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Mandarin flavor and aroma volatile composition are strongly influenced by holding temperature

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ABSTRACT

Mandarin flavor quality often declines during storage but the respective contributions to the flavor disorder of warm versus cold temperature during storage were unknown. To determine this 'W. Murcott Afourer' mandarins were stored for either 6 weeks at a continuous 5 °C or held at 20 °C for either 1 or 2 weeks following 0, 2 or 4 weeks of 5 °C storage. Sensory quality as measured by likeability was maintained throughout the 6 week storage when the fruit were kept at 5 °C, but rapidly declined upon moving fruit to 20 °C. Flavor loss increased as the duration of cold storage prior to the warm temperature holding period was lengthened. The beneficial effect of maintaining mandarins in cold storage was also observed in three of the five other varieties where there was flavor quality loss during storage at a warmer temperature. Soluble solids concentration (SSC) and titratable acidity (TA) were relatively unchanged by holding at 20 °C, but aroma volatiles, with alcohols and ethyl esters being of the greatest importance, were greatly enhanced in concentration and are the likely cause of the off-flavor. The increases in aroma volatile concentration were apparent within one day of holding the fruit at 20 °C, indicating the need to carefully control postharvest storage temperatures. A comparison of 5, 10 and 20 °C holding indicated that it is only at 20 °C that aroma volatiles contributing to off-flavor accumulated. This study suggests that it may be possible in many mandarin varieties to prevent losses in flavor quality by maintaining the fruit at a cold temperature (5–10 °C) following packing and until the time of consumption.

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1. Introduction

Mandarin flavor quality problems have been the focus of a number of recent reports that have investigated the basis for the poor flavor frequently observed following storage (Tietel et al., 2010a, 2011a,b,c,d; Obenland et al., 2011). Although changes in titratable acidity (TA) as a result of storage have been well documented in mandarins as well as other citrus (Obenland et al., 2008; Marcilla et al., 2009), alterations in aroma volatile concentration appears to be the most likely cause for the majority of the poor flavor (Tietel et al., 2010a,b; Obenland et al., 2011). Decreases in certain aldehydes, terpenes and alcohols can occur which may be linked to a diminished mandarin-like flavor (Tietel et al., 2010a,b). Those compounds that increase to the greatest extent during storage of mandarins are most typically alcohols and esters (Tietel et al., 2010a; Obenland et al., 2011), with at least some of the esters being esterification products of ethanol (Tietel et al., 2011b). Ethanol can accumulate to very high concentrations in mandarins that have been commercially waxed due to the initiation of low oxygen-induced fermentation, a process that been also observed in other citrus (Ke and Kader, 1990). An enhancement in the concentration of esters with a fruity, sweet aroma can lead to the perception of the fruit being overripe or having a pronounced off-flavor. Research utilizing gas chromatography/olfactometry has also identified storage-induced enhancements in aroma active compounds leading to potential increases in musty and fatty aromas (Tietel et al., 2011d). Taken together, it is clear that major shifts in aroma volatile composition, in the form of both increases and decreases, occur during mandarin storage and have the potential to cause a major loss in flavor quality.

Storage temperature has been demonstrated to have a strong impact on mandarin flavor quality (Obenland et al., 2011; Tietel et al., 2011c). Although recommended temperatures for mandarin cold storage are between 5 and 8 °C (Kader and Arpaia, 2002), these fruit are often shipped at temperatures of 3–4 °C in an attempt to reduce decay when lower fungicide levels are being used (Tietel et al., 2011c), and it is possible that even lower temperatures would sometimes be used for quarantine disinfestation purposes (APHIS, 2012). Obenland et al. (2011) found that the mandarin variety 'W. Murcott Afourer' had better flavor when stored for up to 6 weeks at 8 °C rather than 0 or 4 °C, while there was no effect seen for 'Owari' using the same temperatures. Improvement of flavor at 8 °C was

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thought to be a result of a higher ratio of soluble solids to titratable acidity that lead to less tartness, as there were no significant differences in aroma volatiles among the storage temperatures. Storage at 2 °C for 4 weeks negatively affected the flavor of the chilling-sensitive variety 'Odem' in comparison to 5 and 8 °C, the most likely cause being the accumulation of terpenes that occurred at this temperature (Tietel et al., 2011c). The flavor of the other tested variety, 'Or', was not affected by storage temperature.

Studies examining the changes in flavor during the postharvest storage of mandarins have typically used a period of cold storage followed by a shorter holding period at a warmer temperature (often 20–25 °C) to simulate the temperatures typically experienced during both storage and marketing (Marcilla et al., 2009; Tietel et al., 2010a). Although this approach provides an approximation of what might occur to the fruit under commercial conditions, it does not allow a determination of the separate effects on fruit quality of warm versus cold temperatures. This is important as it may be possible to more closely regulate the temperatures experienced by the fruit after it leaves the packinghouse if this was proven to be important. In this study we examined independently the contributing roles of both warm and cold temperatures during storage and demonstrated that it is the warm temperatures that appear to be causing the majority of the flavor quality loss. This loss was most closely associated with increases in the amount of certain aroma volatiles.

2. Materials and methods

2.1. Fruit

For experimentation on the effects of warm temperature storage on quality, 'W. Murcott Afourer' mandarins were obtained from a commercial packinghouse in Maricopa, California on February 2 (harvest 1) and March 30 (harvest 2) in 2010. Care was taken on each harvest date to ensure that the fruit had been picked and packed within the previous two days. Wax coatings and fungicides utilized by the packinghouse were those appropriate for mandarins. On April 7, 2011 an additional sample of 'W. Murcott Afourer' mandarins was obtained from the same packinghouse for experimentation designed to examine the timing of the warm temperature-induced aroma volatile changes in more detail. Pearl Lustr (Decco, Monrovia, CA, USA) was the coating applied in combination with 3500 $\mu L\,L^{-1}$ thiabendazole and 2000 $\mu L\,L^{-1}$ imazalil. These fruit had been picked that day and then commercially packed. During the 2010 and 2011 seasons six additional mandarin varieties (China S-9, Frost Owari, Okitsu Wase, Fairchild, Nova and Gold Nugget) were picked at the University of California Lindcove Research and Education Center (LREC) located near Exeter, CA for the purpose of determining if the warm temperature effect on flavor observed for 'W. Murcott Afourer' also occurred in other varieties. Care was taken to harvest the varieties at a time when they would have been commercially mature. After harvest a portion of the fruit were kept unprocessed to act as the initial harvest sample, while the remainder were washed and waxed using an experimental packingline at the LREC. The coating applied was JBT FoodTech (Lakeland, FL) Sta-Fresh 900 in combination with 3000 $\mu L\,L^{-1}$ thiabendazole (Freshgard 598, JBT Foodtech) and 2000 $\mu L\,L^{-1}$ imazalil (Freshgard 700, JBT Foodtech).

2.2. Temperature/storage time treatments

'W. Murcott Afourer' fruit were obtained in 2010 from the packinghouse and transported back to the University of California Kearney Agricultural Center (UCKAC) in Parlier, CA where the fruit were stored at either 20 °C or 5 °C and 85% RH until sampling. Treatments consisted of keeping a portion of the fruit in continuous storage at 5 °C for up to 6 weeks, with samples being removed from 5 °C storage after 2 and 4 weeks and placed at 20 °C for 0, 1 and 2 weeks. These two temperatures were selected because 5 °C is a common storage temperature for mandarins while 20 °C is a warm temperature that may be experienced by the fruit after leaving the packinghouse and prior to consumption. Ninety fruit were placed into storage for each time and temperature combination.

Experimentation conducted in 2011 using packed 'W. Murcott Afourer' fruit to examine the aroma volatile response to warm temperatures in more detail consisted of keeping fruit in storage at 5 °C continuously for 4 weeks and removing another set of fruit from cold storage after 3 weeks and placing them at either 20 °C or 10 °C and 85% RH for a further week. Sampling was conducted daily during the final week of storage for all temperatures. The temperature of 10 °C was chosen as one of the comparisons to ascertain if an intermediate temperature of 10 °C could perform as well as 5 °C in maintaining flavor quality. Sixty fruit were placed into storage for each time and temperature combination. Mandarins utilized to test the effect of warm temperature storage on varieties other than 'W. Murcott Afourer' were subjected to the following temperature regimes: no storage, 1 week at 20 °C or 5 °C, 3 weeks at 5 °C plus 1 week at either 20 °C or 5 °C. The humidity was maintained at 85% RH for all of the treatments. Ninety fruit were randomly selected and placed into storage for each of the treatments for each variety.

2.3. Fruit quality evaluation

Three replications of 30 fruit (20 fruit in the 2011 'W. Murcott Afourer' testing) were evaluated in each treatment combination for visual quality and the number of decayed fruit to provide an overall measure of the quality of the fruit. As the fruit were of excellent quality and as there were no differences among the treatments for visual quality, the data are not presented in the results. Ten fruit per replication were then juiced by using a commercial table-top juicer (Model 932, Hamilton-Beach, Washington, NC, USA) and the juice weighed to provide an estimate of percent juice. A refractometer (AO Scientific, Model 10423, Buffalo, NY, USA) was used to determine SSC from the juice pooled from the 10 fruit. The same juice was titrated (Mettler T50A, Columbus, OH, USA) with 0.1 $mol\,L^{-1}$ NaOH to an endpoint of 8.2 for estimation of TA.

2.4. Sensory analysis

The sensory panel consisted of employees from UCKAC and could be considered semi-expert due to their prior experience with evaluating the flavor of mandarins. For the 2010 'W. Murcott Afourer' evaluation 10–15 panelists were generally present for evaluation each day, with a total of three days of tasting for each of the treatment combinations. Six fruit per treatment were evaluated on each of the three days, with each panelist being given one segment from each of the treatments for that day to evaluate. Just prior to the sensory panel the fruit were prepared by peeling and then placing the separated segments together in a bowl. A small amount of distilled water added to each bowl was used to moisten the segments and prevent them from drying out prior to evaluation. Panelists were seated in individual booths with access to the fruit preparation area through a small door in front of the panelist. Mandarin segments were presented in small soufflé cups in random order to the panelists and the panelists were then asked to place the empty cup on a laminated hedonic score card corresponding to how much the fruit were liked. The card had large numbers on it ranging from 1 (dislike extremely) to 9 (like extremely). The hedonic rating was then recorded along with the random code corresponding to the treatment that was tasted. It is recognized that the panel at UCKAC is not a consumer panel and that care must be taken in the interpretation of the hedonic data. A similar protocol was used in the evaluation of the other mandarin varieties from LREC with the exceptions that only one day of sensory testing was done per treatment (six fruit per treatment, fruit being the replications). In the portion of the experimentation examining the daily changes in aroma volatile concentrations during storage at 5 °C, 10 °C and 20 °C, mandarin segments were presented to the panelists and they were asked to write down comments describing the eating experience. Most commonly mentioned were aspects of sweetness, tartness, off-flavor or texture. These comments were summarized in order to help determine the impact of the storage temperatures on flavor associated with the changes in volatile amount.

2.5. Aroma volatile analysis

Mandarins were removed from storage at the appropriate time, carefully peeled, and juiced using a Hamilton table-top juicer and the juice screened to remove large pieces of pulp. Five replicate samples were taken per treatment with each replicate being composed of the pooled juice from 3 fruit. Five milliliters of the composite juice from each replicate sample was added to 5 mL of saturated sodium chloride in a 12 mm × 32 mm glass vial to which 1-pentanol had been added to a final concentration of 490 μg L^{-1}. The sodium chloride was added to minimize volatile-producing enzymatic activity following juicing. The samples were capped with a Teflon-coated septum and frozen at −20 °C until the time of analysis.

After thawing the vials were placed into a rack that was chilled to 4 °C. Volatile analysis was then conducted by solid phase microextraction (SPME) using a Gerstel MPS-2 robotic system (Linthicum, MD, USA). The fiber used was 75 μm in diameter with a carboxen/polydimethylsiloxane phase (Supelco, St. Louis, MO, USA). Each vial in turn was moved by the robotic system from the cooled rack into a heated (40 °C) agitator, with an agitator speed of 4.2 s^{-1}, where the sample was heated to initiate the analysis. After 15 min the SPME fiber was inserted into the vial and a trapping period of 30 min was conducted. Following this the SPME fiber was removed from the vial and desorbed for 2 min at 280 °C into the splitless inlet of an Agilent 7890 GC (Agilent, Palo Alto, CA, USA) coupled to a 5975 mass selective detector. An Agilent HP-5 ms column was used for the separation using the run conditions described in Obenland et al. (2008). Retention time, retention indices and comparisons to Wiley/NBS library spectra were used to identify the volatiles. Quantification was performed using calibration curves that were made by adding standards from each of the quantified volatiles to deodorized mandarin juice and using the ratios of standard and aroma volatiles to the internal standard for the calculations.

2.6. Statistics

Significance between treatments at a given storage time for the sensory and quality data from the initial study using 'W. Murcott Afourer' in 2010 was determined by a one-way analysis of variance (SPSS, Chicago, IL, USA). The hedonic score data was based upon the average scores from approximately 40 tastings of a total of 18 fruit per treatment at each time point. The soluble solids and titratable acidity data were based upon three replications per treatment at each time point, with each replication being based on the pooled juice from 10 fruit. Aroma volatile data from this portion of the study was transformed by logs for normalization and analyzed by Proc GLM (SAS, Cary, NC, USA) using treatment as a fixed effect and contrast statements to make specific comparisons among the treatment and time combinations. A Bonferroni adjustment was used to perform the multiple comparisons. Each aroma volatile mean was based upon five replicates, each replicate being based upon the pooled juice of three separate fruit.

Table 1
Comparison of storage at 20 °C for 1 week either prior to or after 3 weeks at 5 °C with continuous storage at 5 °C for the effect on likeability (hedonic score) for multiple mandarin varieties.

Variety	Storage	Hedonic score[c]	
	(weeks)	Including 20 °C	Continuous 5 °C
China S-9	0[a]	6.7 (±0.4)	
	1	6.6 (±0.5)a	5.8 (±0.6)a
	4[b]	5.7 (±0.5)a	5.8 (±0.6)a
Frost Owari	0	5.2 (±0.5)	
	1	5.1 (±0.4)a	5.1 (±0.5)a
	4	4.6 (±0.5)b	5.3 (±0.3)a
Okitsu Wase	0	5.3 (±0.5)	
	1	5.1 (±0.4)a	5.5 (±0.4)a
	4	4.3 (±0.5)b	5.9 (±0.5)a
Nova	0	6.8 (±0.3)	
	1	5.5 (±0.5)a	6.4 (±0.3)a
	4	5.8 (±0.4)a	6.7 (±0.3)a
Fairchild	0	6.9 (±0.4)	
	1	6.3 (±0.5)a	7.0 (±0.4)a
	4	6.2 (±0.4)b	7.5 (±0.2)a
Gold Nugget	0	6.6 (±0.2)	
	1	7.2 (±0.4)a	5.6 (±0.6)b
	4	6.4 (±0.4)a	6.3 (±0.4)a

[a] At harvest.
[b] Either 3 weeks at 5 °C + 1 week at 20 °C or 4 weeks at a continuous 5 °C.
[c] Measure of likeability where 1 = dislike extremely and 9 = like extremely. Values are means (±SE); values followed by a different letter are statistically different from each other ($P \leq 0.05$) when comparing temperatures within a storage time.

Data from 10 to 15 tastings per treatment at each storage time were used to calculate the hedonic score means of the mandarin varieties shown in Table 1. Differences between the storage temperatures were determined by one-way analysis of variance using SPSS.

Statistical significance between treatments within each day following the initiation of warm temperature holding from the 2011 'W. Murcott Afourer' testing was determined by one-way analysis of variance using SPSS with Tukey's test to perform the mean comparisons among the three temperatures. Each aroma volatile value represents the mean of five replications, with each replication being composed of the pooled juice from three separate fruit.

3. Results

3.1. Effect of warm temperature storage on mandarin flavor

The hedonic ratings were altered to a greater degree by warm temperature storage in 'W. Murcott Afourer' mandarins from harvest 2 than from harvest 1, and so the two harvests were graphed separately so that the differences could be more easily examined (Fig. 1). For both harvests, fruit that were held at 5 °C were little changed in likeability throughout the 6 weeks of storage in comparison to the values obtained at harvest. Fruit held at 20 °C, however, became less likeable with storage, the amount of decline being more pronounced in fruit that had a previous period of cold storage. In harvest 1 the loss in flavor quality for the 20 °C treatment did not become statistically significant until week 4 following packing, whereas the hedonic score was less than that of the continuous 5 °C treatment for all of the 20 °C treatments for harvest 2.

3.2. Varietal variation in warm temperature flavor response

In order to determine if the response to warm temperatures observed in 'W. Murcott Afourer' was also present in other varieties of mandarin, the fruit of six other mandarin varieties were stored at

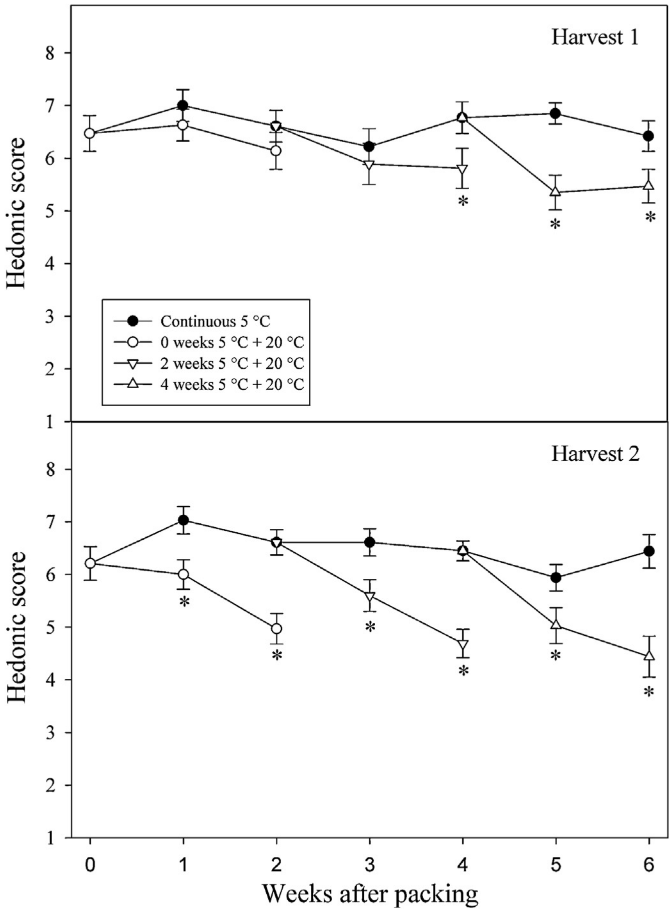

Fig. 1. Influence of exposure to warm temperatures (20 °C) on the likeability of 'W. Murcott Afourer' mandarins following packing as estimated by hedonic ratings for two separate harvests. Fruit were stored for either 0, 2 and 4 weeks at 5 °C and then moved to 20 °C for either 1 or 2 weeks. Fruit were also held at a continuous 5 °C to determine the warm temperature effect. Hedonic ratings were assigned to the fruit samples by the panelists where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. Each data point represents the tasting of 18 fruit. Asterisks signify comparisons of 20 °C storage versus the corresponding storage at 5 °C that were statistically significant from each other ($P \leq 0.05$). Bars indicate standard error.

either continuous 5 °C or exposed to 20 °C for 1 week prior to or after storage at 5 °C for 3 weeks (Table 1). Hedonic testing was used to discern whether panelists found differences in flavor between the storage temperatures. After 4 weeks of storage (3 weeks with and without 1 week at 20 °C) three of the six varieties ('Frost Owari', 'Okitsu Wase' and 'Fairchild') had hedonic scores that were significantly lower for the fruit that included a 20 °C storage period in the storage regime in comparison to that which was stored at a continuous 5 °C. Similar to 'W. Murcott Afourer', the varieties 'Frost Owari', 'Okitsu Wase', 'Nova', and 'Fairchild' all had hedonic scores that were not significantly different ($P \leq 0.05$) following 4 weeks of cold storage at 5 °C in comparison to the scores obtained at harvest.

3.3. Effect of warm temperature storage on SSC and TA

There were no significant differences in SSC between 'W. Murcott Afourer' fruit that had undergone cold storage and either one or two weeks of storage at 20 °C versus fruit that had been continuously stored at 5 °C for the same amount of time (Fig. 2). With the exception of fruit stored for 4 weeks and 2 weeks for harvests 1 and 2, respectively, there were also no statistically significant changes in TA between the storage regimes. Similarly, 'China S-9', 'Frost Owari', 'Nova', and 'Gold Nugget' did not differ in SSC or TA in comparisons of fruit with and without a storage period at 20 °C, while 'Okitsu Wase' and 'Fairchild' mandarins were slightly less acidic as a result of the inclusion of the warmer temperature into the storage protocol (data not shown).

3.4. Effect of warm temperature storage on aroma volatile concentration

Thirty-nine aroma volatiles were identified in juice samples from 'W. Murcott Afourer' (data not shown) of which twenty-one were changed in concentration at some point in the course of the various storage regimes (Table 2). Aroma volatile concentrations are presented separately for each harvest due to the differential response of the two harvests in terms of aroma volatile and flavor change (Table 3). Ethanol, 3-methyl-butanol and 2-methylbutanol were all present in much higher concentrations in fruit that included a holding period at 20 °C in comparison to fruit that had been held continuously at 5 °C. In harvest 1 six weeks of storage at 5 °C led to no significant increases in the amounts of these compounds, while in harvest 2 ethanol was at a higher concentration in the fruit stored solely at 5 °C relative to the value at harvest. Similarly, esters were strongly induced in both harvests by holding the fruit at 20 °C. Some of these compounds, such as ethyl acetate, also accumulated as a result of cold storage alone but the final concentrations were far greater in fruit held at 20 °C. In many cases, ester concentrations were enhanced to an even greater degree if there was 5 °C storage prior to the 20 °C holding period. The response of the quantified aldehydes to temperature was inconsistent. The amounts were greater in mandarins stored at 20 °C versus 5 °C following two weeks of storage in harvest 1, but these differences were only present to a limited degree following the other storage times and not present in harvest 2. In contrast to the other volatile classes, terpenes declined in amount as a result of warm temperature storage, but this was only the case following 6 weeks of storage in harvest 1.

3.5. Timing of the warm temperature-induced changes in aroma volatile concentration

As changes in aroma volatiles appeared to be the most likely causes of the loss in mandarin flavor quality during storage, it was of interest to determine how rapidly the key compounds changed upon introduction of the fruit into storage at 20 °C. Those volatiles that are presented in Fig. 3 are compounds that have a high potential to have a role in the warm temperature-induced flavor changes based upon the amount and consistency of the change observed in experimentation from the prior tests and/or the low odor thresholds of the individual compounds. In a comparison between fruit held at a continuous 5 °C versus that held at either 10 °C or 20 °C following three weeks at 5 °C, the increase in aroma volatile amount due to holding the fruit at 20 °C became significant following only one day for ethanol, ethyl acetate, ethyl propanoate and ethyl 2-methylbutanoate. By the second day of 20 °C holding both ethyl 2-methylpropanoate and 3-methyl-1-butanol were also significantly enhanced in the 20 °C treatment. In contrast, a holding temperature of 10 °C prevented the added accumulation of these aroma volatiles and the concentrations were never statistically significant from those at 5 °C during the entire seven day holding period.

The association of the buildup in aroma volatiles with flavor in this portion of the experimentation was illustrated by results obtained from tasting fruit from the same lot as were analyzed for aroma volatiles following one week of storage at each of the three temperatures. This experimentation found that after 1 week of storage 27% of the responses from the sensory panelists noted the presence of off-flavor in mandarins stored at 20 °C, while 6% and 8% of the responses for fruit stored at 5 °C and 10 °C, respectively, noted this negative flavor characteristic (data not shown).

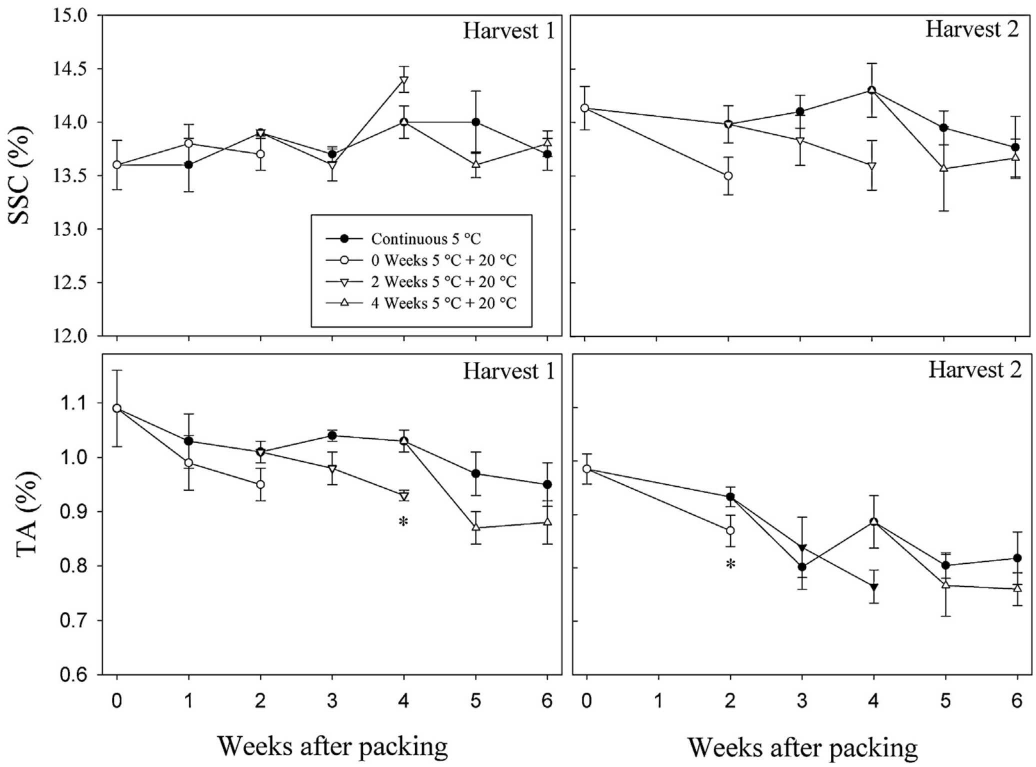

Fig. 2. Influence of exposure to warm temperatures (20 °C) on the soluble solids and titratable acidity of 'W. Murcott Afourer' mandarins for two separate harvests. Fruit were stored for either 0, 2 and 4 weeks at 5 °C and then moved to 20 °C for either 1 or 2 weeks. Fruit were also held at a continuous 5 °C for comparison in order to determine the warm temperature effect. There were three replications per data point with each replication being derived from the pooled juice of 10 fruit. Asterisks signify comparisons of 20 °C storage versus the corresponding storage at 5 °C that were statistically significant from each other ($P \leq 0.05$). Bars indicate standard error.

Table 2
Aroma volatiles that significantly changed in 'W. Murcott Afourer' mandarins due to storage.

Compound	DB-5 LRI[a]	ID[b]	Odor threshold (μg L^{-1})	Descriptors
Alcohols				
Ethanol	528	S, MS	2,000,000[c]	Ethanol
3-Methyl-1-butanol	751	S, MS	1000[d]	Malty
2-Methyl-1-butanol	758	S, MS	320[d]	Malty
Linalool	1105	S, MS	113[e]	Floral, green, citrus
4-Terpineol	1189	S, MS		Woody, earthy
Esters				
Ethyl acetate	618	S, MS	6038[e]	Pleasant, fruity
Ethyl propanoate	720	S, MS	256[e]	Sweet, fruity
Ethyl 2-methylpropanoate	788	S, MS	0.35[e]	Sweet, fruity
Ethyl 2-methylbutanoate	853	S, MS	0.08[e]	Apple
Isoamyl acetate	879	MS	2[f]	Sweet, fruity
2-Methylbutyl acetate	881	MS	5[g]	Sweet, fruity
Aldehydes				
Pentanal	702	S, MS	12[h]	Fruity, nutty
Hexanal	803	S, MS	151[e]	Green, grassy
Heptanal	903	S, MS	3[i]	Oily, fatty
Benzaldehyde	970	S, MS	350[h]	Almond, cherry
Decanal	1210	S, MS	204[e]	Beefy, musty
Terpenes				
α-Terpinene	1025	S, MS		Lemony, citrus
Limonene	1046	S, MS	13,700[e]	Citrus-like, fresh
(E)-β-ocimene	1054	S, MS		Herbaceous, sweet
γ-Terpinene	1068	S, MS	3260[e]	Lemony, citrus
Terpinolene	1097	S, MS		Citrus, pine

[a] Linear retention index (LRI) calculated using n-alkanes with a DB-5 column.
[b] Method of identification where S = standards and MS = mass spectrometry.
[c] Czerny et al. (2008).
[d] Buettner and Schieberle (2001).
[e] Plotto et al. (2004) and Plotto et al. (2008).
[f] Takeoka et al. (1989).
[g] Flath et al. (1967).
[h] Buttery et al. (1988).
[i] Rouseff (2012).

Table 3
Aroma volatile concentrations in 'W. Murcott Afourer' mandarins following continuous cold storage at 5 °C or after either 0, 2 or 4 weeks at 5 °C and 2 weeks at 20 °C. Two separate harvests conducted at different times in the season are presented. Only volatiles with statistically significant changes due to storage for either of the harvest dates are shown. Four separate data contrasts are presented for each of the volatiles: 0 versus 6 week, 2 week, 4 week, and 6 week. Concentrations are $\mu g\,L^{-1}$ with the exception of ethanol which is $mg\,L^{-1}$.[a]

Contrast:	0 versus 6 week		2 week		4 week		6 week	
Weeks 5 °C:	0	6	2	0	4	2	6	4
Weeks 20 °C[b]:	0	0	0	2	0	2	0	2
Harvest 1								
Alcohols								
Ethanol[c]	561.8	1081.7	827.4	1842.6*	876.2	2207.3*	1081.7	1629.8
3-Methyl-1-butanol	0	0	0	2065.7	538.4	4288.0*	0	2778.8
2-Methyl-1-butanol	0	0	0	620.2	0	1334.6	0	774.4
Linalool	16.4	50.3	69.6	33.0	23.8	16.8	50.3	19.9*
4-Terpineol	7.5	13.5*	15.6	11.2	8.9	9.3	13.5	7.1
Esters								
Ethyl acetate	129.1	3020.6*	501.8	10,242.8*	1351.2	20,868.3*	3020.6	20,800.5*
Ethyl propanoate	4.7	19.9*	7.5	147.3*	14.9	231.1*	19.9	171.2*
Ethyl 2-methylpropanoate	0	8.6	0	73.3	9.9	176.8*	8.6	210.7*
Ethyl 2-methylbutanoate	0	1.6	1.0	37.9*	1.6	122.9*	1.6	114.1*
Isoamyl acetate	0	0	0	4.2	3.0	9.5*	0	10.2
2-Methylbutyl acetate	0	0	0	2.2	0	5.6	0	7.5
Aldehydes								
Pentanal	40.7	35.6	19.9	66.2*	64.4	70.2	35.6	53.2
Hexanal	339.6	379.7	175.7	707.1*	611.2	1023.6	379.7	597.0
Heptanal	12.5	18.7	8.1	29.9*	25.6	42.4	18.7	24.2
Benzaldehyde	3.4	4.7	2.9	6.8*	4.3	7.7	4.7	5.3
Decanal	3.3	11.3*	13.1	9.8	7.6	5.2	11.3	4.8*
Terpenes								
α-Terpinene	12.5	27.2	24.8	16.7	16.7	10.2	27.2	5.3*
Limonene	4365.5	9469.2*	6599.7	7160.3	4677.3	3602.7	9469.2	3082.1*
(E)-β-ocimene	1.7	5.9*	5.4	3.5	2.8	2.3	5.9	1.9*
γ-Terpinene	26.0	74.0	67.5	14.0*	17.2	7.7	74.0	5.8*
Terpinolene	26.7	45.8	35.0	41.8	27.7	20.1	45.8	19.3*
Harvest 2								
Alcohols								
Ethanol[c]	360.7	1054.1*	1002.5	1955.3*	1063.6	1962.1*	1054.1	1799.0
3-Methyl-1-butanol	161.3	645.4	224.3	1077.2	318.3	1446.7*	645.4	3370.7*
2-Methyl-1-butanol	0	0	0	325.4	0	376.9	0	914.3
Linalool	23.5	5.2	18.3	42.8	14.8	25.7	5.2	19.1*
4-Terpineol	7.8	7.1	7.1	14.1*	7.7	10.1	7.1	8.3
Esters								
Ethyl acetate	1663.6	3727.3*	4086.5	20,126.0*	3720.8	17,969.5*	3727.3	23,765.4*
Ethyl propanoate	8.6	33.4*	21.7	166.7*	23.9	187.3*	33.4	234.5*
Ethyl 2-methylpropanoate	3.8	14.1*	6.5	106.9*	8.3	186.0*	14.1	418.8*
Ethyl 2-methylbutanoate	1.1	3.8	2.7	64.8*	2.7	103.9*	3.8	233.7*
Isoamyl acetate	0	1.6	0	3.2	0	4.0	1.6	7.2*
2-Methylbutyl acetate	0	0	0	1.8	0	3.0	0	5.4
Aldehydes								
Pentanal	16.3	20.6	15.6	15.0	13.0	22.2	20.6	26.4
Hexanal	138.9	201.4	132.6	135.3	111.9	223.5	201.4	274.5
Heptanal	7.4	10.4	9.9	15.4	7.7	14.6	10.4	16.3
Benzaldehyde	2.1	2.8	3.4	4.6	4.0	5.6	2.8	4.4
Decanal	2.2	2.2	6.9	10.9	6.3	5.1	2.2	3.3
Terpenes								
α-Terpinene	8.3	10.1	20.1	26.3	24.4	15.5	10.1	6.1
Limonene	2702.5	2337.0	7610.7	8697.8	6978.0	5068.9	2337.0	2615.1
(E)-β-ocimene	1.4	1.3	3.6	5.2	3.5	2.5	1.3	1.4
γ-Terpinene	15.8	15.4	85.9	88.3	60.4	30.5	15.4	13.9
Terpinolene	17.5	16.1	39.9	43.7	44.9	32.5	16.1	16.7

[a] Asterisk following concentration value indicates statistical significance ($P \leq 0.05$) for the comparison within the contrast pairs (0 versus 6 week, 2 week, 4 week, and 6 week).
[b] Storage following initial cold storage at 5 °C.
[c] Concentration units are $mg\,L^{-1}$.

4. Discussion

Mandarins are typically placed into cold storage at 5–8 °C immediately after packing but then are subjected to a range of warmer temperatures as the fruit are transported to distribution centers and to the point of sale. At retail the fruit are most commonly displayed at room temperature and may remain so after purchase unless the consumer refrigerates the fruit prior to consumption. The separate effects of warm versus cold temperature storage on flavor has not previously been examined for mandarins, even though it has implications to how the fruit should be commercially handled. We found that 'W. Murcott Afourer' mandarins that are maintained

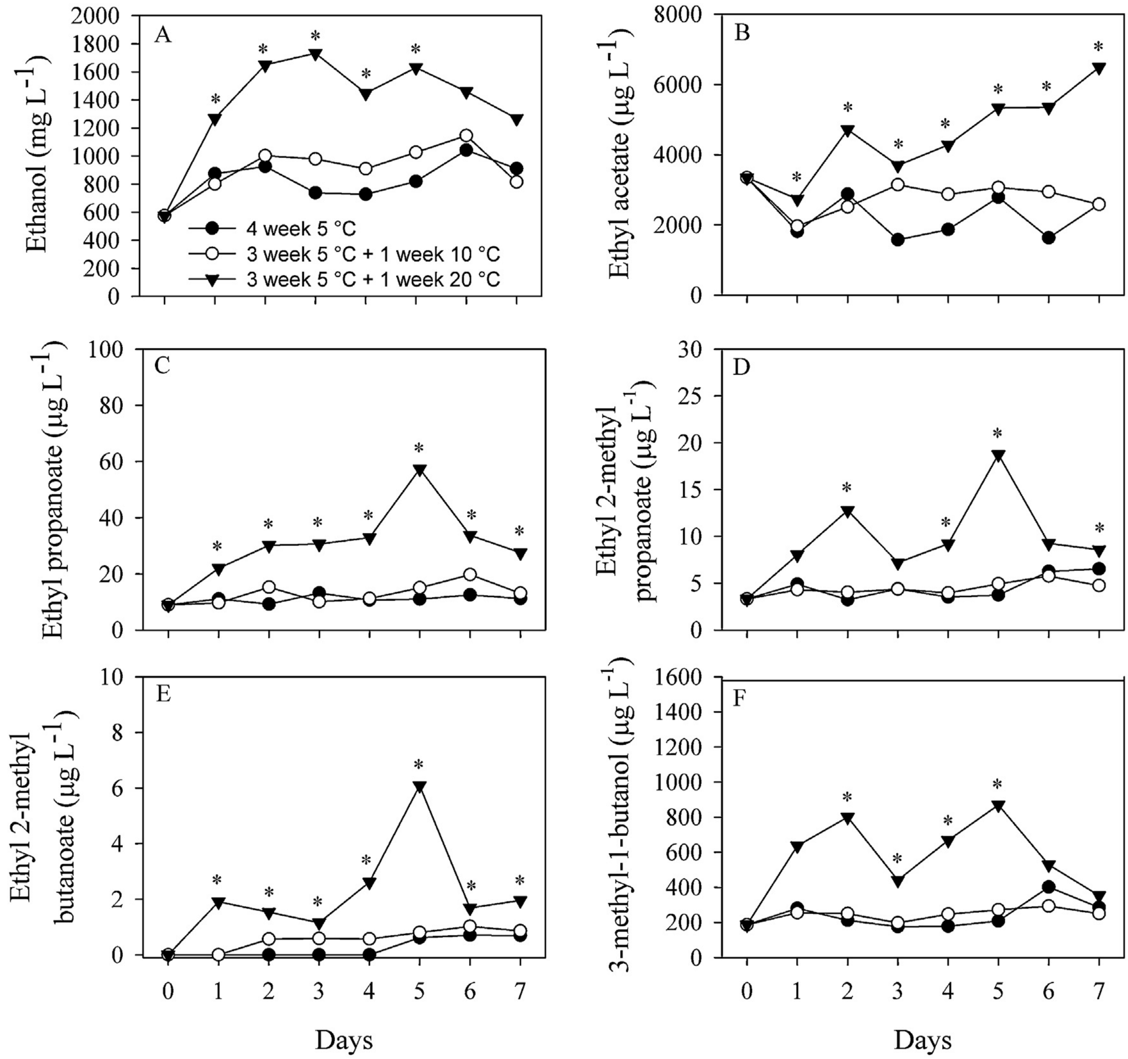

Fig. 3. Daily changes in aroma volatile concentration in 'W. Murcott Afourer' mandarins held at either 5 °C, 10 °C or 20 °C for 1 week following 3 weeks of storage at 5 °C. Only those volatiles that are believed most likely to be involved in the development of off-flavor are shown. Not shown is 2-methyl-1-butanol as its response to holding temperature was nearly identical to 3-methyl-1-butanol. Data points are each based on the mean from five replicate measurements, using the juice from three pooled fruit for each measurement. Asterisks signify comparisons among the three holding temperatures for each day that were statistically significant from each other ($P \leq 0.05$).

at a continuous 5 °C did not decline in sensory acceptability, even after six weeks of storage, while fruit held at 20 °C rapidly lost flavor quality (Fig. 1). The dramatic flavor loss at 20 °C versus the absence of such a loss at 5 °C likely reflects the higher respiratory rate of the warmer fruit that can lead to lower internal oxygen levels. The combined effects of waxing and various storage temperatures on fruit internal atmospheres and citrus quality have been shown previously by other reports in the literature (Eaks and Ludi, 1960; Baldwin et al., 1995; Chun et al., 1998). Oxygen level acts as the initial trigger for fermentation and off-flavor formation (Ke and Kader, 1990; Hagenmaier, 2002) and, although we did not measure it in this study, the oxygen concentration is evidently maintained high enough at 5 °C to prevent or greatly minimize off-flavor formation. Interestingly, the warm temperature flavor loss response apparently is also influenced by the length of prior cold storage (longer enhances) and, potentially, by maturity as is evidenced by the much greater flavor loss observed in fruit from the second harvest (Fig. 1). Although fruit from the second harvest had both higher soluble solids and lower acidity than that from the first harvest, it is unclear why this difference in flavor loss occurred between the two harvests, especially given the relatively high hedonic scores for both harvests for fruit stored at a continuous 5 °C.

As has been previously demonstrated with cold storage temperatures of 0–8 °C (Obenland et al., 2011; Tietel et al., 2011c), it also appears that there are cultivar differences in how mandarins respond in terms of flavor quality to 20 °C with and without prior cold storage. 'Frost Owari', 'Okitsu Wase', 'Nova' and 'Fairchild', all responded in a similar manner as 'W. Murcott Afourer' in that storage at continuous 5 °C acted to prevent the flavor loss that occurs when storage includes a holding period at a warm temperature such as 20 °C (Table 1). On the other hand, the hedonic score was nearly identical after 4 weeks between the different storage regimes with 'China S-9'. In the case of 'Gold Nugget' after 4 weeks of storage there was also little or no impact of holding temperature following cold storage. In this instance, however, the likeability of the fruit did not differ significantly ($P \leq 0.05$) from that at harvest for either of the two storage regimes (with or without 20 °C) after 4 weeks. In a recent screening of mandarin varieties, we reported that 'Gold Nugget' produced relatively low levels of ethanol following waxing and storage in comparison to the other varieties tested (Obenland and Arpaia, 2012). These results, combined with our current findings, suggest that 'Gold Nugget' may be less susceptible to poor flavor following storage as a result of a lesser tendency toward anaerobic fermentation after waxing. A complete comparison of the varietal test results with those from 'W. Murcott Afourer', however, was not possible due to the fact that the waxes utilized for the two studies were from different manufacturers.

Prior research has identified the importance of aroma volatiles to the loss of mandarin flavor quality that occurs after harvest (Tietel et al., 2010a; Obenland et al., 2011). However, the relative impact on these flavor components of cold versus warm portions of the storage regimes that are typically imposed by researchers had not been previously examined. A comparison of the volatile composition for 'W. Murcott Afourer' at harvest versus that after 6 weeks at a continuous 5 °C is instructive as there was little or no reduction in flavor quality that occurred as a result of this storage protocol (Fig. 1) and correspondingly little change in aroma volatile concentration (Table 3). Increases in ethyl acetate and ethyl propanoate concentration were the only two significant changes that occurred consistently in both harvests during 5 °C storage. These two increases may not have contributed to changes in flavor as both compounds were at concentrations below published odor thresholds (Table 2), even at their maximal levels following storage.

The inclusion of a 20 °C holding period following either two, four or six weeks of 5 °C storage leads to large increases in aroma volatile concentration, particularly in the alcohols and esters (Table 3), and a decline in flavor quality (Fig. 1). The lack of any consistent changes in SSC and TA due to holding the fruit at 20 °C indicates a lack of involvement of these quality attributes in the observed flavor loss and supports a role for the aroma volatiles as causal factors. Although ethanol readily accumulates in waxed citrus due to the initiation of anaerobic fermentation (Cohen et al., 1990), the alcohols with the most direct impact on flavor were likely 3-methyl-1-butanol and 2-methyl-1-butanol, compounds with a malty essence that were undetectable in fruit kept at 5 °C but were induced to accumulate during holding at 20 °C. These two compounds are believed to not directly arise through fermentation but rather as products of amino acid catabolism (Schwab et al., 2008). Although these compounds have relatively high odor threshold values, following a 20 °C holding period they were present in amounts well in excess of the thresholds. Esters also appeared to be very important in determining the degree of flavor loss as there were very large increases in all of the ethyl-based esters in the fruit held at 20 °C. It is known that the formation of ethyl esters in fruit can be stimulated by increased ethanol concentration through the action of ethanol as a substrate in subsequent esterification reactions (Mattheis et al., 1991). This is believed to be the link between waxing-induced fermentation and the large-scale increases in these esters that is observed following storage (Tietel et al., 2011b). These compounds are most typically sweet and fruity in aroma but their presence may lead to off-flavor if they become over-abundant in the fruit. Following a holding period at 20 °C, ethyl acetate, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate all were present in concentrations far in excess of their odor threshold values and would be expected to impact flavor. The role of the aldehydes and terpenes is far less clear as the changes were not consistently observed in both harvests and across all the 20 °C storage regimes.

Results from the initial portion of our study made it clear to us that holding mandarins at warm temperatures such as 20 °C can be very harmful to flavor quality. It was, therefore, of interest to determine how long of an exposure at 20 °C is needed to enhance the accumulation of key off-flavor volatiles to estimate the limits of what a "safe" warm temperature exposure might be. Movement of fruit from long-term 5 °C storage to 20 °C induced a doubling of the ethanol content by day 1, as well as significant increases in the concentrations of three of the four ethyl esters that were followed (Fig. 3). The fourth ester, ethyl 2-methyl propanoate, was significantly greater in concentration by the end of the second day as was 3-methyl-1-butanol (Fig. 3). These findings indicate that even a transient exposure of a single day could be enough to negatively alter flavor. The data also show that both 5 °C and 10 °C are equally effective in preventing an increase in concentration of these aroma volatiles. This is a useful finding in that it is often difficult to maintain a strict cold chain at 5 °C throughout the duration of storage and this suggests that there is some allowable leeway in storage temperature. In fact, we have previously reported that 'W. Murcott Afourer' stored at 8 °C was superior in flavor to that stored at either 0 °C or 4 °C (Obenland et al., 2011). Tietel et al. (2011) previously reported with mandarins stored in an anaerobic atmosphere for 0, 4 or 10 d that ethanol accumulation appears to precede that of ethyl esters, suggesting that the increase in ethanol concentration was driving the ethyl ester accumulation by provision of substrate for the esterification reactions. Results from our study using waxed fruit do not provide additional evidence for this (nor do they disprove it) because the warm temperature-induced increase in ethanol occurred simultaneously with increases in ethyl ester concentrations.

In conclusion, we have demonstrated that the flavor loss that occurs for 'W. Murcott Afourer' and a number of other mandarin varieties occurred exclusively during the portion of the storage protocol where the fruit are warmed to room temperature. There may be some specificity among mandarin varieties in this response, however, as most but not all of the mandarins that we tested were influenced by the presence of a 20 °C holding period in the storage regime. Although it may be logistically difficult for retailers, our results indicate that keeping mandarins in a refrigerated case would greatly benefit mandarin flavor quality, especially if consumers would be also be encouraged to keep mandarins cold prior to consumption. Large scale accumulation of aroma volatiles that rapidly occurs at 20 °C, and is largely prevented by cold storage, indicated the involvement of these components in the flavor disorder.

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