Storage temperature and time influences sensory quality of mandarins by altering soluble solids, acidity and aroma volatile composition

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ABSTRACT

Mandarins are very prone to losing flavor quality during storage and, as a result, often have a short shelf life. To better understand the basis of this flavor loss, two mandarin varieties ('W. Murcott' and 'Owari') were stored for 0, 3 and 6 weeks at either 0 °C, 4 °C, or 8 °C plus 1 week at 20 °C, and then evaluated for sensory attributes as well as quality parameters and aroma volatile profile. The experiment was conducted multiple times for each variety over two seasons, using three separate grower lots per experiment. Flavor quality was reduced in 'Owari' following 4 weeks of storage as off-flavor increased, while for 'W. Murcott' the hedonic score decreased after the fruit were stored for 7 weeks. Sensory panelists also noted a decline in tartness during storage for both varieties that was associated with an increase in the ratio of soluble solids concentration (SSC) to titratable acidity (TA). Large increases in alcohols and esters occurred during storage in both varieties, a number of which were present in concentrations in excess of their odor threshold values and are likely contributing to the loss in flavor quality. Thirteen aroma volatiles, consisting mainly of terpenes and aldehydes, declined during storage by up to 73% in 'Owari', only one of which significantly changed in 'W. Murcott'. Although many of these volatiles had aromas characteristic of citrus, their involvement in flavor loss during storage is unclear. 'W. Murcott' stored at 8 °C had slightly superior flavor to fruit stored at either 0 °C or 4 °C, and the better flavor was associated with higher SSC/TA and lesser tartness. Aroma volatiles did not play a role in the temperature effect on flavor as there were no significant differences in volatile concentrations among the three temperatures. There was no effect of storage temperature on the flavor of 'Owari'.

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1. Introduction

Mandarins are a diverse group of thin-skinned, easy-peeling fruit that includes popular citrus types such as satsumas, clementines and tangerines. Mandarins are becoming increasingly popular with consumers, largely due to the ease with which they can be eaten as compared to other types of citrus that are more difficult to peel. This enhancement in popularity has occurred concurrently with a decline in the consumption of navel oranges, long a mainstay of the fresh citrus industry in California. In response, citrus growers in California have been devoting greater amounts of acreage into the production of mandarins.

Although they are popular with consumers, the recommended storage life under optimum conditions is only 2–4 weeks, less than half that of conventional oranges (Kader and Arpaia, 2002). A primary reason for this is that mandarin flavor quality is often rapidly lost after the fruit are picked and commercially packed. Prior work has primarily associated the poor flavor with the internal build-up of anaerobic metabolites due to a lack of oxygen diffusion into the fruit and build-up of internal carbon dioxide caused by the application of waxes (Cohen et al., 1990), but the phenomenon is incompletely understood. Although other types of citrus also have an enhanced accumulation of anaerobic metabolites as a result of the modification of the fruit internal atmospheres by coatings (Davis and Hoffman, 1973; Hagenmaier and Baker, 1994; Obenland et al., 2008), mandarins are known to be especially prone to this problem. Research comparing characteristics of mandarins and grapefruit found that mandarins have a lower peel permeability to gas exchange and higher alcohol dehydrogenase activity in the juice than do grapefruit (Shi et al., 2005, 2007), which suggests possible reasons for this difference. Also, in a further comparison with grapefruit (Shi et al., 2005), mandarins were reported to respond to anaerobic conditions by the production of ethylene, whereas grapefruit did not. This may be of importance to flavor as ethylene has been previously implicated as a compound that enhances the production of off-flavor volatiles (McGlasson and Eaks, 1972).

As with other citrus, the edible portion of the mandarin orange is a rich source of volatile components that contribute greatly to the overall flavor. In a comparison of the juice from a range of mandarin varieties, Moshonas and Shaw (1997) were able to identify 42 volatile constituents. A later study, using gas chromatography/olfactometry, found 38 compounds that were odor-active, with eight of these compounds having odor activity values that indicated that they likely play a role in determining flavor (Schieberle et al., 2003). Other researchers have also presented data linking specific volatile components in mandarins to flavor (Elmaci and Altug, 2005; Pérez-López and Carbonell-Barrachina, 2006). In a recent study (Tietel et al., 2010) identified 13 volatiles believed to be derived from fermentation and amino acid catabolism that increased in amount during storage of waxed 'Mor' mandarins and attributed the observed increase in off-flavor to these changes. The same authors found a further 31 volatiles that decreased by 50% or greater that could be involved in the loss in mandarin flavor that happened concurrently with the off-flavor development.

Marcilla et al. (2009) and Hagenmaier (2002) both presented data linking the degradation of flavor that occurs in mandarins to an accumulation of ethanol in the fruit. Ethanol can be an enhancer of flavor if present in low amounts (Nisperos-Carriedo and Shaw, 1990), but is thought to adversely affect flavor if present in higher amounts, such as $1500 \mu L L^{-1}$ or greater (Hagenmaier, 2002). In the work of Marcilla et al. (2009) off-flavor development and a loss of mandarin-like flavor during storage were found to precede the ethanol threshold, suggesting that at least some of the flavor problems may be due to changes in other uncharacterized chemical constituents. This is consistent with our prior research with navel oranges where we have demonstrated that other aroma-active components change as a result of the packing and storage process that likely impact the subsequent flavor of the fruit (Obenland et al., 2008). It seems probable that this is the case with mandarin oranges as well.

Although prior work has described changes in a range of volatile components and in other flavor attributes, such as sugars and acids, which occur during the storage of mandarins (Tietel et al., 2010), this work only dealt with storage at a single temperature and using a single mandarin variety. Since storage temperatures that are used for mandarins may vary significantly, it was important to understand the impact of temperature on flavor and on the factors that determine flavor. Also, given the wide diversity that exists among mandarin types and varieties, it was also of interest to determine whether or not mandarin varieties differ in these responses by making comparisons within the same study. Our objective was to thoroughly evaluate the changes in the sensory attributes of two important California mandarin varieties following different durations of storage at three temperatures and attempt to understand these changes in light of differences in both volatile and non-volatile flavor components.

2. Materials and methods

2.1. Fruit

Separate lots of 'Owari' mandarins (*Citrus reticulata*) from three growers were harvested (size 138) from groves near the San Joaquin Valley Agricultural Sciences Center in Parlier, CA, taking care to avoid fruit with sunburn, scarring or other external defects. After harvest the fruit were transported to the University of California Lindcove Research and Extension Center (LREC) where the fruit were stored overnight at 11 °C and on the following day washed and waxed on a packing line. The coating that was applied during the packing process was JBT FoodTech (Lakeland, FL) Sta-Fresh 900 combined with the fungicides thiabendazole ($3000 \mu L L^{-1}$) and imazalil ($2000 \mu L L^{-1}$), all as recommended by JBT Foodtech for use for mandarins. The entire experiment was conducted in the same manner on three separate occasions, with harvests occurring on November 1, in 2007 and 2008, and December 12, 2008. The other mandarin variety utilized in this study, 'W. Murcott', was obtained from local packinghouses. All fruit were treated by the packinghouses using carnauba-based coatings and fungicides (imazalil and thiabendazole) appropriate for mandarins. It is recognized that the makeup of the coatings differed among the various mandarin packing houses and compared to what was applied to 'Owari', although all were commercially accepted coatings for mandarins. Previous testing had found there to be no differences in citrus flavor or quality attributes due to manufacturer differences in standard coating formulations (data not shown). Three grower lots were used per test and fruit were obtained for each test on January 8 and March 18 in 2008 and February 5 and April 1 in 2009, as close to the packing date as possible.

2.2. Temperature/storage time treatments

After obtaining the fruit and, in the case of 'Owari', washing and waxing them, they were placed at either 20 °C for tasting on the following days (storage time 0) or at 0 °C, 4 °C, or 8 °C for the long-term storage treatments. The temperatures of 4 °C and 8 °C were selected to span the recommended 5–8 °C for mandarin storage (Kader and Arpaia, 2002) and 0 °C to be a potential quarantine treatment for insect disinfestation should that need arise. Cold storage times were either 3 or 6 weeks, with each treatment including a week at 20 °C afterward to simulate a marketing period. Ninety fruit were placed into storage for each time/temperature combination.

2.3. Fruit quality evaluation

Thirty fruit per grower lot (90 fruit per time and temperature combination) were evaluated after each storage time for surface quality and percent juice to help demonstrate that fruit of good quality was used throughout the duration of the experiment. Fruit were rated for peel quality using a 0–5 rating scale with 0 being no injury and 5 being severe injury, and the percentage of decayed fruit noted. The percent juice in the fruit was calculated by dividing the weight of the juice in each fruit by the weight of the intact fruit.

2.4. Sample preparation

Each fruit was carefully peeled by hand to minimize the transfer of peel oil onto the segments and placed into numbered paper cups. After all of the fruit to be used for that particular day were peeled, the fruit were split in half by hand from stem to stylar end by separating the segments, one half of the segments being used for sensory testing and the other half for volatile, SSC and TA analysis. Generally 10–12 segments were available from each fruit. Fifteen fruit were used per grower lot for each treatment in each of the tests for sensory analysis. Immediately prior to presentation to the sensory panelists the segments were separated and any seeds that were present were removed. The other half of the fruit was juiced using a commercial table-top juicer (Model 932, Hamilton-Beach, Washington, NC, USA) and the juice passed through a screen sieve external to the juicer. Juice for determination of soluble solids concentration (SSC) and titratable acidity (TA) was placed into 15-mL centrifuge tubes, while subsamples of the same juice were placed into 12 mm × 32 mm glass vials sealed with a Teflon-coated septum for measurement of aroma volatiles. All vials were frozen and kept as −20 °C until analysis.

2.5. Sensory analysis

Panelists were employees of the University of California Kearney Agricultural Center and could be classified as being semi-expert due to their previous experience with serving on citrus sensory panels. Generally 15–20 panelists were present for each test. Panelists were seated in individual evaluation booths and samples presented through a small door in the front of the booth. Two segment halves were presented for each sample. Room temperature distilled water was provided to cleanse the palate between samples. Samples were served in random order to the panelists in white, 30-mL soufflé cups that were identified with a unique three-digit number. Each fruit was usually tasted by at least 3–4 panelists. Panelists gave a hedonic score for each sample ranging from 1 to 9, with 1 being dislike extremely and 9 being like extremely. Ratings were also given for sweetness, tartness, richness and the degree of off-flavor present by marking lines on 150-mm line scales. The measured distance from the 0-point indicated the intensity of the sensory attribute, with a greater number indicating more sweetness and richness but less tartness and off-flavor. Richness was a measure of the degree of mandarin-like flavor that was present.

2.6. SSC, TA and volatile analysis

After thawing, SSC was measured for the juice samples from each of the individual mandarin fruit by use of a temperature-compensated refractometer (AO Scientific, Model 10423, Buffalo, NY, USA), while TA was determined by titration with $0.1\,mol\,L^{-1}$ NaOH to an endpoint of 8.1 using a Radiometer Titralab 80 (Lyon, France).

Volatile samples (5 mL) were thawed prior to measurement and 1-pentanol added as an internal standard to a final concentration of $490\,\mu g/L$. Six samples per treatment were measured with each sample being a pooled juice sample from three individual fruit. Volatile analysis was conducted by solid phase microextraction (SPME) with a 75-μm carboxen/polydimethylsiloxane fiber (Supelco, St. Louis, MO, USA) using a Gerstel MPS-2 robotic system (Linthicum, MD, USA). The Gerstel system initiated a sample's analysis by transferring a sample from the cooled (4 °C) holding tray into a heated (40 °C) agitator where the sample was allowed to warm for 15 min. At this point a SPME fiber was inserted into the headspace of the vial to trap volatiles for 30 min at 40 °C with an agitator speed of $4.2\,s^{-1}$. After trapping was completed the SPME fiber was removed from the vial and desorbed for 2 min at 280 °C in the splitless inlet of an Agilent 7890 GC (Agilent, Palo Alto, CA) coupled with a 5975 mass selective detector. The column was an Agilent HP-5ms ultra-inert (30 m × 0.250 mm I.D., 0.25 μm film thickness). Analytical details for the chromatography were as given in Obenland et al. (2008). Identification of the volatiles was made by comparison to Wiley/NBS library spectra and the retention times of standards. Comparisons of retention indices to published values were used to obtain additional confirmation of identities. Quantification was performed by using calibration curves generated from standards added to deodorized mandarin juice and adjusting the resulting values based upon the amount of the internal standard (1-pentanol) present.

2.7. Statistics

Sensory and quality data were analyzed for the effect of temperature by utilizing Proc Mixed (SAS, Cary, NC) for both 4 and 7 weeks of storage with time and temperature being treated as fixed effects and lot and test (date fruit were obtained) as random effects. Reciprocal transformations were performed for SSC and TA prior to analysis. Significance of mean differences was determined using the Bonferroni correction. Analysis for the effect of time on the sensory and quality data was performed using averages over the temperature effect with Proc Mixed (SAS) and using contrast statements with the Bonferroni correction to determine the significance of each time comparison. Analysis of volatiles data for temperature and time effects utilized Proc Rank (SAS) followed by Proc Mixed with time or temperature as fixed effects and lot and test as random effects. Contrast statements with the Bonferroni correction were used to determine the significance of each time or temperature comparison.

3. Results

3.1. Effect of storage time on quality and sensory attributes

Peel quality for both 'W. Murcott' and 'Owari' remained excellent throughout storage with little or no decay present and the quality was not influenced by either storage time or temperature (data not shown).

Storage of 'W. Murcott' resulted in an increase in SSC by the fourth week of storage, the increase from week 0 to week 7 also being statistically significant (Table 1). TA, in contrast, declined, with most of the decline taking place during the first week. Accordingly, SSC/TA became higher, its pattern of change following that of TA. Likeability (hedonic score) was unchanged until the seventh week, by which time the score had declined from 6.1 (slightly above like slightly) at week 0 to 5.3 (between neither like nor dislike). The sensory attributes off-flavor, richness and sweetness were unchanged by storage, although tartness decreased during the first week and remained relatively unchanged thereafter.

SSC was unchanged by storage in 'Owari', but TA began to significantly decline by the fourth week (Table 2). This decline led to increases in SSC/TA over the same time period. Overall likeability (hedonic score) was not altered by storage, although adverse changes in flavor quality were evidenced by increased off-flavor apparent by week 4. Richness and sweetness were unchanged by storage, although tartness declined from week 1 to week 4.

3.2. Effect of storage temperature on quality and sensory attributes

Storage of 'W. Murcott' at 8 °C led to a slightly higher SSC than fruit stored at 0 °C, while temperature had no influence on TA (Table 1). The SSC/TA ratio in the fruit stored at 8 °C was significantly higher than that stored at 0 °C or 4 °C. Fruit stored at 8 °C had a higher hedonic score and so was better liked by the panelists than fruit stored at the other temperatures. Off-flavor was higher at 4 °C than at 8 °C but similar to that at 0 °C. Temperature had no effect on richness or sweetness but higher temperature led to fruit that was less tart. There was no significant influence of temperature on any of the quality and sensory attributes of 'Owari' (Table 2).

3.3. Effect of storage time and temperature on volatiles

Forty-six volatiles were identified and quantified for both 'Owari' and 'W. Murcott' mandarins (data not shown). No statistically significant differences in the amounts of these volatiles were found among the three storage temperatures for either of the varieties. Storage time, on the other hand, was found to greatly alter the volatile profiles of both mandarin varieties. Nine of the 46 identified volatiles were significantly changed ($P \leq 0.05$) in 'W. Murcott', with 7 alcohols and esters increasing by more than 50%, and a ketone (carvone) decreasing by the same amount (Table 3). Twenty-one volatiles had significant changes ($P \leq 0.05$) in 'Owari', with 3 alcohols and 5 esters increasing and 8 hydrocarbons, 2 aldehydes, a ketone (carvone), an alcohol (4-terpineol), and an ester

Table 1
Influence of storage time (0, 3, 6 weeks cold storage + 1 week at 20 °C) and temperature on quality and sensory attributes of 'W. Murcott' mandarins.

	SSC	TA	SSC/TA	Hedonic[a]	Off-flavor[b]	Richness[b]	Sweetness[b]	Tartness[b]
Storage (weeks)								
0	12.7	1.17	11.56	6.1	106.6	92.8	97.0	83.9
1	12.8	1.00	13.31	6.0	101.2	92.3	99.2	92.3
4	13.0	0.96	14.30	5.9	98.3	91.7	99.4	94.4
7	13.4	0.91	15.40	5.3	91.6	85.9	96.3	97.1
Storage contrasts[c]								
0 vs. 1	NS[d]	*	*	NS	NS	NS	NS	*
0 vs. 4	*	**	**	NS	NS	NS	NS	**
0 vs. 7	*	**	**	*	NS	NS	NS	**
1 vs. 4	NS	NS	NS	NS	NS	NS	NS	NS
1 vs. 7	NS	NS	NS	*	NS	NS	NS	NS
Temperature (°C)								
0	13.0b[e]	0.95a	14.42a	5.5b	93.8ab	88.4a	96.9a	92.7b
4	13.1ab	0.95a	14.62a	5.3b	89.8b	86.6a	96.8a	95.2ab
8	13.4a	0.95a	15.32b	6.0a	102.8a	92.4a	100.5a	98.9a

Each temperature value is based upon means from a minimum of 288 fruit. Each storage value is based upon means from a minimum of 144 fruit (0 or 1 week) or 432 fruit (4 or 7 weeks).

[a] Hedonic ratings were on a 1–9 scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.
[b] Ratings using a 150-mm line scale where a higher number indicates less off-flavor and tartness, but more richness and sweetness.
[c] Contrasts for storage time effect were performed across temperatures. Cold storage treatments (4 and 7 weeks) included 1 week at 20 °C to simulate a marketing period.
[d] Contrasts are indicated as nonsignificant (NS) or significant at either the *$P \leq 0.05$ or **$P \leq 0.01$ level using the Bonferroni correction.
[e] Values with a different letter are statistically different ($P \leq 0.05$) as estimated by determining the significance of least square mean differences using the Bonferroni correction.

(octyl acetate) decreasing (Table 4). In both varieties 4–7 weeks were required for the changes in volatile amounts to occur.

4. Discussion

As has been reported previously for mandarins (Cohen et al., 1990; Hagenmaier, 2002; Tietel et al., 2010), storage was deleterious to the flavor of both cultivars, with an increase in off-flavor ('Owari') and a decrease in overall hedonic score ('W. Murcott') being indicative of the loss in flavor quality (Tables 3 and 4). There was no specific decrease in richness, or mandarin-like quality, of the fruit as a result of storage as has been noted in prior studies (Marcilla et al., 2009; Tietel et al., 2010), although a loss of mandarin-like flavor could also have been manifested in a decline in hedonic score as was observed for 'W. Murcott'.

Fruit from both cultivars was also found by the panelists to become progressively less tart as storage progressed. The decrease in tartness during storage was likely due to increases in SSC/TA that occurred during storage as a consequence of loss in TA (both varieties) and a gain in SSC ('W. Murcott'). The loss in acidity during storage is a consequence of the respiratory activity of the fruit and has been often reported to occur in citrus (Davis et al., 1973; Echeverria and Ismail, 1987; Purvis, 1983). Marcilla et al. (2009) reported in a recent paper on the storage of 'Clemenules' mandarins that the level of TA was closely related to flavor, with the decline in acidity being associated with the development of poor flavor. At least in the case of 'Owari' it seems unlikely that the loss of acidity had a negative influence on flavor given the relatively low SSC/TA values throughout the storage period and, in fact, the loss in acidity may have benefited flavor. Obenland et al. (2009) reported that sensory panelists increasingly liked navel oranges as

Table 2
Influence of storage time (0, 3, 6 weeks cold storage + 1 week at 20 °C) and temperature on quality and sensory attributes of 'Owari' mandarins.

	SSC	TA	SSC/TA	Hedonic[d]	Off-flavor[e]	Richness[e]	Sweetness[e]	Tartness[e]
Storage (weeks)								
0	10.9	1.39	8.18	5.3	97.7	82.5	71.1	61.8
1	11.2	1.35	8.70	5.2	91.6	79.6	77.5	62.7
4	11.3	1.29	9.26	5.1	81.8	75.8	80.4	72.0
7	11.2	1.26	9.67	4.9	79.7	73.5	78.7	75.7
Storage contrasts[a]								
0 vs. 1	NS[b]	NS	NS	NS	NS	NS	NS	NS
0 vs. 4	NS	*	**	NS	**	NS	NS	**
0 vs. 7	NS	**	**	NS	**	NS	NS	**
1 vs. 4	NS	NS	NS	NS	*	NS	NS	*
1 vs. 7	NS	*	*	NS	*	NS	NS	**
Temperature (°C)								
0	11.1a[c]	1.27a	9.39a	4.7a	75.5a	72.0a	78.3a	74.3a
4	11.2a	1.30a	9.26a	5.1a	85.1a	77.2a	79.7a	72.5a
8	11.4a	1.26a	9.68a	5.1a	82.1a	75.1a	80.9a	74.1a

Each temperature value is based upon means a minimum of 216 fruit. Each storage value is based upon means from a minimum of 108 fruit (0 or 1 week) or 324 fruit (4 or 7 weeks).

[a] Contrasts for storage time effect were performed across temperatures. Cold storage treatments (4 and 7 weeks) included 1 week at 20 °C to simulate a marketing period.
[b] Contrasts are indicated as nonsignificant (NS) or significant at either the *$P \leq 0.05$ or **$P \leq 0.01$ level using the Bonferroni correction.
[c] Values with a different letter are statistically different ($P \leq 0.05$) as estimated by determining the significance of least square mean differences using the Bonferroni correction.
[d] Hedonic ratings were on a 1–9 scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.
[e] Ratings using a 150-mm line scale where a higher number indicates less off-flavor and tartness, but more richness and sweetness.

Table 3
Influence of storage time (0, 3, 6 weeks cold storage + 1 week at 20 °C) on aroma volatiles of 'W. Murcott' mandarins.

Compound	Descriptor	Orthonasal threshold[a] (μg/L)	Retronasal threshold[a] (μg/L)	Concentration (μg/L)			
				Weeks of Storage			
				0	1	4	7
Ethanol[b]	–			648.5b	744.9ab	1012.2a	1135.5a
Ethyl acetate	Pleasant, fruity	6038[c]	3554[c]	189.2b	618.0ab	1343.1a	2106.0a
Ethyl propanoate	Sweet, fruity	256[c]	146[c]	2.1c	7.5b	15.5ab	26.8a
3-Methylbutanol	Malty	1000[d]	250[d]	5.1b	30.4ab	89.7ab	194.9a
Ethyl 2-methylpropanoate	Sweet, fruity	0.35[c]	0.11[c]	0.0c	0.3c	0.7b	1.7a
Ethyl 2-butenoate	Pungent, allicious	–	–	0.0c	0.2bc	1.1ab	3.3a
Ethyl 2-methylbutanoate	Apple	0.080[c]	0.055[c]	0.0c	0.1bc	0.6ab	2.3a
Heptanal	Oily, fatty	3[e]	21[e]	14.0ab	12.0b	15.9a	15.0ab
Carvone	Spearmint or carraway	–	–	65.2a	50.1ab	47.5ab	32.8b

Only those volatiles with statistically significant changes due to storage are shown. Each volatile concentration value is the mean taken across tests (dates fruit were obtained), grower lots, and storage temperatures. Volatile concentrations followed by a different letter are statistically significant as determined by contrast statements using the Bonferroni correction.

[a] Orthonasal perception of odors occurs through the nostrils while retronasal perception is from the inside of the mouth.

[b] Concentration in mg/L.

[c] Aroma thresholds based upon values published by Plotto et al. (2004, 2008).

[d] Buettner and Schieberle (2001).

[e] Rouseff (2010).

TA declined and SSC/TA rose during navel orange maturation and that the increase in likability did not plateau until SSC/TA values of 18 or more were reached. Also, results from a consumer sensory evaluation suggested that 'Owari' mandarins were highly liked by consumers until SSC/TA reached 15 or more (Campbell et al., 2008). As 'W. Murcott' had much higher SSC/TA values than 'Owari' and exceeded 15 after 7 weeks of storage, it is possible that the loss of acidity had some role in the decline in flavor quality in this cultivar, although this cannot be conclusively determined.

A more important cause of the flavor loss that occurred during storage is likely the extensive changes in aroma volatiles that were found to occur. Although 46 volatiles were identified in both of the varieties, many were not altered in amount during storage. Empha-

sis was placed on the volatiles that significantly changed in amount ($P \leq 0.05$) and so were more likely to have a role in causing loss in flavor quality as a result of storage (Tables 3 and 4).

The most prominent changes occurred in a group of eight alcohols and esters that increased in amount during storage. The most abundant in this group was ethanol, whose accumulation in waxed mandarins and other citrus is due to low oxygen-induced fermentation and correlates with the development of off-flavor (Cohen et al., 1990; Ke and Kader, 1990; Marcilla et al., 2009). A substantial amount of ethanol was present for both varieties at the initial sampling (week 0), although Cohen et al. (1990) reported even higher levels in 'Murcott' mandarins at harvest. Ethanol can act in a beneficial manner at low to moderate concentrations to accentuate other

Table 4
Influence of storage time (0, 3, 6 weeks cold storage + 1 week at 20 °C) on aroma volatiles of 'Owari' mandarins. Only those volatiles with statistically significant changes due to storage are shown.

Compound	Descriptor	Orthonasal threshold[a] (μg/L)	Retronasal threshold[a] (μg/L)	Concentration (μg/L)			
				Weeks of storage			
				0	1	4	7
Ethanol[b]	–			692.9b	808.1b	1018.7b	1068.6a
Ethyl acetate	Pleasant, fruity	6038[c]	3554[c]	67.4b	306.5ab	595.5a	532.6a
Ethyl propanoate	Sweet, fruity	256[c]	146[c]	3.4b	10.2b	27.7a	18.4a
3-Methylbutanol	Malty	1000[d]	250[d]	0.0b	62.5b	255.7b	366.0a
2-Methylbutanol	Malty	320[d]	–	0.0b	39.9b	184.0b	263.1a
Ethyl 2-methylpropanoate	Sweet, fruity	0.35[c]	0.11[c]	0.1b	0.3ab	2.0a	2.1a
Ethyl 2-butenoate	Pungent, alliaceous	–	–	0.0b	0.2ab	0.9a	1.0a
Ethyl 2-methylbutanoate	Apple	0.080[c]	0.055[c]	0.1b	0.7ab	3.6a	2.6a
β-Myrcene	Musty, wet soil	773[c]	500[c]	169.3a	194.5a	87.8b	76.8b
Octanal	Fatty, citrus-like	233[e]	97[e]	43.2a	42.1ab	32.0b	26.7c
α-Terpinene	Lemony, citrusy	–	–	32.0a	32.7ab	20.2b	20.1b
p-Cymene	Solvent, citrus	–	–	198.0a	223.1a	139.4b	125.2b
β-Ocimene	Herbaceous, sweet	–	–	13.1a	16.1ab	6.7bc	6.2c
γ-Terpinene	Lemony, lime-like	3260[c]	2140[c]	28.5a	38.8a	20.6c	21.1bc
Terpinolene	Citrus, pine	–	–	90.5a	88.1ab	61.3bc	55.4c
1,3,8-Para-menthatriene	Oily, woody, pine	–	–	0.7a	0.7a	0.5b	0.4c
4-Terpineol	Woody, earthy	–	–	22.7a	22.8a	19.5b	18.9b
Octyl acetate	Fruity, slightly fatty	2767[c]	3540[c]	0.4a	0.5a	0.2b	0.2b
Carvone	Spearmint or carraway	–	–	21.9a	16.3a	14.0a	7.4b
Perillaldehyde	Green, oily, cherry	–	–	15.1a	12.6a	7.2a	4.1b

Each volatile concentration value is the mean taken across tests (dates fruit were obtained), grower lots, and storage temperatures. Volatile concentrations followed by a different letter are statistically significant as determined by contrast statements using the Bonferroni correction.

[a] Orthonasal perception of odors occurs through the nostrils while retronasal perception is from the inside of the mouth.

[b] Concentration in mg/L.

[c] Aroma thresholds based upon values published by Plotto et al. (2004, 2008).

[d] Buettner and Schieberle (2001).

[e] Rouseff (2010).

aromas in the fruit (Nisperos-Carriedo and Shaw, 1990), but can also act at higher concentrations, such as those occurring in the mandarins in this study, as a substrate to stimulate ester synthesis (Mattheis et al., 1991; Rudell et al., 2002). In both mandarin varieties there were large increases in five different esters as a result of storage, results similar to the recent report of Tietel et al. (2010). Concentrations of ethyl acetate and ethyl propanoate were below published odor thresholds, and these volatiles may have a lesser influence on flavor. Ethyl 2-methylpropanoate and ethyl 2-methylbutanoate, on the other hand, had measured concentrations well above their odor thresholds and likely have a noticeable impact on flavor. Increases in 3-methylbutanol ('Owari' and W. Murcott') and 2-methylbutanol ('Owari') were also substantial during storage and exceeded (at least in the case of 3-methylbutanol) the odor threshold. The malty character of these volatiles combined with the sweet, fruity, ethereal, wine-like odor of the esters could have altered the flavor profile of both of the varieties and been at least partly responsible for the storage-induced flavor loss.

Although 'Owari' and 'W. Murcott' had similar increases in esters and alcohols during storage, there was a large difference between the varieties in volatiles that declined in amount during the same period. In 'W. Murcott' only carvone showed any meaningful decline, while in 'Owari' 13 compounds, represented primarily by terpenes, showed significant losses over the storage period. These volatiles had aromas such as citrusy, pine-like and fruity, the loss of which could conceivably alter the overall flavor profile. The impact of these losses, however, is unclear given the lack of odor thresholds determined in deodorized citrus juice for many of these compounds. Those thresholds that were available (Tables 3 and 4) indicated that those compounds were present at sub-threshold levels. Tietel et al. (2010) suggested that the losses in volatile amount that they observed during the storage of 'Mor' mandarins was likely responsible for the decline in mandarin-like flavor that they observed, although only the change in β-ocimene was statistically significant from time 0. In this study panelists did not detect any significant change in mandarin-like flavor (richness), but noted instead a decline in likeability (hedonic score) in 'W. Murcott' and an increase in off-flavor in 'Owari'. It also should be noted that storage-induced flavor loss in 'W. Murcott' still occurred in the absence of any large-scale decline in aroma volatiles.

The recommended storage temperature for mandarins is 5–8 °C (Kader and Arpaia, 2002), although the potential exists that they may be stored at 2.22 °C or below if quarantine cold treatments for fruit fly disinfestation are utilized (Burns, 2004). In this study mandarins were stored at 0 °C, 4 °C or 8 °C in order to determine if storage temperature influences flavor quality. This had not been previously determined as prior research had been focused primarily on the influence of temperature on external peel quality (Sala and LaFuente, 2000; Sanchez-Ballesta et al., 2000) and had not addressed the issue of flavor. Marcilla et al. (2006) evaluated the influence of storage temperature on Valencia orange flavor, but examined a range of temperatures that exceeded those potentially utilized by the mandarin industry in California. In this study it was found that the temperature at which 'Owari' mandarins were stored had no influence on any of the measured sensory parameters and, accordingly, there were also no significant changes in SSC, TA, SSC/TA or measured volatiles due to storage temperature. For 'W. Murcott', however, panelists slightly preferred fruit that were stored at 8 °C, in comparison to that stored at either 0 °C or 4 °C. This preference was associated with a perception of lesser off-flavor and reduced tartness. This may have been due to the fruit stored at 8 °C having a higher SSC/TA ratio than that at either 0 °C or 4 °C, although the difference in SSC/TA was fairly small. Aroma volatile concentration did not appear to be a factor in the flavor differences as storage did not significantly alter ($P \leq 0.05$) any of the measured volatiles.

The higher SSC/TA of 'W. Murcott' stored at 8 °C was due to the greater concentration of SSC at this temperature as TA was the same for all temperatures. Similarly, in lemons (Eaks, 1961) and Valencia oranges (El-Zeftawi, 1976) it was noted that the concentration of SSC increased during storage, and that warmer storage temperatures enhanced the amount of increase. It is possible that the concentration difference was a result of the lower water content of the fruit, due to the higher rate of moisture loss that likely occurred at 8 °C. This idea is not supported, however, by percent juice data that indicated that there were no statistical differences in percent juice among the three storage temperatures after 7 weeks of storage (data not shown).

In summary, this test conducted over two seasons and using multiple fruit lots, found that storage of two varieties of mandarins acted to decrease flavor quality, as evidenced by increased off-flavor ('Owari') or a decline in overall likeability ('W. Murcott'). It should, though, be noted that this test was conducted using a small panel that can be considered as semi-expert due to their familiarity with tasting citrus and that these panelists likely had an enhanced ability to detect flavor change relative to the average consumer. Although storage caused SSC/TA to increase and the perceived tartness to decrease, large increases in the amounts of alcohols and esters appears to be the factors most likely to be responsible for the loss in flavor quality during storage. Storage as 8 °C resulted in better tasting fruit for 'W. Murcott' than storage at either 0 °C or 4 °C, a potential cause being that SSC/TA is higher at 8 °C and the fruit less tart than at the lower temperatures. Temperature did not affect the flavor of 'Owari'.

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