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Effect of 1-Methylcyclopropene on Volatile Emission and Aroma in Cv. Anna Apples

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The rapidly ripening summer apple cultivar Anna was treated with 0.1 μL L^{-1} and 1 μL L^{-1} 1-methylcyclopropene (MCP) at harvest and kept at 20 °C, or stored for 5 weeks at 0 °C and then transferred to 20 °C. Total volatiles were not reduced by treatment with 0.1 μL L^{-1} MCP, but were 70% lower in fruits treated with 1 μL L^{-1} MCP than in untreated fruits. Ethylene production was 50% and 95% inhibited by 0.1 μL L^{-1} and 1 μL L^{-1} MCP, respectively. The volatiles produced by fruit at harvest were predominantly aldehydes and alcohols, with some acetate esters as well as 2-methyl butyl acetate and β-damascenone. During ripening, the acetate and butyrate esters increased greatly and alcohols and aldehydes decreased. MCP-treated apples retained more alcohols, aldehydes, and β-damascenone volatiles than did untreated apples. Sensory evaluation found that control and 0.1 μL L^{-1} treated apples developed more fruity, ripe, and overall aromas, but the preference was for the 1 μL L^{-1} treated apples with a less ripe aroma.

KEYWORDS: Ethylene; ripening; *Malus sylvestris*

INTRODUCTION

Volatile compounds increase dramatically as fruit ripening progresses (*1*−*3*). These compounds include esters, alcohols, aldehydes, ketones, and terpenoids. The largest change in volatile compound production during apple ripening is an increase in ester production (*4*). Reports differ as to whether the onset of biosynthesis of volatile compounds is concurrent with, or precedes, and perhaps plays a role in the initiation of the climacteric rise in fruit respiration (*13*). Brown et al. (*4*) showed that ethylene production and the climacteric rise in respiration occurred before the large increase in production of volatiles. Ethylene action inhibitors, such as diazocyclopentadiene and 1-methylcyclopropene (MCP) inhibit ethylene production in ripening apples (*5*, *6*). Volatile production was inhibited by MCP in banana (*7*) and plum (*8*) fruits, although it was unclear whether production of all volatile compounds was inhibited to the same degree. A study of the effect of MCP on volatile accumulation in cv. Fuji apples found that individual alcohols and esters appeared to be differentially inhibited by MCP (*9*).

Anna apples are an early ripening summer cultivar which produces copious amounts of ethylene and softens rapidly even at 0 °C storage (*10*). It was developed in Israel as a low-chilling-requirement apple, and is planted widely in subtropical regions around the world (*11*). The fruit has a crisp texture at harvest which quickly becomes mealy, and usually this cultivar is marketed directly after harvest or stored for only a short period of time. However, because it ripens in June and the next cultivar does not ripen until August there is a need for longer storage. The current work examined the effect of MCP applied to Anna apples on their volatile production and aroma when held at 20 °C for 12 days, or when stored for 5 weeks at 0 °C after the treatment and then removed to 20 °C.

MATERIALS AND METHODS

MCP Treatment. Cv. Anna apples were harvested from a commercial orchard in Israel. They were treated with 0 (control), 0.1 μL L^{-1}, and 1 μL L^{-1} MCP. Each group of apples (120 fruit) was placed in a 30-L plastic jar. The MCP (4.5 mg, or 45.3 mg of Ethylbloc, a.i. 0.14%, for 0.1 μL L^{-1} or 1 μL L^{-1}, respectively) was weighed out into a capped test tube. Just before treatment, 1 mL of 40 °C water was added to the test tube. The tube was closed, vortexed, placed in the jar with the apples, and opened just prior to sealing the jar. After 20 h at 20 °C, apple fruit were removed from different jars and held at 20 °C for 12 days. A second experiment was conducted for fruits to be stored. Half the fruits (100 fruit per treatment) were placed directly in storage, and half were treated with 1 μL L^{-1} MCP as above. The fruits were stored for 5 weeks at 0 °C and then held for 10 d at 20 °C.

Volatile Analysis. This analysis was done on harvested fruit, on untreated fruit, and on fruit treated with 0.1 μL L^{-1} or 1 μL L^{-1} MCP after 6 and 12 days shelf life. Another analysis was done of fruits following storage, both at removal and after 6 and 12 days at 20 °C. The extraction and analysis procedures were according to ref *12*. A 200-g composite sample from 5 apples was blended for 2 min in 400 mL of 20% NaCl. The slurry was filtered through cheesecloth, centrifuged at 10000*g* for 30 min. To 10 mL of supernatant was added

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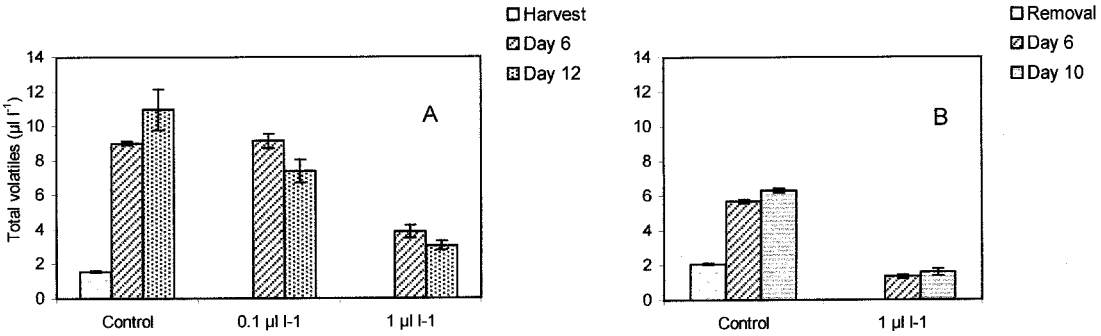

Figure 1. Total volatiles from control cv. Anna apples and fruit treated with 0.1 μL L^{-1} or 1 μL L^{-1} MCP at harvest and held at 20 °C for 6 and 12 days (A), or (B) stored for 5 weeks at 0 °C and then removed to 20 °C for 10 days (control and 1 μL L^{-1} MCP-treated apples only).

2,6-dimethyl-5-hepten-2-ol (0.1 μL L^{-1} v/v) (Merck) as an internal standard. This sample was transferred to a 20-mL vial containing 2 g of NaCl to inhibit enzymatic reactions and sealed. The samples were held at 4 °C until analysis. Each treatment was prepared in duplicate.

The volatiles were sampled by manual headspace solid-phase microextraction (SPME) at ambient temperature. After 30 min of sampling, the fiber (75-μm PDMS/DVB, Supelco) was placed in the injection port of a GC−MS (Hewlett-Packard GCDplus system equipped with a 30 m × 0.25 mm i.d. Rtx-5SIL MS column) and analyzed in the splitless mode. The oven had an initial temperature of 70 °C, and was heated at 4 °C/min to 165 °C. The carrier gas was helium with a constant flow of 1 mL min^{-1}; the injector was held at 250 °C; the electron ionization detector (at 70 eV) was set at 280 °C; and the mass range was 45 to 300 *m/z*. Compound identification was made by comparison of spectra of sample compounds with those from the database NIST 98 and by comparing retention indices of sample compounds from standards and from literature. The volatiles were quantified by calculating the relative concentrations in relation to those of the internal standard. The areas of the volatile component peaks were normalized by the area of the internal standard peak.

Ethylene Measurement. Five fruits from each of the 20-h treatments (control, 0.1 μL L^{-1}, and 1 μL L^{-1}) were weighed and placed in 0.6-L jars at 20 °C. The jars were sealed 1 h each day with a cap containing a rubber septum. Gas samples were removed with a syringe and injected into the gas chromatograph. Ethylene was determined using a FID detector on a GC and an alumina column. The carrier gas was helium at 30 mL min^{-1}, the injection port and oven were set at 100 °C, and the detector was set at 155 °C.

Aroma Assessment. The aroma assessment was done on control fruit and on fruit treated with 0.1 μL L^{-1} and 1 μL L^{-1} MCP amd control, after 6 and 12 days shelf life, as well as on newly harvested fruit. To assess all the samples simultaneously, fruit were sampled at the different time points and kept at 0 °C until the day of the sensory test (after 12 days of shelf life). They were equilibrated to room temperature prior to sensory evaluation. The same procedure was conducted during ripening after storage on control and 1 μL L^{-1} MCP-treated fruit at removal, and after 6 and 12 d at 20 °C.

The descriptive analysis was performed by a 10-member panel of employees of the ARO (5 women and 5 men). A first session allowed agreement on the following aroma descriptors: overall intensity, flowery, fruity, ripe, green, alcohol, or off-flavors. The panel was trained on these descriptors by profiling 4 kinds of apples: Red Delicious, Granny Smith, Anna "unripe" (just harvested), and Anna "ripe" (5 days shelf life), covering the variation in most descriptors. Each sample was assessed 3× and panelists were evaluated for reliability and validity.

For each aroma test, at least 5 apples per treatment (approximately 500 g) were cut into small pieces (with the peel), pooled, and put into glasses (Duralux). The glasses were covered and left at room temperature 30 min for acclimation before the test. The sample presentation was randomized per panelist and per replicate. Each panelist assessed 3 or 4 samples labeled with three digits, using an unstructured 10-cm scale with anchor points "very weak" on the left-hand side and "very strong" on the right-hand side for each of the descriptors. The panelists were also asked to express their preference among the samples. All assessments were replicated 2× in a randomized block experiment.

RESULTS

Volatile Analysis. The total volatiles from freshly harvested apples were low and increased 6-fold in control apples after 6 days at 20 °C (**Figure 1**). The apples treated with 0.1 μL L^{-1} MCP also increased in volatile production 6-fold after 6 days, while volatiles from 1 μL L^{-1} MCP-treated apples doubled. After 12 days at 20 °C, there was a further rise in total volatiles in control fruit, but the MCP-treated fruit did not show any increase.

Freshly harvested fruit had only a small amount of acetates, and 50% of the total volatiles were aldehydes. A number of volatiles greatly decreased or disappeared during ripening of control apples (**Table 1**). These included the cyclic, ether 2-ethylfuran, as well as the aldehydes, hexanal, 2-hexenal, 2-heptenal, and benzaldehyde and the terpene β-damascenone. Two alcohols present in freshly harvested apples, 2-methylbutanol and hexanol, decreased during ripening, but other alcohols which were not present in these fruit appeared in ripening fruit.

All the fruit held at 20 °C showed large increases in ester volatiles, particularly the acetate esters, which comprised over 50% of the total volatiles of the control apples. The butyrate esters, together with the methyl butyrate esters, also comprised a major group of volatiles and were close to 25% of the total volatiles of ripe control apples.

Volatile production of the MCP-treated apples differed from that of the control apples. In addition to the decreased amount of volatiles produced by apples treated with 1 μL L^{-1} MCP, the proportion of volatiles which were esters was lower than that from other fruits. Hexanol and 2-hexenal remained high, as did hexanal and β-damascenone. All these compounds were found in high amounts in the freshly harvested fruit, indicating that the 1 μL L^{-1} MCP retained the volatile synthesis pathway of the freshly harvested fruit, while developing to a smaller extent the pathways of ripening apples. The apples treated with 0.1 μL L^{-1} MCP had ester volatile accumulation similar to that of the control fruit. However, they also produced higher levels of hexanol than did control fruits.

Ethylene production was low in freshly harvested apples, but increased greatly during the first 6 days at 20 °C and showed a further, though less dramatic, rise between 6 and 12 days (**Figure 2**). The fruits treated with 0.1 μL L^{-1} MCP had about 50% of the ethylene production rate of the control fruits, and fruits treated with 1 μL L^{-1} MCP showed almost no ethylene production.

At removal from 5 weeks of 0 °C storage, ethylene production of the control apples was already quite high, and it continued to increase during the 10 days that the fruit were held at 20 °C (**Figure 2**). The final rates were 16% lower than the ethylene

Table 1. Volatiles from Cv. Anna Apples[a]

		6 days at 20 °C			12 days at 20 °C				6 days at 20 °C		10 days at 20 °C	
volatile	harvest	control	0.1 μL L^{-1}	1 μL L^{-1}	control	0.1 μL L^{-1}	1 μL L^{-1}	removal	control	1 μL L^{-1}	control	1 μL L^{-1}
Acetate Esters												
isobutyl acetate										0.2		
butyl acetate	1.5	15.3	14.0	14.5	16.3	17.5	0.7	15.9	14.0	7.2	15.5	7.2
2-methylbutyl acetate	6.2	12.4	6.8	12.1	12.5	13.0	19.9	5.4	17.6	10.8	10.7	17.0
pentyl acetate	0.6	1.6	1.7	1.4	0.5	1.0	0.9	1.5	1.3	0.9	0.7	0.7
hexenyl acetate	3.1	1.0	0.3	0.4	0.3	0.5	0.8	1.1	1.3	1.9		1.3
hexyl acetate	6.4	25.0	27.7	16.6	27.1	22.5	10.7	15.3	16.0	8.1	18.6	8.2
2-hexenyl acetate		0.7		0.8		1.3		1.8	0.7	0.9	0.8	2.5
benzyl acetate										1.0		
total	17.8	56.0	50.5	45.8	56.1	55.6	39.1	41.0	50.9	31.0	46.3	36.9
Propanoate Esters												
butyl propanoate		1.3	0.9	1.0	0.9	1.1			1.5		0.5	
hexyl propanoate			0.8	0.4	0.2	0.4			0.5		0.2	
total		1.3	1.7	1.3	1.1	1.5			2.0		0.7	
Butyrate Esters												
methyl butyrate		0.4	0.6	0.6	0.2	0.4	1.6		0.2		0.4	
ethyl butyrate		3.9	0.3	2.9	8.9	5.1			6.5	1.2	11.0	0.4
propyl butyrate		1.5	0.7	1.2	2.8	1.9			1.8		2.2	
butyl butyrate		4.5	5.7	2.6	4.1	3.9	2.2	3.5	8.8	1.0	4.0	1.8
pentyl butyrate			0.2		0.2						0.2	
hexyl butyrate		2.0	2.3	1.1	1.0	0.7	0.6	0.5	2.2		1.3	0.6
total		10.9	9.8	8.4	17.0	12.0	4.3	4.0	15.3	2.2	19.1	2.8
Hexanoate Esters												
methyl hexanoate		0.5	0.3	0.3		0.4	0.4				0.1	
ethyl hexanoate		0.6		0.4	1.5	0.7			0.5		1.2	
butyl hexanoate		1.4	2.3	0.9	1.0	0.7	0.4	0.5	2.2		1.3	0.5
hexyl hexanoate		0.6	1.8	0.4	0.3	0.2			0.3		0.4	
total		3.1	4.4	2.0	2.8	2.0	0.8	0.5	3.0		3.0	0.5
Methyl Butyrate Esters												
methyl 2-methylbutyrate		0.3	0.2	0.4	0.2	0.2	0.7		0.5	0.6	0.2	
ethyl 2-methylbutyrate		1.0	0.2	1.0	2.8	1.1			0.5		3.8	
butyl 2-methylbutyrate		2.3	1.6	1.2	1.7	2.1	0.7		0.8		1.6	0.3
propyl 2-methylbutyrate		0.8			0.4	0.3					0.7	
hexyl 2-methylbutyrate		1.9	3.7	1.5	1.9	0.9	1.5		0.8		0.9	0.7
total		6.3	5.7	4.1	7.0	4.6	2.9		2.6	0.6	7.2	1.0
Alcohols												
butanol		1.2	1.0	1.0	1.0	1.6	0.5	1.7	0.7	0.7	0.8	0.6
2-methylbutanol	3.9	1.1	0.9	4.2	1.0	1.6	4.7	1.4	0.6	3.1	0.6	3.5
hexanol	10.8	9.2	15.7	12.5	6.8	11.3	11.5	23.1	9.3	9.3	8.7	10.7
octanol		0.2	0.3	0.3		0.2			0.2		0.1	
decanol		0.5		1.2		0.3						
total	14.7	12.2	17.9	19.2	8.8	15.0	16.7	26.2	10.8	13.1	10.2	14.8
Aldehydes												
hexanal	2.1	0.9	0.6	1.0	0.6		3.1	1.8	2.0	3.1	2.1	4.6
2-hexenal	45.4	3.9	6.2	14.1	3.0	4.7	24.4	20.2	8.7	38.4	4.2	34.0
2-heptenal	1.0		0.1				0.6					
benzaldehyde	0.9			0.4			0.5					
total	49.4	4.8	6.9	15.5	3.6	4.7	28.9	22.0	10.7	41.5	6.3	38.6
2-ethylfuran	7.6		0.2	1.0			0.9	1.0		4.3		1.2
2-pentylfuran		0.2	0.2	0.2		0.2					0.2	
vitispirane				0.4			0.5					
β-damascenone	5.7	1.2	1.3	1.7	0.8	1.4	4.9	2.8	0.5	3.0	0.6	2.3
α-farnesene	1.9	1.2	0.8		0.4	0.2		1.8	1.2	1.0	3.2	
others	2.9	2.8	0.9	0.4	2.4	2.8	1.0	0.7	3.0	3.3	3.2	1.9

[a] Values are the average of the results of two samples from each treatment and are expressed as percent of total volatiles of that sample.

produced from apples without storage. Apples treated with 1 μL L^{-1} MCP at harvest were inhibited in their ethylene production following the 5-week storage period.

The increase in volatiles in the ripening apples after storage was less than in fruit that was analyzed directly after harvest (**Figure 1**). On day 6 stored apples had only 60% of the total volatiles that were present in unstored apples after that length of time at 20 °C. Moreover, the volatile profile of control apples at removal from storage was quite different from that of freshly harvested fruit (**Table 1**). Esters made up a large proportion of the volatiles. The acetate esters were 2.3-fold more abundant in fruit at removal from storage than from harvested fruit, although the other ester classes were less prominent in these fruit at removal. However, the proportion of alcohols present in fruit at removal was greater than that in harvested fruit, mainly due to a large proportion of hexanol. During ripening, the control fruit increased in ester volatiles and decreased in the alcohols and aldehydes, similar to fruit that was not stored. The MCP-treated fruit after storage behaved similarly to MCP-treated fruit without storage, with a smaller increase in esters than control fruit and a higher proportion of alcohols and the aldehyde 2-hexenal.

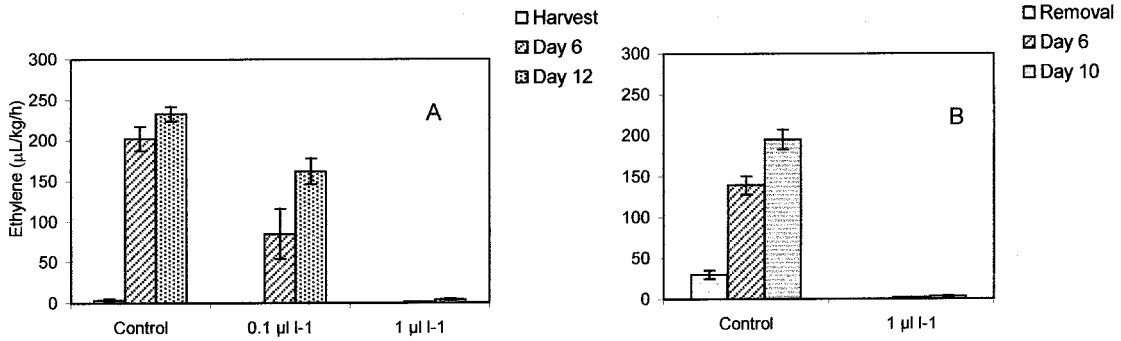

Figure 2. Ethylene evolution from control cv. Anna apples and fruit treated with 0.1 μL L^{-1} or 1 μL L^{-1} MCP at harvest and held at 20 °C for 12 days (A), or (B) stored for 5 weeks at 0 °C and then removed to 20 °C for 10 days (control and 1 μL L^{-1} MCP-treated apples only).

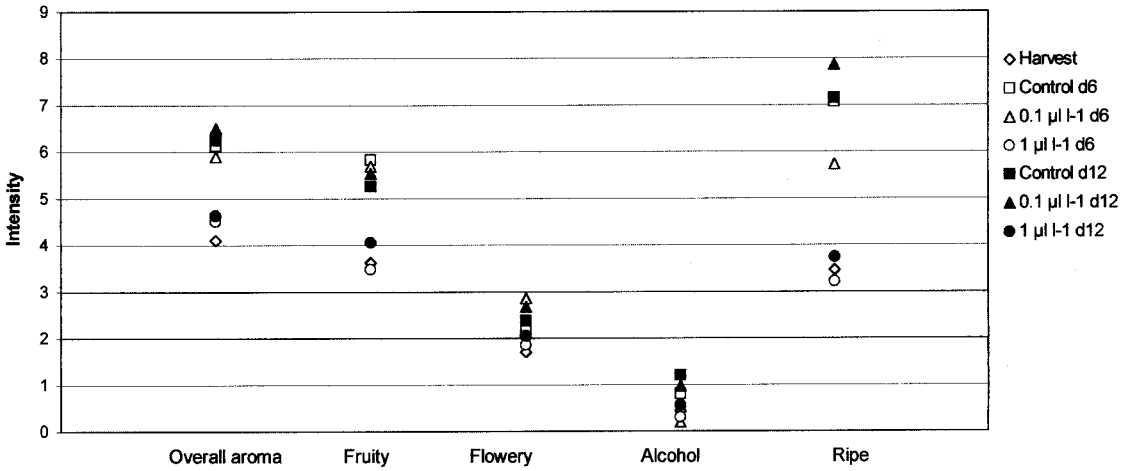

Figure 3. Intensity of aroma descriptors from control cv. Anna apples and fruit treated with 0.1 μL L^{-1} or 1 μL L^{-1} MCP at harvest and held at 20 °C for 12 days.

Aroma Assessment. Panelists were able to distinguish differences in overall aroma intensity, as well as fruity and ripe aromas, among the different treatments of nonstored apples (**Figure 3**). The other descriptors (flowery, alcohol, and off-flavors) were indistinguishable among the different samples, either because there were actually no differences as far as those qualities were concerned, or because the panel members were unable to distinguish the differences. Harvested fruits had the lowest overall aroma intensity and the lowest ratings in most of the other descriptors. Aroma changes occurred very rapidly during the shelf life of Anna, as the overall aroma intensity and ripe aroma of control fruit after 6 and 12 days were very similar, whereas there were big aroma differences between apples at harvest and control day 6. The fruits treated with 0.1 μL L^{-1} MCP had aroma profiles similar to those of control fruits, whereas the fruits treated with 1 μL L^{-1} MCP showed lower ratings for overall aroma intensity, and fruity and ripe aromas.

After storage, a similar pattern emerged whereby the control fruit at removal had low overall aroma intensity and little fruity aroma (**Figure 4**). During ripening at 20 °C, the control fruits increased in overall, fruity, and ripe aromas, whereas fruit treated with 1 μL L^{-1} MCP at harvest remained very similar to stored fruits for the first 6 days of ripening, and only after 10 days was there an increase in the overall and fruity aromas.

The preference of the panelists for unstored fruit was for 1 μL L^{-1} MCP 12 d > 1 μL L^{-1} MCP 6 d > harvest. The control fruit and 0.1 μL L^{-1} MCP-treated fruit after 6 or 12 days were not preferred, with panelists preferring "green" or harvest-like apple aroma more than the ripe and fruity aroma.

DISCUSSION

The temporal sequence of volatile biosynthesis in apples has been studied in some detail. Paillard (*12*) found that as ripening progressed in cv. Golden Delicious apples degreening occurred. This was due to disassembly of chloroplasts which released membrane galactolipids rich in linolenic and linoleic acids. As these lipids were oxidized, biosynthesis of the aldehydes hexenal and 2-hexanal occurred. Upon onset of the respiratory climacteric, aldehyde production decreased, and alcohol biosynthesis ensued. Ester biosynthesis also closely followed advancement of the climacteric.

This progression of volatile biosynthesis according to molecular class was also observed in Bisbee Delicious apples, with aldehydes being prominent in preclimacteric fruit, and alcohols and then esters increasing as the internal ethylene of the fruit increased (*2*). In Rome apples the total acetates accumulation increased as fruits ripened from the mid- to postclimacteric stage (*1*), whereas in Fuji apples, acetates began to accumulate as apples approached harvest maturity (*14*).

The accumulation of volatiles in untreated Anna fruit followed the pattern found in other apple cultivars (*1*, *2*, *13*, *14*). At harvest there were almost no esters present but a great deal of the aldehyde, 2-hexenal, and the alcohols hexanol and 2-methylbutanol. These decreased in proportion of total volatiles during ripening while esters accumulated.

Over 300 volatiles have been isolated from apples (*15*). Of these, three esters, butyl acetate, 2-methylbutyl acetate and hexyl acetate, are considered major contributors to the characteristic apple-like aroma (*14*). In addition to these esters, each cultivar

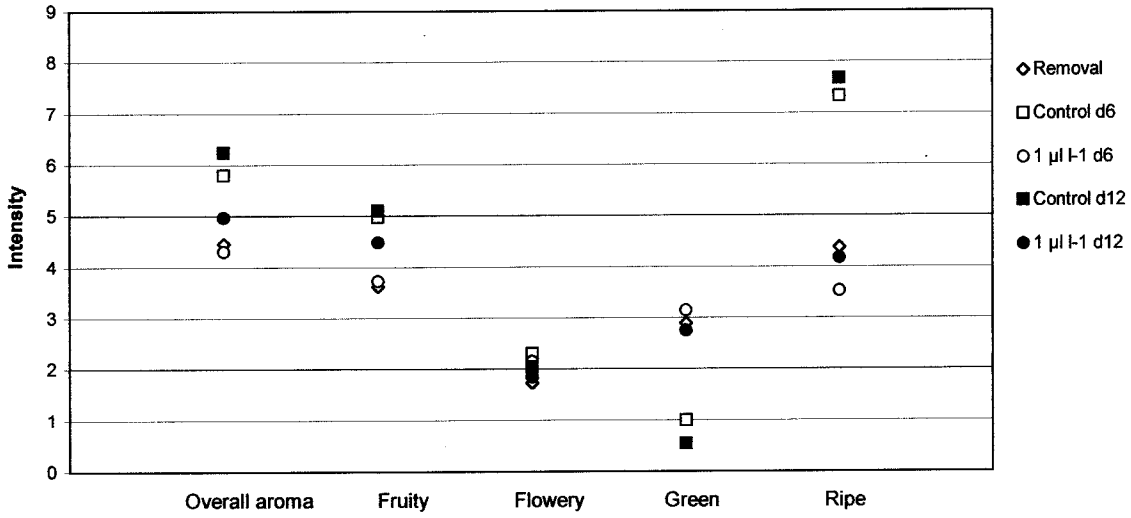

Figure 4. Intensity of aroma descriptors from control Anna apples and fruit treated with 1 μL L^{-1} MCP at harvest, stored for 5 weeks at 0 °C, and removed to 20 °C for 12 days.

has varying proportions of other esters which give each cultivar a slightly different and distinctive aroma (2, *16*−*20*). Control Anna apples contained small amounts of butyl acetate, hexyl acetate, and 2-methylbutyl acetate at harvest, when almost all other esters were absent, and these three esters accounted for 53% of the total volatiles on day 6, and 56% of the total on day 12. The 1 μL L^{-1} MCP-treated apples also contained comparable proportions of these three volatiles: 43% on day 6 and 31% on day 12. However, the total amount of volatiles from 1 μL L^{-1} MCP-treated apples was 57% less than control apples on day 6 and 72% less on day 12.

Treating apples with MCP inhibited the production of ethylene, with 0.1 μL L^{-1} being less inhibitory than 1 μL L^{-1} MCP. Considering MCP also decreased volatile production, it appears that development of full aroma in apples depends on ethylene, as Fan and Mattheis (*9*) found in cv. Fuji apples. However, in Fuji apples ethylene production was not detectable after MCP treatment. In Anna apples, ethylene is progressively inhibited as the concentration of MCP increases, but even at the highest concentration it is still measurable. At 50% inhibition, as with 0.1 μL L^{-1} MCP, volatile production levels are similar to those of control fruit, yet alcohols are a higher proportion of the volatiles than they are in control fruits. In apples treated with 1 μL L^{-1} MCP, where ethylene is inhibited over 95%, both alcohols and aldehydes remain a high proportion of the total volatiles, even while acetate esters are being accumulated.

Panelists could clearly distinguish aroma differences between control and 1 μL L^{-1} MCP-treated apples, both during ripening without storage and following 5 weeks of storage. The control fruits increased in overall, fruity, and ripe aromas, while the aroma of MCP-treated apples remained similar to that of harvested fruit. Interestingly, the preference of the panelists was for the MCP-treated fruit over the ripened control fruit. The accumulation of large quantities of volatiles in the control fruit led to a perception of very ripe fruit which was less preferred than the partially ripened aroma of 1 μL L^{-1} MCP-treated fruits.

A partial explanation of this preference may be that MCP fruits continue to accumulate the volatiles of freshly harvested apples, along with the esters of ripe apples. One compound which is present in the freshly harvested apples and is retained in 1 μL L^{-1} MCP-treated apples is β-damascenone, which imparts a flowerlike aroma. This compound, together with 2-hexenal, which has a green odor, gives the MCP-treated apples an aroma which is different from that of the control apples. In conclusion, 1 μL L^{-1} MCP inhibited both ethylene and total volatile production in ripening cv. Anna apples, and these apples were perceived to be less ripe, yet more acceptable, than control apples.

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