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Production of Volatile Compounds by Fuji Apples Following Exposure to High CO_2 or Low O_2

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The emission of volatile compounds by Fuji apples following short- or long-term exposure to high CO_2 was studied. The production of ethanol, methyl and ethyl esters, octanal, nonanal, and decanal was enhanced while the production of C_3-C_6 alcohols, propyl, butyl, pentyl, and hexyl esters and butanal decreased in fruit exposed to 10 kPa O_2 + 20 kPa CO_2 at 20 °C for up to 12 days. The impact of high CO_2 exposure on volatile production was dependent on fruit maturity at harvest. Apples stored for 8 months in an ultralow O_2-controlled atmosphere (CA) (0.5 kPa O_2 + 0.05 kPa CO_2) or high CO_2 CA (1.5 kPa O_2 + 3 kPa CO_2) at 0.5 °C had reduced production of most volatiles, especially butyl and hexyl esters, as compared to fruit stored in air. Two exceptions were ethanol and ethyl acetate for which the production was enhanced by both CA regimes. Treatment with the antioxidant diphenylamine prior to storage prevented most of the high CO_2-induced and ultralow O_2-induced changes in volatile production. The results of this study do not indicate that changes in volatile production following the exposure of Fuji apples to high CO_2 are causally related to the development of CO_2 injury.

KEYWORDS: Apple maturity; controlled atmosphere storage; diphenylamine; esters; alcohols; aldehydes

INTRODUCTION

The storage of apple fruit in a low O_2 and/or high CO_2 controlled atmosphere (CA) delays the deterioration of texture, color, flavor attributes, and nutritional value (*1*, *2*). However, prolonged storage in a CA can reduce and/or delay poststorage production of volatile compounds that contribute to fruit aroma (*3*−*5*). Esters, alcohols, and aldehydes are quantitatively the major volatile compounds that contribute to apple aroma (*6*, *7*). Factors influencing the production of these volatile compounds after CA storage include cultivar, fruit maturity at harvest, O_2 and CO_2 partial pressures during storage, storage duration, days of ripening in air after storage, and other preharvest factors including environment, cultural practices, and agrichemicals (*5*, *8*−*13*).

The suppression of aroma production in apples after CA storage may be related to low rates of alcohol (*4*) and fatty acid synthesis and/or degradation (*14*). The syntheses of alcohols (through β-oxidation of fatty acids) and esters (via esterification of alcohols and carboxylic acids) are oxygen-dependent processes (*4*, *13*, *15*). Consequently, the production of some alcohols and esters by apple fruit is reduced during and after storage in low O_2 CA conditions (*5*, *13*, *16*). In contrast, increased production of ethyl esters by apple fruit following anaerobic storage conditions is likely due to accumulation of ethanol (*17*).

High CO_2 during storage or a prestorage treatment can be an effective means to preserve fruit quality (*18*, *19*) and increase fruit tolerance to disorders induced by chilling temperatures (*20*, *21*). Brief periods of high CO_2 and/or hypoxia are also potential treatments to reduce decay and insect infestation (*22*). However, high CO_2 treatments can alter volatile production enhancing ethanol, acetaldehyde, and ethyl esters while suppressing the production of acetate esters (*19*, *23*−*25*). The tolerance of apple fruit to brief high CO_2 and/or low O_2 treatments and the qualitative changes in volatile production in response to these treatments are cultivar-dependent (*25*−*27*).

Storage in ultralow O_2 (0.5−1.0 kPa) maintains higher firmness, acidity, and soluble solids in Fuji apples as compared to conventional (2.0 kPa O_2) CA (*28*, *29*). However, Fuji apples produce a considerable amount of ethanol and acetaldehyde even under aerobic conditions, especially when the fruit is harvested at an advanced maturity (*30*). Both ultralow O_2 (0.5 kPa) and high CO_2 (3 kPa) at 0.5 °C or 20 kPa at 20 °C enhance accumulation of fermentative metabolites while only high CO_2 atmospheres induce development of an internal browning disorder in Fuji apples (*30*).

The application of the antioxidant diphenylamine (DPA) prior to exposure to high CO_2 reduces ethanol and acetaldehyde

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production and prevents development of internal CO_2 injury in Fuji (*30*) and other apple cultivars (*31*). The synthesis of some esters by apple fruit requires continuous ethylene action and production (*33*). Ethylene production in apple fruit can decrease following DPA application (*32*); however, impacts of DPA on Fuji apple volatile production are unclear. Although the mechanism of CO_2 injury development has not been elucidated, there are indications that this disorder may be related to an oxidative stress (*34*). Because oxidative stress can be associated with peroxidation of fatty acids from cell membranes (*35*) and many volatile compounds are synthesized from fatty acid degradation (*36*), the development of CO_2 injury could be accompanied by or associated with changes in volatile production.

The objective of this study was to characterize volatile emissions from Fuji apples after exposure to CO_2 sufficient to cause internal browning and O_2 low enough to induce anaerobic metabolism. The impact of a prestorage treatment with DPA to prevent internal browning on volatile compound emissions was also evaluated.

MATERIALS AND METHODS

Apples [*Malus sylvestris* var. *domestica* (Borkh.) Mansf. cv. Fuji] were harvested 162, 173, and 188 days after full bloom (DAFB) from a commercial orchard near Wenatchee, WA. Fruit maturity at harvest was determined by analyses of firmness and starch index as described previously (*30*). Treatments with DPA were applied the day of harvest by submerging apples for 1 min in 2000 μL L^{-1} DPA using a formulated Shield-DPA 15% emulsion (Shield-Brite Corp., Kirkland, WA).

Storage Conditions. Two experiments were performed. The apples were held at 20 °C for 12 days (short-term storage) in air or 10 kPa O_2 + 20 kPa CO_2 in air (*30*). The fruit were enclosed in 76 μm thick plastic bags within 24 h of harvest. The bags were purged at 6 L h^{-1} with compressed air or a mixture of air and compressed CO_2 (10 kPa O_2 + 20 kPa CO_2) at 20 °C for 3, 6, 9, or 12 days. The O_2 and CO_2 concentrations were monitored by electrochemical and infrared gas analyzers (California Analytical Instruments, Orange, CA), respectively. For the second experiment (long-term storage), fruit was cooled to 0.5 °C within 24 h of harvest and then enclosed in 0.145 m^3 gastight chambers and stored 4 or 8 months in 0.5 kPa O_2 + 0.05 kPa CO_2 (ULO) or 1.5 kPa O_2 + 3 kPa CO_2 (high CO_2 CA). The O_2 concentration was reduced beginning 36 h after harvest. The storage chamber atmospheres were established within 60 h after harvest and monitored at 90 min intervals (Techni-Systems, Chelan, WA). Semistatic chamber atmospheres (purged only when the atmosphere was adjusted) were maintained with N_2 generated from a membrane system (Permea, St. Louis, MO), compressed air, and CO_2. In addition to modification of chamber gas composition, the low (0.05 kPa) CO_2 concentration was maintained by adding 0.1 kg of hydrated lime [$Ca(OH)_2$] per kg of fruit.

Volatile Sampling. Headspace volatile compounds were collected 36 and 24 h after removing fruit from short- or long-term storage, respectively. This sampling delay allowed fruit internal O_2 and CO_2 concentrations to equilibrate with those of ambient air. Intact fruit (3−4 fruits per jar, ~1 kg) were placed into 4 L glass jars sealed with Teflon lids and supplied with compressed air at 100 mL min^{-1}. The compressed air passed through traps containing activated charcoal, molecular sieve, $KMnO_4$ (Purafil Inc., Atlanta, GA), and Tenax GC (Alltech Assoc., Deerfield, IL) prior to entering the sealed jars. The jars containing apples were equilibrated for 1 h, and then sample collection was performed at 20 °C for 15−60 s. The volatile compounds in effluent air were adsorbed onto 50 mg of Tenax GC packed in glass tubing (17.5 cm × 0.4 cm i.d.).

Analyses and Quantification of Volatiles. The volatile compounds were desorbed from the Tenax traps using an automated thermal desorption and cryofocusing autosampler (Teckmar Associates, Cincinnati, OH) and then injected into a gas chromatograph (HP 5890; Hewlett-Packard, Palo Alto, CA) equipped with a mass selective detector (HP 5971A; Hewlett-Packard) for qualitative and quantitative analysis (*11*, *17*). The initial identification of compounds was by matching spectra using the Wiley NBS library. Confirmation and identification were made by comparison of sample retention indices and mass spectra with those of authentic standards (Sigma-Aldrich, Milwaukee, WI). Quantification was performed using selected ion monitoring for base peaks, and quantitative values were calculated using response factors generated with authentic standards.

Esters detected were pooled by adding the production rates of individual esters as follows: (i) methyl esters: methyl butanoate and methyl 2-methylbutanoate; (ii) branched chain methyl esters: 2-methylpropyl acetate, 2-methylbutyl acetate, and 2-methylbutyl-2-methylbutanoate; (iii) ethyl esters: ethyl-acetate, -propanoate, -butanoate, -2-methylbutanoate, -pentanoate, -hexanoate, and -octanoate; (iv) propyl esters: propyl-acetate, -propanoate, and -hexanoate; (v) butyl esters: butyl-acetate, -propanoate, -butanoate, -2-methylbutanoate, and -hexanoate; (vi) pentyl esters: pentyl-acetate and -butanoate; and (vii) hexyl esters: hexyl-acetate, -propanoate, -butanoate, -2-methylbutanoate, and -hexanoate.

Statistical Analyses. The two experiments were conducted using a completely randomized design, with four replicates of three or four fruit per storage period per treatment for volatile analyses and 20 fruit for analyses of fruit maturity. Data were subjected to analysis of variance using the Statistical Analysis System (SAS Institute, Inc., Cary, NC), and the treatment mean separation was determined by Fischer's least significant difference (LSD) or Duncan's multiple range tests ($p = 0.05$).

RESULTS

The values for fruit firmness averaged 82, 78, and 75 N, and the starch indices (1−6 scale) averaged 3.3, 4.3, and 5.4 at harvest 162, 173, and 188 DAFB, respectively. The fruit harvested 173 DAFB was considered at optimum maturity for long-term CA storage based on firmness and starch index values (*28*).

Volatiles Produced after Short-Term Exposure to High CO_2 (20 kPa) at 20 °C. The emission of most alcohols and esters by Fuji apples increased during 12 days in air at 20 °C (**Figures 1** and **2**). Exposure to 20 kPa CO_2 for 3 or 12 days enhanced the production of ethanol approximately 9- and 16-fold, respectively, as compared to fruit held in air. In contrast, ripening-related increases in emissions of 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 2-methyl-1-butanol were inhibited by high CO_2 treatment after 9 or 12 days at 20 °C.

The emission of methyl and ethyl esters was enhanced following exposure to high CO_2, while the emission of propyl, butyl, hexyl, and branched chain methyl esters was lower than that from fruit stored in air. Ester production decreased with increased duration of high CO_2 exposure, and the reduction in ester production was greater than the reduction in corresponding alcohols. The pattern of changes for individual esters following exposure to high CO_2 was the same as that for total methyl, ethyl, propyl, butyl, pentyl, and hexyl esters (data not shown) with one exception. Exposure to high CO_2 resulted in a larger increase in ethyl acetate emission as compared to nonacetate ethyl esters after 9 and 12 days (**Figure 3**).

The stimulation of ethanol, methyl, and ethyl ester production resulting from exposure to high CO_2 was highest in fruit harvested 173 or 188 DAFB (**Table 1**). In contrast, the inhibitory effect of high CO_2 exposure on the production of C_3−C_6 esters decreased as harvest DAFB increased. Reduced emission of C_3−C_6 alcohols following high CO_2 exposure was greatest for fruit harvested at optimum harvest maturity (i.e., 173 DAFB).

The emission of butanal after 9 or 12 days exposure to high CO_2 was reduced as compared to fruit held in air (**Figure 4**). In contrast, depending on the duration of high CO_2 exposure, the production of heptanal, octanal, nonanal, and decanal

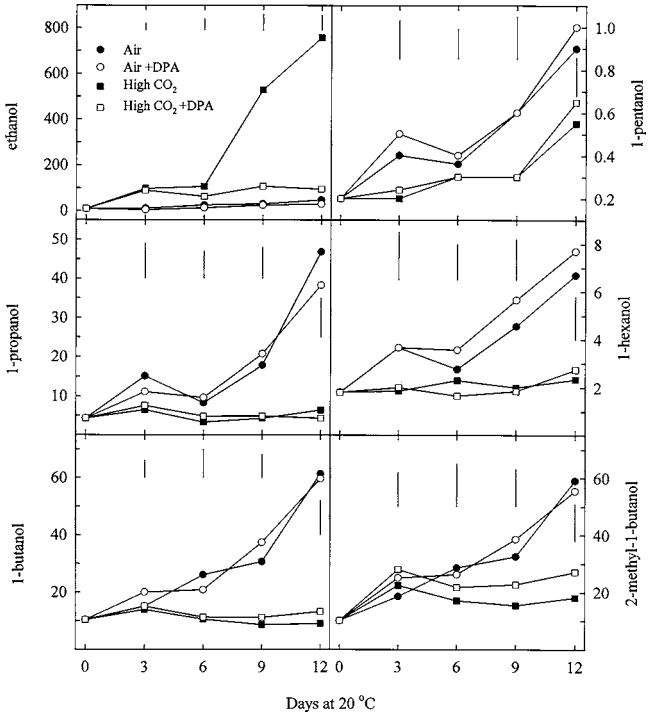

Figure 1. Production (nmol kg^{-1} h^{-1}) of alcohols by Fuji apples following harvest. Fruits harvested 173 DAFB were treated with DPA (0 or 2000 μL L^{-1}) and then held 12 days at 20 °C in air or 20 kPa CO_2. Volatile samples ($n = 4$) were collected from intact fruit held for 36 h at 20 °C after removal from air or high CO_2. Vertical bars represent the LSD ($p = 0.05$).

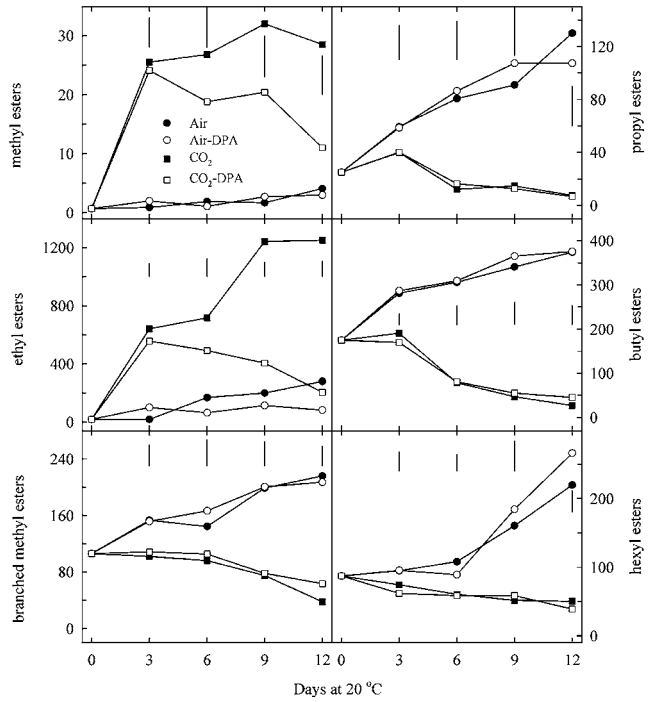

Figure 2. Production (nmol kg^{-1} h^{-1}) of esters by Fuji apples following harvest. Fruits harvested 173 DAFB were treated with DPA (0 or 2000 μL L^{-1}) and then held for 12 days at 20 °C in air or 20 kPa CO_2. Volatile samples ($n = 4$) were collected from intact fruit held for 36 h at 20 °C after removal from air or high CO_2. Vertical bars represent the LSD ($p = 0.05$).

increased or was not affected by high CO_2. Emissions of pentanal (data not shown) and hexanal were not significantly affected by high CO_2 treatment. The impact of exposure to high

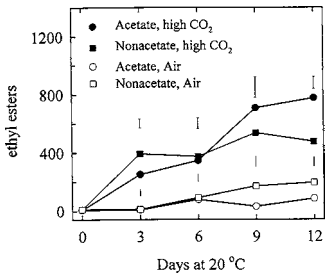

Figure 3. Production (nmol kg^{-1} h^{-1}) of ethyl acetate and nonacetate ethyl esters by Fuji apples following harvest. Fruits harvested 173 DAFB were treated with DPA (0 or 2000 μL L^{-1}) and then held for 12 days at 20 °C in air or 20 kPa CO_2. Volatile samples ($n = 4$) were collected from intact fruit held for 36 h at 20 °C after removal from air or high CO_2. Vertical bars with and without caps represent the LSD ($p = 0.05$) for acetate and nonacetate ethyl esters, respectively.

Table 1. Relative Difference between Air Control and High CO_2 (20 kPa) Treatment in Volatile Production by Fuji Apples after 6–12 Days at 20 °C Following Harvest[a]

	harvest date (DAFB)					
volatiles	162		173		188	
ethanol	122.6	***[c]	431.1	***	578.7	***
$\sum C_3$–C_6 alcohols	–65.0	***	–82.6	***	–64.6	***
methyl esters	4.7	**	14.2	***	22	***
ethyl esters	227.4	***	855.1	***	891.3	***
$\sum C_3$–C_6 yl esters[b]	–590.7	***	–524.1	***	–285.4	***
butanal	–1.2	***	–0.9	*	–0.6	NS
$\sum C_7$–C_{10} aldehydes	5.9	***	3.4	*	14.2	***

[a] Combined data for 6, 9, and 12 days CO_2 exposure were subtracted from those for air control. [b] Includes propyl, butyl, pentyl, hexyl, and branched 2-methyl esters. [c] NS, ***, **, * nonsignificant or significant at $p = 0.1$, 1, and 5%, respectively.

CO_2 on the production of butanal decreased with the harvest date; however, the emission of C_7−C_{10} aldehydes increased with the harvest date and CO_2 exposure (**Table 1**).

Effects of Long-Term Exposure to High CO_2 CA (1.5 kPa O_2 + 3 kPa CO_2) or ULO (0.5 kPa O_2 + 0.05 kPa CO_2) at 0.5 °C. Fruit stored in either CA regime had reduced production of most volatiles as compared to fruit stored in air for 8 months (**Table 2**). An exception was a higher production of ethanol and ethyl acetate for fruit stored in either CA environment, and pentanal production was not significantly affected by storage in CA as compared to storage in air.

Production of 1-butanol, 1-pentanol, and 1-hexanol were 11−35-fold higher from fruit stored in air as compared to CA-stored fruit. The production of most aldehydes was reduced 1.5−3-fold following CA storage, whereas emanation of hexanal was more than 40-fold lower in CA- than in air-stored fruit. Similarly, the production of butyl and hexyl esters was 15−80-fold lower in CA- than in air-stored fruit whereas production rates of ethyl esters (except ethyl acetate) were 1.2−3-fold lower in CA- than in air-stored fruit. Conversely, after 8 months of storage, production of ethanol and ethyl acetate were 2.8−9.8-fold and 2.2−3.2-fold higher, respectively, in CA- than in air-stored fruit. Productions of 1-propanol, 2-methyl-1-propanol, propyl acetate, methyl esters, heptanal, and octanal were not consistently reduced by CA storage, depending on DPA treatment, CA regime, and duration of storage. Emission of most alcohols and aldehydes by non-DPA-treated fruit stored in ULO or high CO_2 CA for 4 months were similar. Exceptions were 1-butanol, 2-methyl-1-butanol, and butanal for which production was enhanced following storage in ULO. After 8 months, the production of most alcohols by fruit stored in ULO was higher

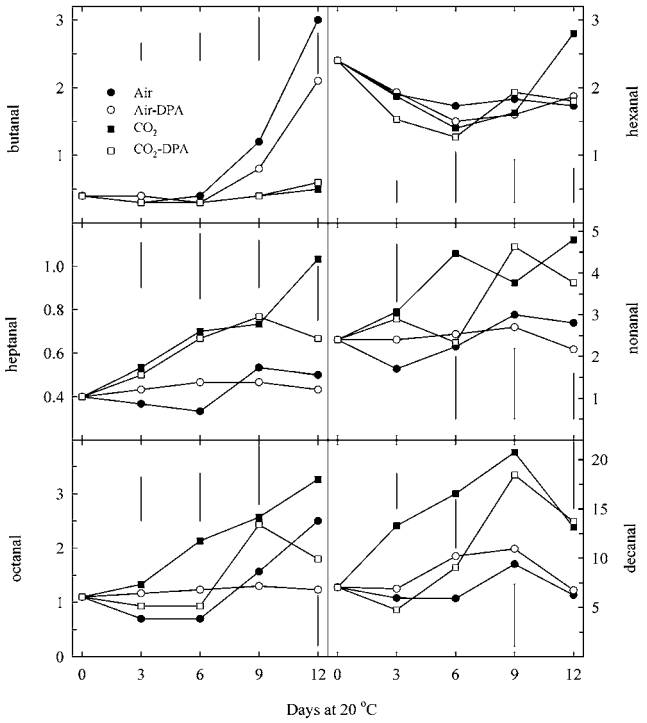

Figure 4. Production (nmol kg^{-1} h^{-1}) of aldehydes by Fuji apples following harvest. Fruits harvested 173 DAFB were treated with DPA (0 or 2000 μL L^{-1}) and then held for 12 days at 20 °C in air or 20 kPa CO_2. Volatile samples ($n = 4$) were collected from intact fruit held for 36 h at 20 °C after removal from air or high CO_2. Vertical bars represent the LSD ($p = 0.05$).

as compared to fruit stored in high CO_2 CA. For fruit not treated with DPA, the CA regimes had similar impacts on emission of 1-pentanol and C_4-C_7 aldehydes, while production of octanal, nonanal, and decanal were higher after storage in high CO_2 CA than in ULO.

Fruit stored in ULO had higher production of ethyl, propyl, pentyl, and hexyl acetates after 4 months and ethyl, propyl and hexyl acetate after 8 months compared to production by fruit stored in high CO_2 CA. In contrast, the production of methyl esters and nonacetate ethyl, butyl, and hexyl esters by fruit not treated with DPA was similar for both CA treatments regardless of storage period. The production of nonacetate propyl esters and branch chain methyl esters by non-DPA-treated fruit was higher after storage in high CO_2 CA as compared to ULO.

Impact of DPA Treatment Prior to Storage. The application of DPA prior to storage prevented the increase in the production of ethanol (**Figure 1**) and methyl and ethyl esters (**Figure 2**) induced during 9 or 12 days exposure to high CO_2. The presence of DPA did not affect emission of C_3-C_6 alcohols, propyl, butyl, hexyl, or branched chain methyl esters by fruit exposed to 20 kPa CO_2. DPA also delayed the CO_2-induced increase in octanal, nonanal, and decanal but did not affect emanation of butanal and heptanal (**Figure 4**). Pre-storage DPA treatment resulted in increased production of hexyl esters and reduced production of ethyl esters, butanal, and octanal for fruit stored in air at 20 °C for 12 days.

Fruit treated with DPA then stored in high CO_2 CA at 0.5 °C produced less ethanol, ethyl acetate, methyl esters, nonacetate ethyl and propyl esters (regardless of storage period), 1-propanol, and 1-butanol (after 4 months) than non-DPA-treated controls (**Table 2**). However, fruit treated with DPA and stored in ULO at 0.5 °C produced less 1-butanol and propyl acetate (regardless of storage period), 2-methyl-1-butanol, hexyl acetate, methyl esters, nonacetate ethyl and hexyl esters (after 4 months), and ethanol (after 8 months) than non-DPA-treated controls. Fruit treated with DPA had enhanced production of 2-methyl-1-butanol after being stored in high CO_2 CA and higher production of nonacetate propyl esters after storage in ULO as compared to untreated controls. Pre-storage DPA treatment resulted in increased C_7-C_9 aldehyde production and decreased decanal production after 8 months storage in ULO and high CO_2 CA, respectively.

DISCUSSION

The exposure of Fuji apples to 20 kPa (at 20 °C) or 3 kPa CO_2 (at 0.5 °C) induces the development of internal CO_2 injury (brown-heart) (*30*) and changes in volatile production. Fruits exposed to high CO_2 had a higher production of methanol, ethanol, acetaldehyde (*24*, *30*), and straight chain methyl and ethyl esters (especially ethyl acetate) but a lower production of C_3-C_6 alcohols and the corresponding propyl, butyl, pentyl, and hexyl esters. Similar qualitative changes in the production of alcohols and esters were observed in Delicious apples after 30 days of low O_2 hypoxia at 1 °C (*17*) and in several apple cultivars exposed to brief (24 h) high CO_2 (~100 kPa) hypoxia (*25*).

Ethanol accumulation in apples and other fruit is a characteristic response to low O_2 (*17*, *37*, *38*) and/or high CO_2 stresses (*23*, *39*). The increase in ethanol and ethyl esters indicates that anaerobic metabolism was induced in Fuji apples exposed to high CO_2. The increased activity of pyruvate decarboxylase and alcohol dehydrogenase in response to low O_2 or high CO_2 has been demonstrated in pear (*39*), strawberry (*23*), tomato (*40*), avocado (*41*), and sweet potato (*42*).

Whole apple fruit exposed to vapors of short chain aliphatic alcohols produce the corresponding esters (*4*, *43*, *44*) indicating that alcohols are substrates for ester production in apples (*15*). On the basis of the results herein and the suggestion that ester production can be driven by the endogenous alcohol concentration (*4*), increased production of methyl and ethyl esters is driven in part by the enhanced pool of methanol and ethanol available after anaerobic metabolism.

The exposure of Fuji apples to high CO_2 had less impact on 1-propanol, 1-butanol, 1-pentanol, and 1-hexanol production than on the production of the corresponding esters, especially after 9 and 12 days of high CO_2 exposure or in fruits harvested immaturely (162 DAFB). In addition, when apple fruit is exposed to 100 kPa CO_2 for 24 h at 20 °C, ethanol production is enhanced, C_3-C_6 alcohol production is unaffected or increases depending on apple cultivar, and C_3-C_6 acetate production is unaffected or decreases, again depending on apple cultivar (*25*). These results support the hypothesis that the large amount of ethanol produced in response to low O_2 hypoxia may displace other alcohols as a substrate for the ester-forming enzyme(s) (*17*). Therefore, the reduced production of some esters, including propyl, butyl, pentyl, and hexyl esters following exposure to high CO_2 can also be related in part to the competition between ethanol, methanol, and C_3-C_6 alcohols for the esterification reaction.

The exposure to high CO_2 suppresses ethylene production by Fuji apples (*30*), and the synthesis of some ripening related volatiles is regulated by ethylene (*33*). Phospholipid degradation increases, and some free fatty acids are converted into alcohols and esters in ripening apple fruit (*7*, *45*). Inhibition of ethylene production and fruit ripening by aminoethoxyvinylglycine (*46*) or storage in CA environments (*14*) reduce free fatty acid concentrations as well as volatile production in apple fruit. These

Table 2. Production Rates of Alcohols, Esters, and Aldehydes (nmol kg^{-1} h^{-1}) by Fuji Apples Following Air or CA Storage at 0.5 °C[a]

	4 months								8 months									
	0.5 O_2 + 0.05 CO_2 (kPa)				1.5 O_2 + 3 CO_2 (kPa)				0.5 O_2 + 0.05 CO_2 (kPa)				1.5 O_2 + 3 CO_2 (kPa)				air	
volatile	−DPA		+DPA		−DPA		+DPA		−DPA		+DPA		−DPA		+DPA		−DPA	
ethanol	1032	b[b]	1489	a	1091	b	395	c	4067	a	2665	b	1163	c	780	d	414	d
1-propanol	47	a	47	a	32	a	22	b	47	b	50	ab	17	c	15	c	61	a
2-methyl-1-propanol	7.4	a	7.0	a	6.5	a	6.6	a	16	a	116	a	9.8	b	11	b	15	a
1-butanol	46	a	33	b	27	b	19	c	19	b	15	c	7.7	d	5.7	d	255	a
2-methyl-1-butanol	28	a	23	b	17	b	43	a	40	b	32	b	26	c	35	b	74	a
1-pentanol	0.8	a	0.9	a	0.9	a	1.3	a	0.9	b	0.5	b	0.9	b	0.1	b	31	a
1-hexanol	7.5	a	8.2	a	9.1	a	8.0	a	6.4	b	5.3	b	3.6	c	2.9	c	72	a
ethyl acetate	1570	a	1415	a	1132	b	681	c	1565	a	1240	ab	1055	b	601	c	490	c
propyl acetate	61	a	37	b	18	c	16	c	22	a	12	b	19	b	4.9	c	39	a
butyl acetate	104	a	77	b	49	c	37	c	25	b	22	b	18	b	7.9	c	398	a
pentyl acetate	11	a	8.3	ab	7.7	b	6.7	b	4.8	b	4.1	b	2.8	bc	1.5	c	14	a
hexyl acetate	84	a	58	b	50	bc	39	c	22	b	20	bc	14	c	7.3	d	55	a
methyl ester[c]	21	a	10	b	17	a	10	b	15	ab	11	b	23	a	5.2	c	26	a
branched methyl esters[d]	74	c	93	b	108	ab	128	a	69	b	71	b	93	a	73	b	105	a
ethyl ester [e]	1590	a	1164	b	1461	a	922	b	528	b	506	b	507	b	215	c	1170	a
propyl ester[e]	12	c	39	b	70	a	29	bc	3.0	c	21	b	87	a	0.6	c	65	a
butyl ester [e]	15	a	9.8	ab	9.7	ab	9.5	b	5.2	b	5.2	b	2.7	bc	1.7	c	237	a
hexyl ester[e]	14	a	9.8	b	13	ab	15	a	5.6	b	5.5	b	4.4	b	3.3	b	205	a
butanal	5.0	a	3.5	ab	3.5	b	2.7	b	2.8	b	2.9	b	1.7	bc	1.5	c	4.9	a
pentanal	1.5	a	1.6	a	1.6	a	1.7	a	1.0	a	1.4	a	1.2	a	1.2	a	1.4	a
hexanal	7.1	a	6.4	a	5.5	a	7.3	a	3.2	b	5.1	b	2.9	b	3.3	b	135	a
heptanal	3.2	a	3.8	a	2.4	a	3.9	a	1.3	b	2.9	a	1.9	ab	1.5	b	3.9	a
octanal	9.6	ab	13	a	7.7	b	9.3	ab	2.4	b	5.3	a	4.1	a	3.1	ab	4.7	a
nonanal	19	ab	23	a	15	b	22	ab	5.1	c	9.8	b	8.3	b	5.8	bc	17	a
decanal	55	a	77	a	49	a	51	a	10	c	17	bc	20	b	14	c	38	a

[a] Fruit harvested 173 DAFB, treated with 0 (−DPA) or 2000 μL L^{-1} diphenylamine (+DPA). Apples were removed from storage, and volatile samples ($n = 4$) were collected from intact fruit after 24 h at 20 °C. [b] Means with the same letter within a row for a given storage period are not significantly different ($p = 0.05$). [c] Sum of methyl butanoate and methyl 2-methylbutanoate. [d] Sum of 2-methylpropyl acetate, 2-methylbutyl acetate, and 2-methylbutyl-2-methylbutanoate. [e] Sum of ester-propanoate, -butanoate, -2-methylbutanoate, -pentanoate, and -hexanoate.

factors may contribute to reduced production of C_3-C_6 alcohols and the corresponding esters following exposure to high CO_2. In contrast, exposure to high CO_2 reduces ethylene production while production of some esters (e.g., methyl and ethyl esters) increases, an indication that the production of these esters may be less related to ethylene production and action, at least when anaerobic metabolism has occurred previously.

The reduction in volatile production, especially C_3-C_6 alcohols and esters by Fuji apples following long-term CA storage, confirms a pattern previously identified (*3*−*5*). A typical response of Fuji apples to both CA regimes was the consistent enhancement of ethanol and ethyl acetate relative to fruit stored in air. In contrast, the production of ethanol and ethyl acetate is suppressed by CA regimes in many other apple cultivars including McIntosh (*16*), Rome (*47*), Bisbee Delicious (*48*), and Starking Delicious (*49*). This different response of Fuji apples to CA treatment can be attributed in part to differences in the low O_2 limit where anaerobic metabolism is intensified. The anaerobic compensation point of Fuji apples is about 1.4 kPa O_2, which is higher than those of Rome (0.8 kPa) and Delicious (0.7 kPa) although it is lower than that of McIntosh (2.9 kPa) (*50*).

The high production of ethyl acetate in Fuji apples stored in ULO and high CO_2 CA indicates that the esterification system is operative and may not be limiting ester production following CA storage. However, the possible effects of low O_2 and high CO_2 on the esterification enzyme alcohol acyl transferase (AAT) may also influence ester production (*13*). The activity of esterification enzymes in CA-stored apples is similar to that of fruit stored in air (*4*, *51*). In contrast, the activity of AAT is markedly reduced in Gala apple fruit following low O_2 CA (*13*), but it is enhanced in strawberry fruit during high CO_2 exposure (*52*). It has been suggested (*25*) that high CO_2 treatment could induce new isozymes, which preferentially use ethanol as a substrate for ester production, as new isozymes associated with anaerobic metabolism are induced by hypoxia (*53*).

Enhanced volatile production is associated with climacteric fruit ripening, and substrates for volatile synthesis may arise in part from membrane catabolism (*13*). Therefore, as DPA delays fruit senescence and reduces oxidative stress including oxidation of membrane lipids as suggested (*32*, *54*), then the presence of DPA could reduce the availability of fatty acid precursors of volatile synthesis. However, the only changes in volatile production detected in DPA-treated apples during ripening in air at 20 °C following harvest were an increased production of hexyl esters and a decreased production of ethyl esters and butanal after 12 days. Prestorage application of DPA prevented the increase in respiration and production of acetaldehyde, ethanol (*30*), and methyl and ethyl esters following high CO_2 exposure. Additionally, the prestorage application of DPA resulted in decreased fruit production of ethanol and ethyl esters following 8 months in 0.5 kPa O_2 as compared to fruits not receiving DPA. These results indicate that DPA effects on volatile production by Fuji apples exposed to high CO_2 or low O_2 could result, at least in part, from partial amelioration of anaerobic metabolism. Other impacts of DPA on apple volatile production include enhanced ester production (including hexyl esters) accompanying prevention of superficial scald symptoms in Cortland apples (*55*) when there was no evidence of anaerobic metabolism.

In general, the nature of changes in alcohol and ester production induced by long-term exposure to high CO_2 CA was similar to that induced by ULO, except that alcohols and esters were in some instances enhanced by ULO. For example, production of 1-butanol (after 4 months), ethanol (after 8 months), C_3-C_6 acetates (after 4 and 8 months), acetaldehyde,

and methanol (*30*) was higher in fruit stored in ULO than in fruit stored in high CO_2 CA. In contrast, fruit not treated with DPA and stored in high CO_2 CA (i.e., those that developed CO_2 injury) for 8 months had higher emission of nonacetate propyl esters, octanal, nonanal, and decanal than fruit stored in ULO. Similarly, fruit exposed to short-term high (20 kPa) CO_2 emitted higher octanal, nonanal, and decanal than fruit held in air. However, long-term storage in either ULO or high CO_2 CA resulted in similar or reduced production of these C_8-C_{10} aldehydes and nonacetate propyl esters relative to fruit stored in air. These results indicate that changes in Fuji apple emission of alcohols, esters, and aldehydes following exposure to high CO_2 are not clearly identified as a possible cause of CO_2 injury development in Fuji apples.

While ULO and/or high CO_2 CA conditions improve maintenance of Fuji apple firmness, acidity, and sugar content (*28*, *29*), it is not clear how CA-induced changes in volatile production impact fruit aroma and/or flavor. The present study has shown that both ULO and high CO_2 CA conditions enhance the production of ethyl acetate while reducing the production of butyl and hexyl esters more intensely than methyl and nonacetate ethyl esters relative to fruit stored in air. Sensory analyses of Fuji apples after air, regular CA, and ULO CA storage indicate that "well-accepted" fruit have more ethanol, ethyl butanoate, 1-hexanol, hexyl acetate, and butyl hexanoate than "little-accepted" fruit (*29*). Gas atmospheres used for quarantine treatments of apples (<0.5 kPa O_2 for 24 h) following long-term storage induce a higher production of ethyl butanoate and ethyl 2-methylbutanoate indicating eventual enhancement of fruit aroma (*26*). However, ethanol and acetaldehyde can reduce fruit quality contributing to off-flavor development when present at amounts greater than their flavor threshold values (*56*). Additional sensorial analyses are necessary to define maximum acceptable amounts of ethanol, acetaldehyde, and esters produced from ethanol for Fuji apples.

In summary, exposure to high CO_2 may alter volatile production in Fuji apples, primarily through increased production of ethanol, ethyl esters, and some aldehydes and reduced production of C_3-C_6 alcohols and correspondent esters. The prevention of CO_2 injury by DPA treatment is accompanied by a reduced impact on volatile production, mainly ethanol and ethyl esters. However, results of this study do not support a causal role for volatile compounds induced during CO_2 treatment with development of CO_2 injury.

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