FINAL PROJECT REPORT

Project Title: Fruit metabolic responses to controlled atmosphere O_2 and CO_2 stress

PI:	Jim Mattheis	**Co-PI**:	Dave Rudell
Organization:	USDA, ARS	**Organization**:	USDA, ARS
Telephone:	509-664-2280 x 249	**Telephone**:	509-664-2280 x 245
Email:	james.mattheis@ars.usda.gov	**Email**:	david.rudell@ars.usda.gov
Address:	1104 N. Western Ave	**Address**:	1104 N. Western Ave
City:	Wenatchee	**City**:	Wenatchee
State/Zip:	WA, 98801	**State/Zip**:	WA, 98801

Cooperators: Chris Watkins, Cornell University
Michael Young, Glade Brosi, Stemilt Growers

Other funding sources

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Amount awarded (Federal + non-Federal): $2.4 million
Notes: D. Rudell is Project Director, J. Mattheis is a Co-PI. The Standard Research and Extension Project, "A diagnostic toolbox for integrated management of postharvest apple necrotic disorders" was submitted (01/12/10) for the current funding cycle. WTFRC and AgroFresh, Inc. are co-sponsors.

Total Project Funding:

Budget History:

Item	2010	2011	2012
Salaries	$43,278	$44,176	$45,093
Benefits	$18,457	$18,933	$19,326
Wages			
Benefits			
Equipment			
Supplies	$2400	$2400	$2400
Travel			
Plot Fees			
Miscellaneous			
Total	$64,225	$65,509	$66,819

Objectives:

1. Identify volatile compounds that accumulate during CA storage.
2. Characterize volatile compound dynamics during storage in atmospheres that induce low O_2 and/or high CO_2 injury.
3. Determine if recognition of changes in volatile compound production during low O_2 or high CO_2 stress has utility for CA system management.
4. Develop sampling protocols to enable detection of biomarkers for scald and other disorders.

The goal of this research is to develop an active fruit monitoring system that alerts storage operators to undesirable CA conditions in real or near-real time. This system would incorporate some if not all existing technologies for storage room monitoring while providing additional measures to identify abnormal fruit metabolism. While the bulk of the proposed research will utilize GC-MS as the analytical system, we anticipate commercialization of this concept could utilize existing instruments and/or expertise already in place at some warehouses, consulting businesses, and ag chemical supply companies. Successful system development and implementation would reduce storage disorder risk that results from CA gas concentrations outside the range tolerable by apple fruit.

Significant Findings

1. CA chamber volatile content differed with cultivar ('Delicious', 'Fuji', 'Granny Smith'), chamber O_2 and CO_2 concentration, and storage duration.
2. Ethanol and ethyl ester accumulation increased with decreased O_2 content ('Delicious') while accumulation of typical 'Delicious' volatiles decreased.
3. 'Fuji' volatile accumulation peaked within 30 days regardless of atmosphere, 'Delicious' within 25 days (CA) or 82 days (RA).
4. Accumulation of ethyl- and methyl esters increased with increased CO_2 content ('Fuji') soon after harvest and prior to an increase in ethanol.
5. CA chamber ethylene concentration decreased with decreased % O_2 or increased % CO_2 concentration; chamber nitric oxide content decreased as storage duration increased.
6. Volatile compounds in commercial CA rooms containing 'Delicious', 'Fuji', and 'Granny Smith' apples were similar to those detected in research CA chambers, and compounds previously identified as possible predictors of 'Granny Smith' superficial scald were detected in both research chambers and commercial CA rooms.

Results and Discussion

'Delicious' low O_2 storage. Fermentation due to insufficient O_2 during CA storage can result in off-flavor development and internal browning. As 'Delicious' is often stored at low O_2 to limit superficial scald development, studies were conducted to characterize volatile compound accumulation during CA storage to assess the potential for non-invasive CA room monitoring for low O_2 stress. Fruit was obtained from the same commercial grower throughout the study. Maturity at harvest was similar in both years except for internal ethylene concentration, higher in 2010 (Table 1).

year	starch	firmness lbs	soluble solids %	titratable acidity %	internal ethylene ppm	weight g
2010	2.3±0.2	15.5±0.4	11.2±0.2	0.250±0.005	10.3±5.57	234±15
2011	2.2±0.2	15.8±0.2	11.9±0.2	0.906±0.330	0.91±0.33	223±7

Table 1. Maturity and quality of 'Delicious' apples at harvest in 2010 and 2011. Fruit obtained from a commercial orchard near Monitor, WA.

The lowest CA O_2 setpoint was determined by monitoring fruit chlorophyll fluorescence using the HarvestWatch system, and the O_2 % where a change in fluorescence was observed was 0.2 and 0.3 in 2010 and 2011, respectively. Fruit were held at these O_2 concentrations to induce fermentation and low O_2 injury as well as at two higher settings and air (2011). Fifty one volatile compounds other than ethylene and nitric oxide were detected and monitored (supplementary tables). Chamber volatile content was impacted by O_2 concentration with compounds associated with low O_2 metabolism, ethanol and ethyl acetate in particular, accumulating as O_2 concentration decreased (Figure 1). Ethanol accumulation occurred sooner after harvest in 2011 compared to 2010, possibly reflecting less capacity for fruit to use ethanol for ester volatile production in 2011 compared to 2010. In both years, CA chamber concentrations for both compounds exceeding 10 nmol L^{-1} were reached for the lowest O_2 setpoints but not in chambers held at higher O_2 settings. Consistent accumulation of ethanol and ethyl acetate above a threshold value when held at an O_2 concentration inducing fermentation would enable a CA room monitoring system to operate with target values for compounds produced during fermentation. Room ethylene and nitric oxide concentrations did not provide an indication of low O_2 stress (Figure 2).

The accumulation pattern for ethanol, ethyl acetate and other volatile compounds varied with harvest year. This difference in relation to typical harvest maturity tests indicates no simple relationship exists between fruit harvested at maturity suitable for long-term CA and response to anaerobic storage conditions. Assuming a consistent headspace threshold value for ethanol and/or ethyl acetate exists that only rooms held under low O_2 stress would exceed, a monitoring system based on the threshold value could alert the storage operator of a problem. In the two years of this study, that alert would occur in less than 3 weeks (2011) compared to 2 months (2010). Responding to the alert (by increasing room O_2) even at 2 months is likely to avoid fruit quality problems as fruit ethanol production would decrease and ethyl volatile production, a source of off-flavors, would also decrease as ethanol is depleted. If a consistent threshold value indicative of low O_2 stress does not exist, a monitoring system based on room to room comparisons could be feasible.

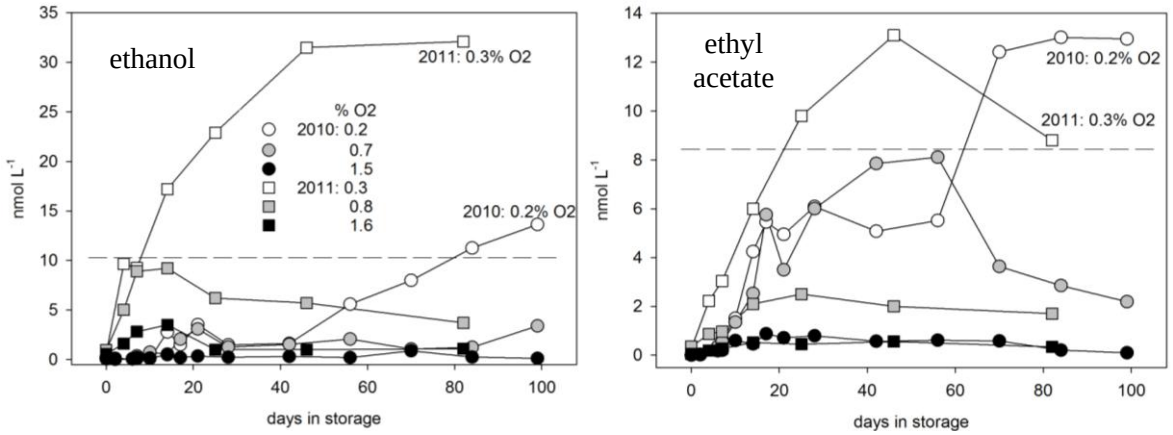


Figure 1. Ethanol and ethyl acetate in CA chambers holding 'Delicious' apples. Fruit harvested in: 2010 stored in 0.2, 0.7, or 1.5% O_2; 2011 stored in 0.3, 0.8, or 1.6% O_2. All fruit held at 33 ^oF with 1.5% CO_2. Gas samples for analysis obtained directly from CA chambers.

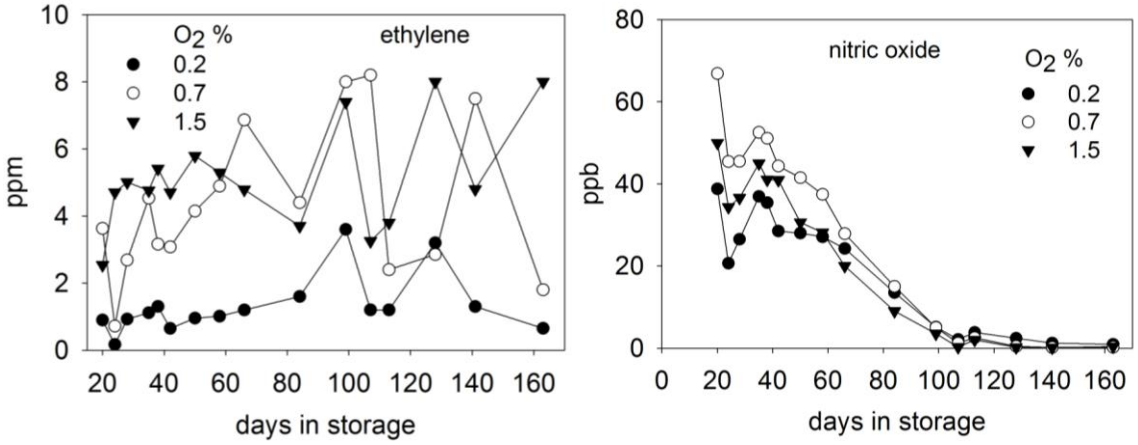


Figure 2. Ethylene and nitric oxide in CA chambers holding 'Delicious' apples. Fruit harvested in 2010 stored in 0.2, 0.7, or 1.5% O_2. All fruit stored at 33 ^oF with 1.5% CO_2. Gas samples for analysis obtained directly from CA chambers.

Apple volatile production proceeds in part based on the amounts of alcohols present in fruit tissue. Fermented fruit accumulate ethanol and therefore produce higher quantities of ethyl-volatiles. These fruit also produce less quantity of volatiles typical of normal ripening as typical alcohols are available in lower amounts, and also due to the lower O_2 setpoint. The combination of more ethyl-volatiles and less typical volatiles leads to off-flavor. This pattern is illustrated by a prominent 'Delicious' aroma volatile, 2-methylbutyl acetate (Figure 3) for which production decreases with decreased O_2 concentration and also with storage duration. The seasonal impact on volatile production is also evident for 2MBA as amounts peaked earlier in storage and to a higher amount in 2010 compared to 2011.

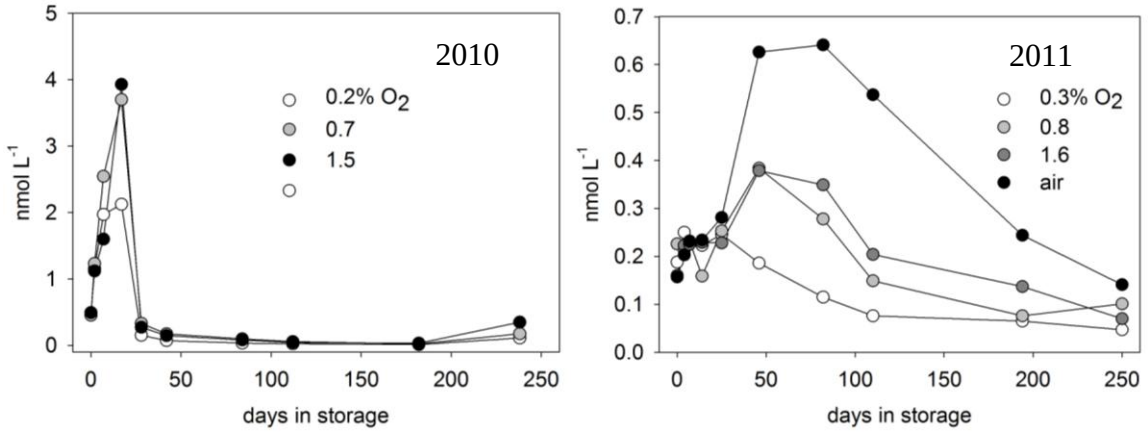


Figure 3. 2-methylbutyl acetate in CA chambers holding 'Delicious' apples. Fruit harvested in 2010 stored in 0.2, 0.7, or 1.5% O_2; 2011 stored in 0.3, 0.8, or 1.6% O_2. All fruit stored at 33 ^oF with 1.5% CO_2. Gas samples for analysis obtained directly from CA chambers.

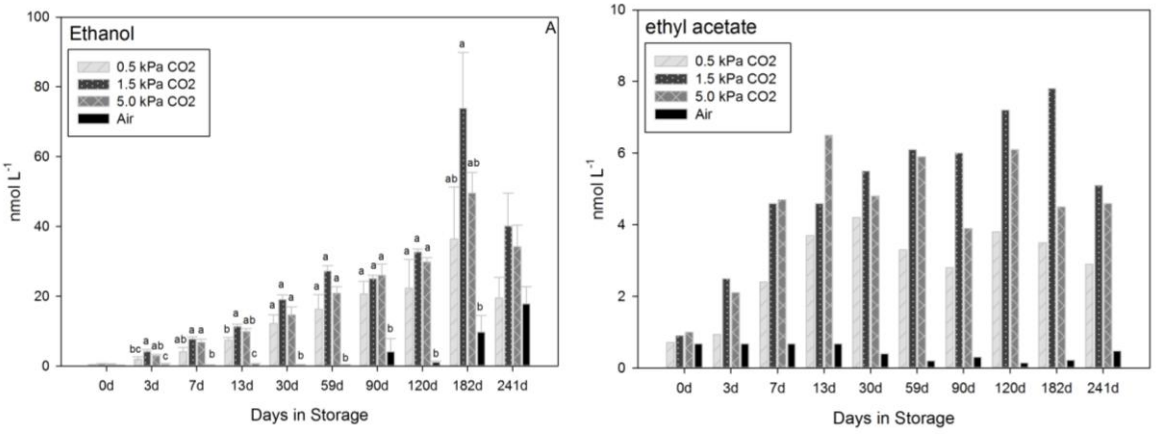
Low O_2 stress conclusions: accumulation of ethanol and ethyl acetate in CA chamber headspace was evident in chambers held at O_2 concentrations that promoted fermentation. A threshold amount for each compound exceeded only in chambers held at 0.2 or 0.3% O_2 was identified based on the 2 study years. This threshold value (10 nmol L^{-1}) could serve as a validation point for further studies to assess the potential for CA room monitoring for the potential for low O_2 injury. Low O_2 setpoints and generation of excess ethanol both limit production of typical 'Delicious' apple ester volatiles.

'Fuji'high CO_2 storage Internal browning due to high CO_2 exposure during CA storage of 'Fuji' occurs via undescribed mechanisms, however, high CO_2 CA storage is known to alter fruit volatile compound production after storage, so studies were conducted to assess volatile accumulation during CA with high CO_2 setpoints. Fruit was obtained from the same commercial grower throughout the study. Fruit at harvest had higher starch, lower firmness, titratable acidity and internal ethylene in 2011 compared with 2010 (Table 2).

year	starch	firmness lbs	soluble solids %	titratable acidity %	internal ethylene ppm	weight g
2010	4.1±0.1	17.2±0.4	16.7±0.1	0.482±0.010	3.01±0.45	279±7
2011	4.9±0.2	15.8±1.9	15.0±0.1	0.405±0.010	1.93±0.57	271±13

Table 2. Maturity and quality of 'Fuji' apples at harvest in 2010 and 2011. Fruit obtained from a commercial orchard near Orondo, WA.

The CA CO_2 settings, 0.5, 1.5, and 5%, were chosen based on previous work where 0.5 and 1.5 did not induce injury whereas injury usually occurs at 5%. All fruit was held at 33 ^{o}F and CA O_2 was 1.5% for all CO_2 treatments. Chlorophyll fluorescence of fruit held in high CO_2 has not proven to provide an indication of stress similar to that occurring as low O_2 conditions are imposed. Internal browning had developed in fruit stored in 5% CO_2 after 1month (2010) and 4 months (2011) but was not observed in other CA treatments. Chambers in which 'Fuji' apples were stored accumulated 51 volatile compounds other than ethylene and nitric oxide. While differences in volatile accumulation were evident due to CA treatment, the magnitude of the differences was not sufficient to clearly indicate the imposed CA conditions were causing stress. Examples include ethanol, ethyl acetate, ethyl butyrate, and methyl butyrate (Figure 4). While ethanol accumulated over time in CA storage, a pattern linking accumulation to stress at 5% CO_2 was not evident. Accumulation of other esters occurred at a faster rate, but no clear patterns associated with 5% CO_2 and injury were observed.



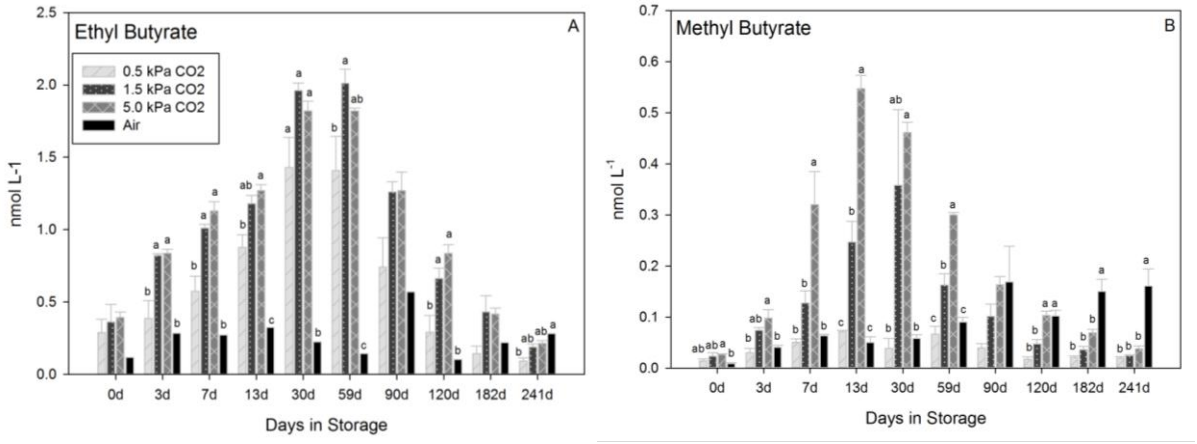


Figure4. Ethanol, ethyl acetate, ethyl butyrate, and methyl butyrate in CA chambers holding 'Fuji' apples. Fruit harvested in 2011 stored in 0.5, 1.5, or 5.0% O_2. All fruit stored at 33 ^oF with 1.5% O_2. Gas samples for analysis obtained directly from CA chambers.

While total accumulation of ethyl and methyl esters was somewhat higher in chambers held at 5% CO_2 compared to lower values, large quantitative differences due to CA treatment were not observed. This finding in both years of the study limits the potential for development of a CA room volatile monitoring system for alerting storage operators to ongoing CO_2-induced fruit stress. The results of the 2 year study are limited in part due to a relatively low incidence of CO_2 injury, even at 5% CO_2. It is unknown what differences if any in volatile accumulation would occur prior to or after CO_2 in situations where a high incidence of disorders occurs.

The results do indicate a pattern of reduced accumulation of some volatiles, illustrated by ethyl- and methyl butyrate, over time in storage. This tendency contributes to the well known diminution of aroma in fruit previously held in long term CA. Should procedures be identified that could enhance the capacity for aroma production during CA, such as manipulation of room O_2 and/or CO_2, monitoring systems similar to that used in this study could provide the basis for enhanced fruit quality management resulting in greater aroma after storage.

'Granny Smith' apple volatile accumulation during air or CA storage. Fruit were stored in air or CA (1% O_2, 1% CO_2) with or without pre-storage DPA (2000 ppm) treatment. Through 8 months storage, volatile compounds typical of 'Granny Smith' were detected with amounts lower in CA chambers. Several volatiles previously related to scald development were detected from fruit stored in air or CA. Accumulation of some esters including butyl butyrate, 2-methylbutyl acetate, and butyl 2-methylbutyrate was greater in chambers held at 1/1 O_2/CO_2 containing DPA-treated fruit. Contrary to what was observed for 'Delicious' and 'Fuji', ester accumulation increased throughout the first 8 weeks CA storage of 'Granny Smith'. Superficial scald symptoms developed on fruit stored in air or CA and volatile accumulation indicative of scald preceded symptom development.

Volatiles accumulating in commercial CA storage. Gas samples from one room each of 'Delicious', 'Fuji', and 'Granny Smith' apples were collected beginning in mid-December from a Stemilt facility in East Wenatchee. All volatiles detected (46) in commercial rooms have been previously identified in research CA chambers containing the same cultivars. Amounts of volatiles detected in all commercial room samples were low compared to samples collected from CA chambers. Of the 3 cultivars, volatile content in the 'Fuji' room was highest followed by 'Delicious' and then 'Granny Smith'. 2-methyl-1-butanol was the most abundant volatile collected in all three rooms, and ester accumulation was low in all rooms. Volatile compounds that have been associated

with development of superficial scald were detected in the commercial ‘Granny Smith’ room. As similar gas volumes were collected from research CA chambers and the commercial rooms, volatile production or accumulation in commercial rooms may be less compared to CA chambers, or headspace volume in rooms versus chambers may be different. Additional sampling from rooms is needed to determine optimum sampling conditions, and additional room sampling of ‘Granny Smith’ rooms at this facility is ongoing in 2012-13.

Executive Summary

A detailed report of the low O_2 and high CO_2 portions of this project is available, send request to james.mattheis@ars.usda.gov.

The low O_2 and high CO_2 studies demonstrate the capacity to identify and quantify apple volatile compounds produced during rather than after CA storage. CA gas composition has a marked impact on volatile accumulation as does storage duration and season of fruit production. For chambers held at very low O_2 concentrations with a risk of fermentation, patterns of volatile accumulation indicative of stress are identifiable. This part of the study demonstrates proof of concept for using storage chamber volatile compound monitoring to identify situations that can result in fruit quality issues leading to postharvest loss. Consistent patterns of ethanol and ethyl acetate accumulation over the initial 2 years of the project support a threshold value for these compounds that could be the subject of validation under scaled up commercial conditions. If validated, a monitoring system could be developed that would alert storage operators when either of these compounds exceeded the threshold value. High CO_2 stress did not result in similar results, possibly due to a low incidence of CO_2 injury even though 'Fuji' apples were held in 5% CO_2.

Both cultivars showed diminution of volatile accumulation over time, a result similar to post storage analyses of fruit volatile production. The capacity to monitor fruit during storage provides a potential means to time alteration of room gas conditions to enhance capacity to produce aroma currently lost as duration in low O_2 increases. Our lab previously demonstrated the utility of this type of dynamic CA where brief periods of increased O_2 during storage resulted in enhanced aroma production after storage. What was lacking in the earlier work was a basis on which to decide when to add oxygen to the room. A decision to add O_2 based on CA room volatile content could potential enhance the effectiveness of this protocol while minimizing loss of other quality components, particularly firmness, in susceptible cultivars. This system may have greater utility in cultivars where firmness management is not the primary objective of the postharvest system. The commercial room sampling to date shows volatile accumulation patterns are similar to those observed in research CA chambers.

The capacity to detect volatiles shown to be likely predictors of superficial scald risk for 'Granny Smith' was also demonstrated in research as well as commercial CA chambers and rooms. Validation of the use of this analysis by Dave Rudell is ongoing. Current work with 2012 crop fruit held initially in 4 now 3 rooms in the Stemilt East Wenatchee facility will provide additional information regarding the dynamics of particular volatiles as a function of storage duration.

Supplementary Table 1. Volatiles monitored in storage chambers containing 'Delicious' apples. Compounds listed in groups as clustered by response patterns during storage.

ethyl propanoate	butyric acid	2-methylbutyl propanoate	methyl hexanoate
ethyl-2-methylbutyrate	pentanal	2-methylbutyl butyrate	1-pentanol
ethyl hexanoate	heptanal	propyl 2-methylbutyrate	1-butanol
ethyl pentanoate	hexanal	propyl propanoate	acetone
ethyl butyrate	propyl hexanoate	propyl acetate	1-hexanol
1-propanol	hexyl propanoate	butyl butyrate	butyl 2-methylbutyrate
methyl butyrate	butyl hexanoate	butyl propanoate	decanal
methyl acetate	hexyl butyrate	pentyl acetate	6-methyl-5-hepten-2-one
methyl-2-methylbutyrate	hexyl 2-methylbutyrate	propyl butyrate	6-methyl-5-hepten-2-ol
ethyl acetate	ethyl octanoate	2-methylpropyl acetate	2-methyl-1-propanol
ethanol	estragole	butyl acetate	2-methyl-1-butanol
acetaldehyde	octanal	2-methylbutyl acetate	
	2-ethyl-1-hexanol	hexyl acetate	
	nonanal		
	benzaldehyde		

Supplementary Table 2. Volatiles monitored in storage chambers containing 'Fuji' apples. Compounds listed in groups as clustered by response patterns during storage.

2-methylbutyl butyrate	ethyl butyrate	1-pentanol
butyl butyrate	ethyl propanoate	1-butanol
2-methylbutyl propanoate	ethyl pentanoate	2-methyl 1-butanol
hexyl acetate	ethyl hexanoate	1-hexanol
pentyl acetate	ethyl 2-methyl butyrate	1-propanol
2-methylbutyl acetate	ethyl acetate	2-methyl 1-propanol
butyl acetate	methyl butyrate	acetone
butyl propanoate	methyl hexanoate	butyl 2-methylbutyrate
2-methylpropyl acetate	methyl 2-methylbutyrate	6-methyl-5-hepten-2-ol
propyl acetate	pentanal	benzaldehyde
propyl propanoate	heptanal	acetaldehyde
propyl butyrate	hexanal	ethanol
propyl 2-methyl butyrate	butyric acid	methyl acetate
6-methyl-5-hepten-2-one	2-ethyl-1-hexanol	decanal
hexyl propanoate	nonanal	
propyl hexanoate	octanal	
butyl hexanoate		
hexyl butyrate		
hexyl 2-methylbutyrate		
ethyl octanoate		
estragole		