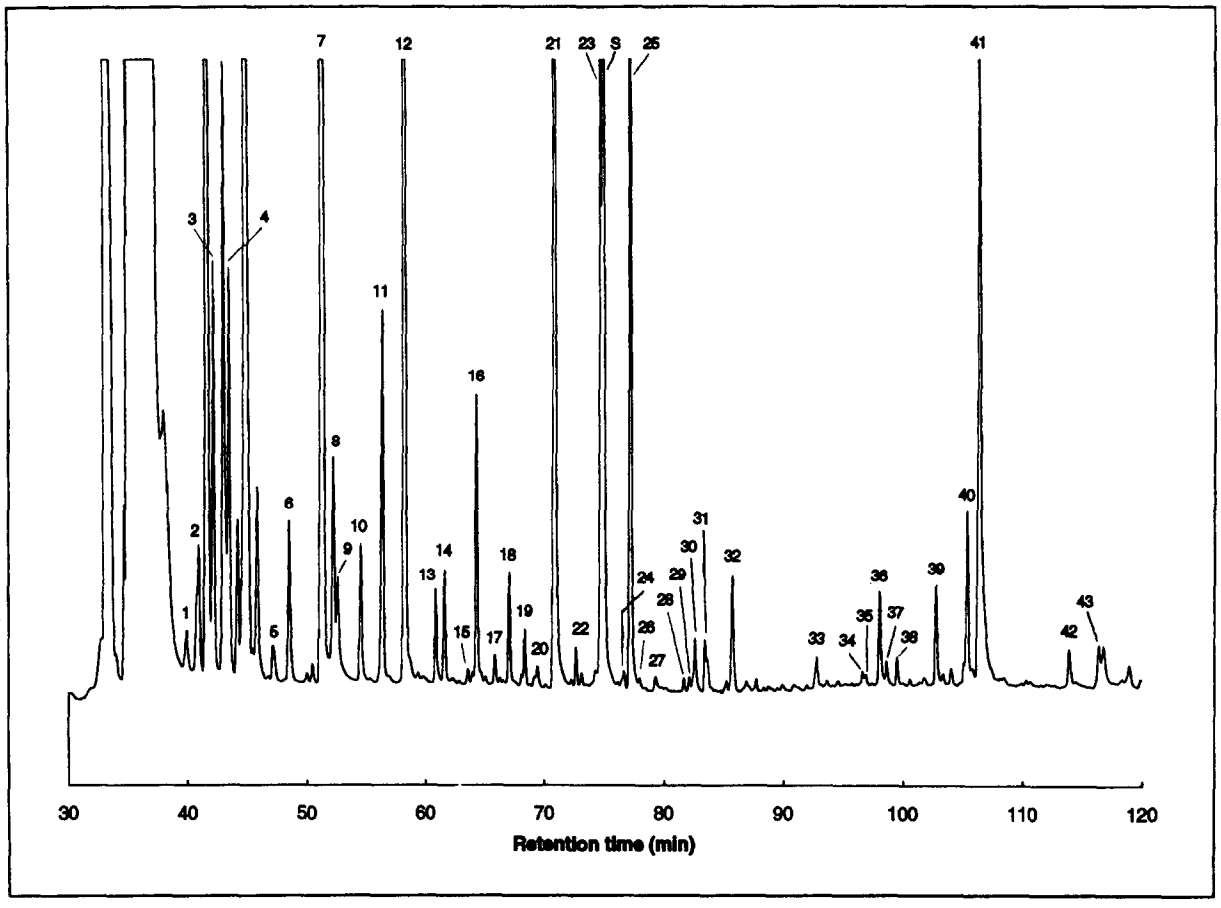

Fig. 1. Typical capillary gas chromatogram of solvent extracted volatiles from 'Jonagold' apple stored for 6 months in air. The peak numbers correspond to the volatile numbers in Table 3.

CO_2 fixation, an inhibition of the respiratory metabolism, and a lower consumption of acid under CA (Metlitskii *et al.*, 1977; Calderon & Barkai-Golan, 1990). Previous research investigated the changes in textural properties of stored apples in relation to pectin; retardation of protopectin transformation to soluble pectin by CA was shown for several apple cultivars (Seipp, 1974).

Forty three compounds were found in the volatile concentrates from 'Jonagold' apples. Identification, retention index, and average area count of the volatiles are listed in Table 3 for both storage modes. A typical chromatogram of air-stored fruit is shown in Figure 1. Profiles included 18 esters, 10 alcohols, four aldehydes, four hydrocarbons, three ketones, two phenols, one acid and one sulfur-containing compound.

Harvest date, ground skin colour and storage regime influenced the volatile profiles to different extents. Storage regime followed by harvest date were the most important factors. Ground colour had a smaller effect. Total volatile production in CA-stored apples was only half that of air-stored fruits (Fig. 2). This effect was illicited through reduced levels of esters, alcohols, and hydrocarbons. Volatile production was higher with successive harvest dates (Fig. 3). Esters and alcohols accounted for the 14% difference in cumulative response. The results show that harvest date and storage regime have an effect on the main groups of volatiles — esters, alcohols and hydrocarbons. In comparison, aldehydes were found in lower concentration. This reflects the process of ester synthesis. Apples contain enzymic pathways reducing aldehydes to alcohols that are subsequently esterified with available carboxylic acids (Salunkhe & Do, 1976; Knee & Hatfield, 1981; DePooter *et al.*, 1987).

Figures 4 and 5 display histograms of volatiles that were affected by harvest date and storage regime, respectively. CA reduced many of the acetates (propyl, butyl, pentyl and hexyl) by approximately 75%. These esters have been identified as odour active compounds in apples (Cunningham *et al.*, 1986). C3 to C6 alcohols (propanol, butanol, 2-methyl-1-butanol, hexanol and (Z)-3-hexenol) and the hydrocarbon α-farnesene also decreased by 45% to 75% in CA. α-Farnesene has been linked to the development of storage scald. Lower concentration of this unsaturated sesquiterperne hydrocarbon in CA-stored 'Jonagold' apples confirms the work of Morozova *et al.* (1974) on the reduced accumulation in fruit of farnesene and its oxidation products under low O_2 environment resulting in a decreased susceptibility

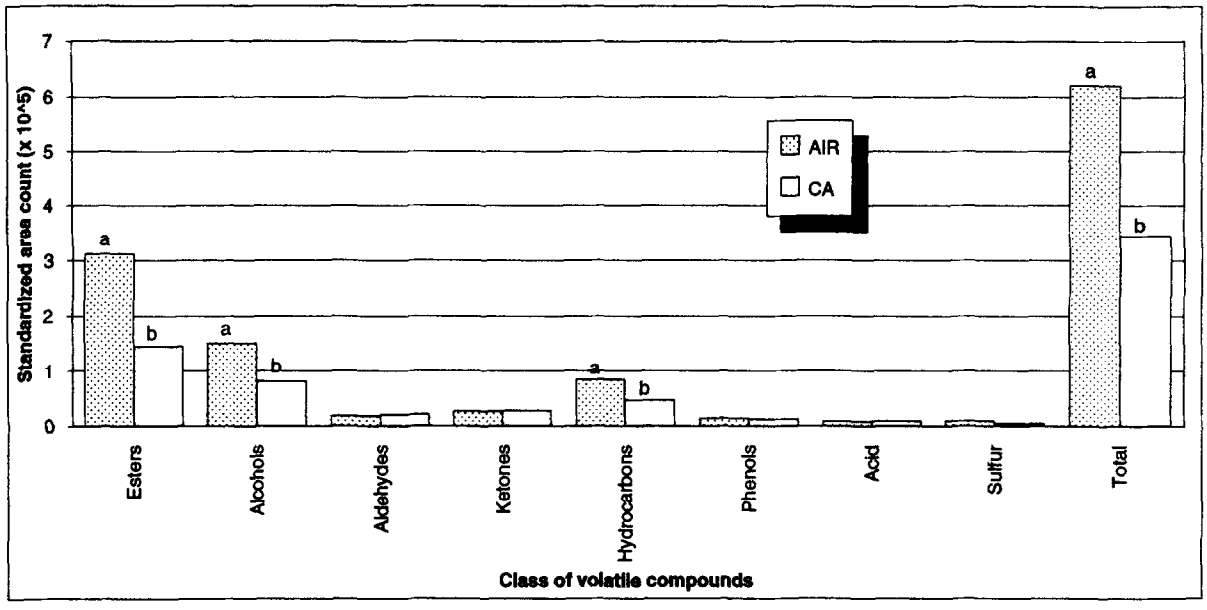

Fig. 2. Effect of storage regime on grouped volatile compounds in 'Jonagold' apple after 6 months of air or CA (1·5% + 1·5% CO_2) storage. Storage regimes within volatile classes with different letters are significantly different at $P \leq 0{\cdot}05$.

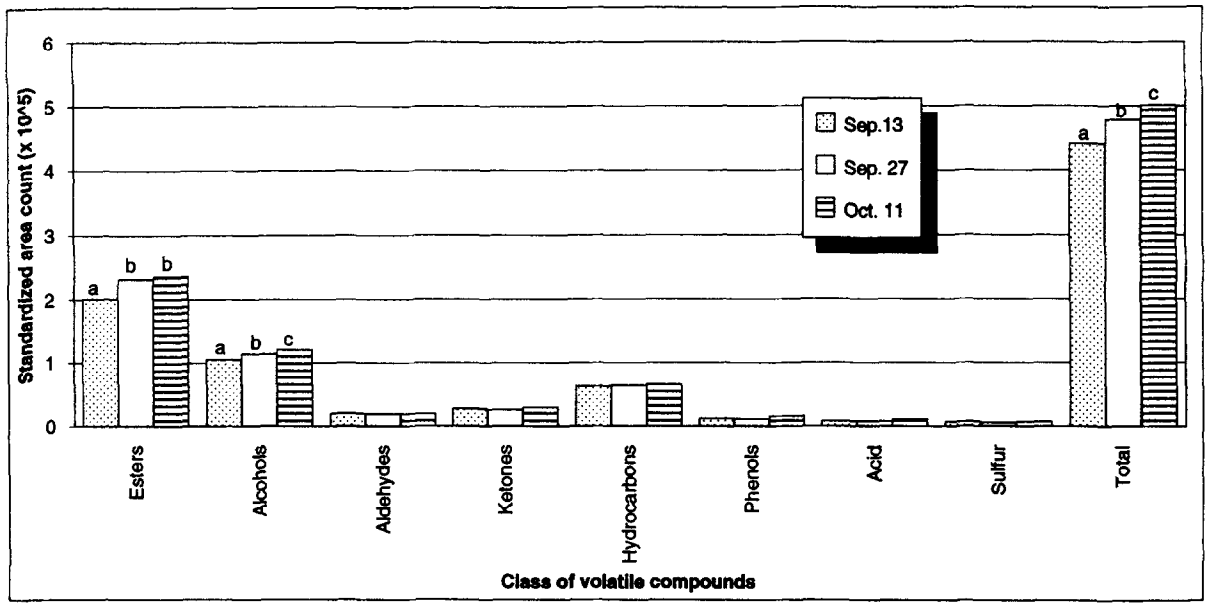

Fig. 3. Effect of harvest date on grouped volatile compounds from 'Jonagold' apple stored for 6 months (EH, 13 September; MH, 27 September; LH, 11 October). Harvest dates within volatile classes with different letters are significantly different at $P \leq 0{\cdot}05$.

to scald (Mellenthin *et al.*, 1980; Little *et al.*, 1985). Other miscellaneous odour-active compounds such as anethole and 3-methylthio-1-propanol also declined by 35% to 40% under CA as compared to air storage.

The last harvest (11 October) increased several esters containing C2 to C4 acid moieties and butyl 2-methylbutyrate by 10 to 20% when compared to the first harvest (13 September) (Fig. 5). The concentration of (Z)-3-hexenol and 6-methyl-5-hepten-2-ol increased in late-picked fruit (50%). Hexanal contributed to more than 50% of the aldehydes but was not influenced by either harvest date or storage mode. As harvest period progressed, esters and their precursors were produced in larger numbers and concentration, and increased at the climacteric stage (Mattheis *et al.*, 1991). The result of this differential state for esterification due to harvest date was carried through after six months of storage.

Presence of esters in all treatments indicates the enzymes catalyzing ester synthesis are functional. Smaller amounts of esters in the CA-stored apples may represent a decreased esterase activity (Gorin *et al.*, 1981) coupled with lower availability of substrate for esterification (Knee & Hatfield, 1981; Mattheis *et al.*, 1991). Long chain fatty acids can be precursors to

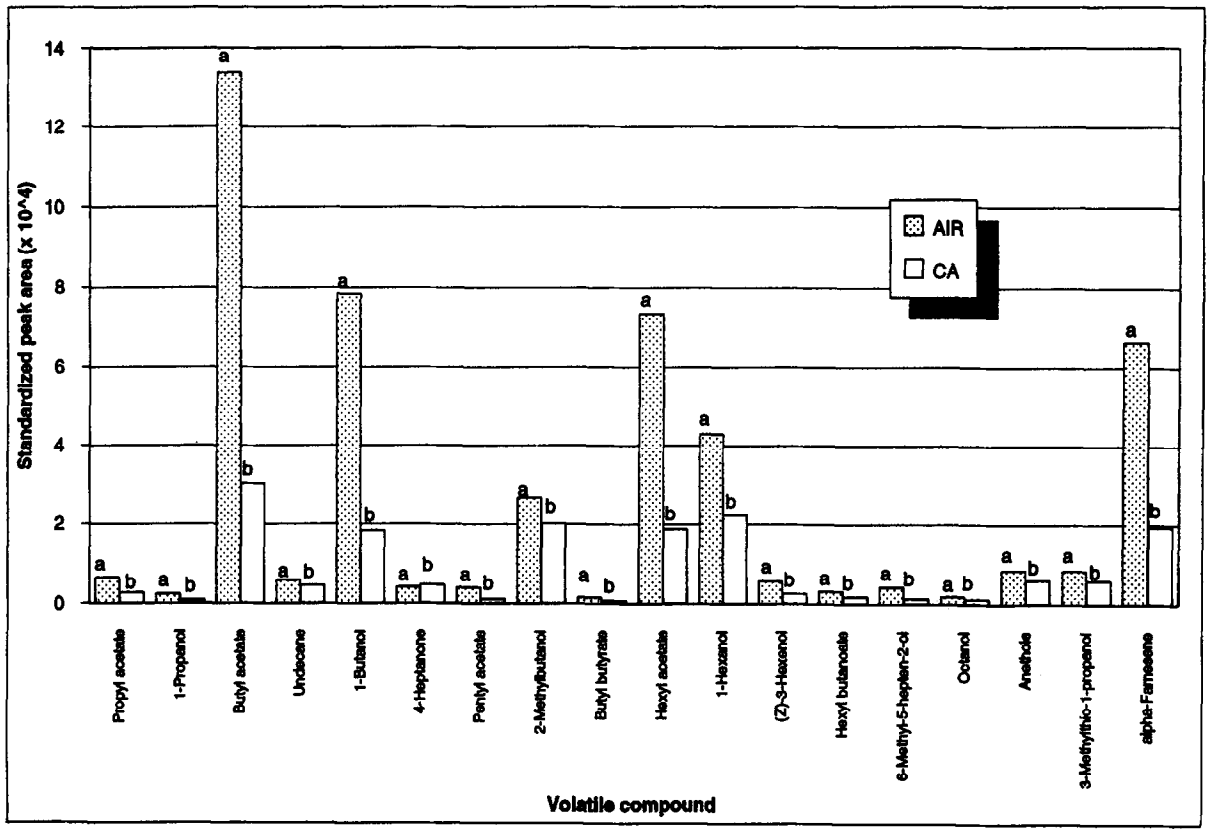

Fig. 4. Influence of storage regime on volatiles from 'Jonagold' apple. Storage regimes within volatile classes with different letters are significantly different at $P \leq 0{\cdot}05$.

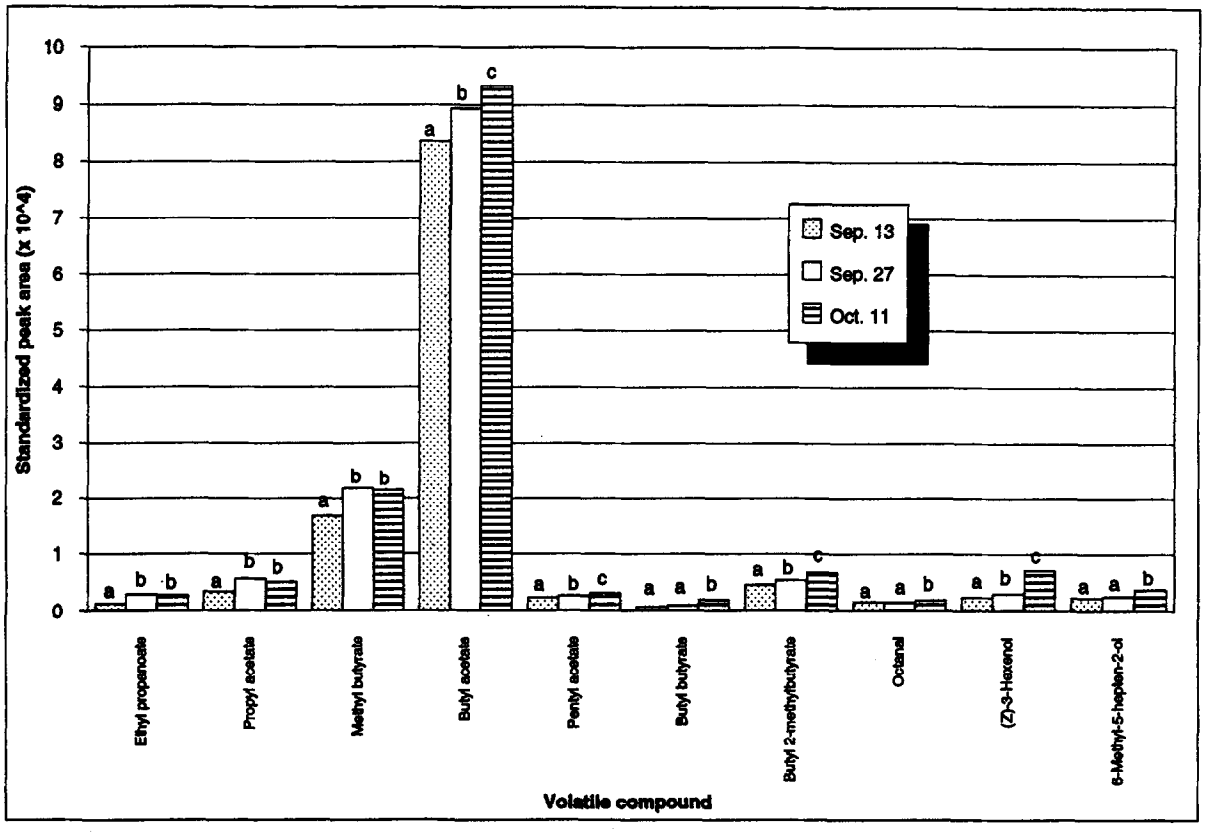

Fig. 5. Influence of harvest date on volatiles from 'Jonagold' apple. Storage regimes within volatile classes with different letters are significantly different at $P \leq 0{\cdot}05$.

straight chain alcohols, aldehydes, and acids contributing to the pool of intermediates for ester formation (Bartley, 1986). 2-Methyl-1-butanol, a branch-chain alcohol, is thought to arise from the degradation of the amino acid leucine (Myers *et al.*, 1970). In a similar manner, the sulfur-containing compound 3-methylthio-1-propanol may be derived from methionine. Since CA suppressed the production of aroma compounds with unbranched and branched C-chains, Brackman *et al.* (1993) concluded that CA affects the metabolism of both fatty acids and amino acids. Pathways of ester synthesis from fatty acid and amino acid metabolism, and their response to CA environments are not completely understood, and the mechanisms behind their regulation need to be elucidated.

CONCLUSIONS

In summary, the quantity of solvent extracted volatiles from 'Jonagold' apples varied considerably between harvests and storage regimes. Groupings of fruit at harvest based on skin ground colour did not translate in flavour volatile differences after six months of storage. Esters, alcohols, and hydrocarbons were reduced almost by half in stored 'Jonagold' by CA while fruits harvested at the climacteric and postclimacteric stages permitted an increase of 14% in the amount of volatile compounds. Flesh firmness and TA decreased at the latter dates but remained at acceptable levels (FF > 50N and TA > 300 mg malate/100 ml). Starch index was a simple tool for determining harvest maturity. The decision of harvesting fruit at a more advanced maturity (e.g. SI of 8·5) to gain in flavour volatile concentration should be weighed against the loss in firmness and acidity.

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