

# Commercial packing and storage of navel oranges alters aroma volatiles and reduces flavor quality

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## Abstract

Navel oranges were sampled either from the harvest bin, after the washer, after the waxer or at the end of the packing process in a commercial packing house and stored for 0, 3 or 6 weeks at 5 °C followed by 4 d at 13 °C and 3 d at 20 °C. Individual oranges were analyzed for percent juice, SSC, TA and ethanol concentration and a portion of each fruit tasted and rated for freshness, tartness, sweetness and likeability (hedonic score). Ethanol levels increased in the fruit as a result of storage and as a result of the waxing step of the packing line in both of the two tests. In one of the tests there was a significant increase in ethanol caused by each of the packing line steps, indicating a physiological effect on the fruit of the packing line itself. The freshness and likeability rating both decreased as a result of storage and packing, although packing had a lesser effect. The individual packing line steps could not be differentiated between each other in terms of an effect on flavor but the waxing step seemed to have the most impact. The SSC/TA ratio increased significantly during storage, mainly due to a decline in TA. In the third test navel oranges were sampled from the harvest bin and after the packing line and stored for 0, 3 or 6 weeks at 5 °C followed by 4 d at 13 °C and 3 d at 20 °C. Quality and sensory attributes were evaluated as in the previous two tests and fruit were also characterized for changes in aroma-active volatiles using GC–olfactometry. Freshness and likeability decreased as a result of storage, but only in packed fruit. Percent juice, SSC and TA did not change as a result of any of the treatments. Ethyl butanoate, ethyl hexanoate, and four constituents with uncertain identification were aroma-active compounds that increased, while limonene decreased in amount to a greater degree in the packed fruit and may be at least partially responsible for the observed flavor changes. Ethanol was not identified by GC–olfactometry but was more abundant in packed fruit and may have influenced flavor.

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## 1. Introduction

During the commercial packing of navel oranges the fruit are subjected to a number of processes on the packing line which include washing, rinsing, waxing, drying, sizing and placement into boxes. It is commonly believed in the citrus industry, and has been documented in the older literature (Biale, 1961), that the packing process, combined with subsequent storage of the fruit, acts to reduce the eating quality of the fruit.

Waxing or application of non-wax based coatings that occurs during commercial packing can alter the internal atmosphere in

citrus fruit, leading to the production of anaerobic metabolites such as ethanol and acetaldehyde (Davis and Hoffman, 1973; Hagenmaier and Baker, 1994; Hagenmaier and Goodner, 2002). Accumulation of these metabolites has been linked to poor flavor in waxed citrus (Ahmad and Khan, 1987; Cohen et al., 1990; Hagenmaier, 2002) and in citrus exposed to long-term controlled atmosphere storage (Ke and Kader, 1990). Ethanol is naturally present in unwaxed fruit and is thought to be an enhancer of flavor if present in low to moderate amounts (Nisperos-Carriedo and Shaw, 1990), although high amounts (in excess of 2000  $\mu\text{L L}^{-1}$ ) appear to cause off-flavor (Cohen et al., 1990; Ke and Kader, 1990). Mandarin oranges are especially very prone to the accumulation of ethanol and off-flavors following waxing (Hagenmaier, 2002). Shi et al. (2005), in a comparison of different citrus types, found mandarins to be much more sensitive

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to anaerobic stress than grapefruit and speculated that this may be a major reason for the relatively poor storability of mandarin oranges.

A limited number of studies have also documented that alcohol and acetaldehyde are not the only flavor-related volatiles that are altered in amount by the storage of waxed oranges. Nisperos-Carriedo et al. (1990) compared the effects of five different coatings during the storage of pineapple oranges stored at 21 °C for 12 d and found the coated fruit to have increased levels of at least five volatile components, some of them being potentially beneficial to the flavor of the fruit. In a study that attempted to more closely simulate commercial conditions Baldwin et al. (1995) reported changes in numerous flavor-related volatiles as a result of waxing and storage. Patterns of change varied depending on the compound, some increasing and others decreasing during storage with waxing and type of wax being key factors in determining the amounts present. As in the previously mentioned study, some of the volatiles could have positively influenced flavor while others may have had a negative influence. Controlled atmosphere storage can also rapidly influence flavor volatiles in oranges and was proposed as a means to increase the flavor strength of the aqueous essence that is used in the production of frozen juice concentrate (Shaw et al., 1992).

Although storage of oranges generally acts to increase the SSC/TA ratio (El-Zeftawi, 1976; Schirra and Cohen, 1999), there are mixed reports on the effect of altering internal atmospheres on either SSC or TA. Short term anaerobic treatments of oranges and grapefruit prior to storage were found to reduce TA and increase the SSC/TA ratio (Bruemmer and Roe, 1969; Pesis and Avissar, 1989), while maintaining ‘Valencia’ oranges under low oxygen for 20 d or high CO<sub>2</sub> for up to 14 d did not significantly influence SSC or TA (Ke and Kader, 1990). Evaluations of stored ‘Valencia’ oranges (Baldwin et al., 1995) or grapefruit (Hagenmaier and Goodner, 2002) did not find any effect of waxing on either SSC or TA.

Numerous steps on the packing line have the potential to induce physiological changes in the fruit that could potentially result in flavor changes. Washing, which often utilizes a set of brushes combined with a low or high-pressure water spray can cause enhanced water loss from the peel (Hagenmaier and Baker, 1993). If the peel begins to dry as a result of this, the resistance to gas exchange may increase and alter the internal atmosphere of the fruit (Ben-Yehoshua, 1969). Washing, if aggressive enough, can also induce a wounding effect in the fruit and lead to an increased respiratory rate and an increased accumulation of ethylene (Petracek et al., 1998). Dropping or squeezing of citrus fruit, as may occur on a packing line, can also cause similar wound responses (Eaks, 1961; Vines et al., 1968). In recognition of this fact, researchers had proposed using CO<sub>2</sub> evolution as a physiological test to detect damage to citrus following packing (Eaks, 1961; Parker et al., 1984).

Prior work on the effects of waxing on orange flavor and quality have for the most part been performed under non-commercial situations that do not adequately mimic the conditions that commonly exist in an actual packing house or during subsequent storage. Hagenmaier (2002) did use commercially packed fruit

for a portion of a study but did not examine the effect of storage time and did not evaluate other quality parameters related to flavor. In addition, this study used mandarin oranges which may respond differently to the packing process than navel oranges. Also, there has never to our knowledge been a study conducted to sort out and examine both the mechanical and waxing effects on flavor and quality of the packing process. The goal of this study was to determine the influence of the different aspects of the commercial packing process and influence of subsequent storage on navel orange quality attributes, flavor-related volatiles and overall flavor.

## 2. Materials and methods

### 2.1. Fruit

Three separate grower lots of navel oranges (*Citrus sinensis* (L.) Osbeck) were run on commercial packing lines for each of the three tests. All fruit were commercially harvested from orchards in the San Joaquin Valley of California in the vicinity of the packing houses on the day of the tests. For all three tests commonly available strains (size 88) were utilized.

### 2.2. Packing house tests

The term “packing” in this paper refers to the entire process of commercially preparing fruit for market on a packing line by washing, grading, waxing and placement into boxes, while “packed” is the final end stage where processed fruit are in boxes. A citrus industry representative was consulted to help select packing houses that were set up and operated in a manner typical of other California commercial orange packing houses. Test 1 took place on 11 January 2005, with lot 3 being processed at a different packing house (House 2) than lots 1 and 2 (House 1). Both packing houses utilized FMC Stay Fresh 227 shellac to wax the fruit, the wax containing either 1500  $\mu\text{L L}^{-1}$  imazalil, 5000  $\mu\text{L L}^{-1}$  TBZ and 50  $\mu\text{L L}^{-1}$  gibberellin (lots 1 and 2) or 2000  $\mu\text{L L}^{-1}$  imazalil (lot 3). Test 2 was completed on 7 March 2005 and all of the lots were run at House 1 using the same wax, fungicides and packing house conditions as for Test 1. These packing house runs were not specifically staged for the purposes of this test, but were part of the normal operations of the packing house. To determine the effect of the packing process on fruit quality, at least 90 fruit (size 88) each were taken for each grower lot from the following locations on the packing line: (1) harvesting bins just prior to bin dumping; (2) after washing; (3) after waxing; (4) after packing (packed). Test 3 was completed on 11 April 2006 at House 1 and utilized the same wax formulation as in the previous tests at that packing house. Fruit were only collected from the harvesting bins just prior to the bin dump and after packing (packed). Following each of the tests, fruit were transported to the University of California Kearney Agricultural Center and placed into cold storage at 5 °C. Evaluations of the fruit were conducted within 24–48 h of harvest and following storage at 5 °C for 3 and 6 weeks, followed by 4 d at 13 °C and 3 d at 20 °C to simulate a period of storage and

marketing. Relative humidity during storage was maintained at 85–90%.

### 2.3. Sensory evaluation

The top and bottom third of each fruit were removed and discarded, leaving a 2.5-cm center section of the fruit for testing. Each center section was then cut in half cross-wise with one half used for sensory analysis and the other half used for volatile and other quality measurements. The half used for sensory analysis was peeled and cut into six small wedges. It had been previously determined that these sizes of wedges were sufficiently large for the purposes of sensory evaluation. The evaluation area consisted of individual, three-sided booths fitted with small doors through which the samples were presented. Panelists were served fruit wedges in white, 30-mL soufflé cups. Distilled water was used to cleanse the palate between samples. Generally, 12–20 panelists were available for each day of sensory testing. The panelists routinely perform sensory evaluations of oranges and were well versed in detecting flavor differences in oranges and can be considered an experienced panel. For each storage time fruit were tasted over a 3-d period, tasting 1 grower lot per day and 6 fruit per treatment for Tests 1 and 2 and 12 fruit per treatment for Test 3. A portion of each fruit was tasted by at least four panelists and each panelist tasted at least two separate fruit from each treatment. Responses to the tasting were recorded using a 9-point hedonic scale, where 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely. In addition, panelists were asked to place a mark on three 150-mm line scales to record perceived sweetness, tartness and freshness for the flavor of each sample.

### 2.4. Quality and ethanol determinations

The unpeeled portion of the fruit section reserved for the quality and volatile analyses was weighed and then separately juiced using a Hamilton-Beach Commercial Juicer (model 932). All of the quality and ethanol determinations utilized individual fruit, the number of fruit being previously described in the sensory section. In the second year of the study (Test 3) the fruit were carefully peeled prior to juicing to prevent peel oil components from entering the juice and disrupting the results of the juice volatile analysis. In both cases the juice was filtered through a screen sieve and collected in screw top vials. Percent juice was calculated by weighing the juice for each sample and dividing by the weight of the unpeeled section. Soluble solid concentration (SSC) was determined in the juice using a temperature compensated refractometer and titratable acidity (TA) by use of an automatic titration system, and both were expressed as a percentage. A small portion of each juice sample was centrifuged ( $12,000 \times g$ ) and used for ethanol quantification using a kit based upon the enzymatic conversion of ethanol to acetaldehyde using alcohol dehydrogenase (Diagnostic Chemicals Limited, Oxford, CT). The remaining juice was divided into 9 mL portions and placed into 12 mm  $\times$  32 mm glass vials which were then capped with a Teflon-coated septum and frozen at  $-80^\circ\text{C}$  for later gas chromatography/olfactory (GCO) analysis (Test 3).

### 2.5. Gas chromatography/olfactometry (GCO)

Immediately prior to analysis the juice was thawed for 15 min at  $40^\circ\text{C}$  and the vial was then transferred to another  $40^\circ\text{C}$  water bath in which the level of the water was maintained just over that of the juice in the vial. Solid phase microextraction (SPME) utilizing a fiber with  $75\ \mu\text{m}$  carboxen/polydimethylsiloxane coating was used to trap volatile components present in the vial headspace. During the 30 min trapping period the juice was slowly stirred by means of a stir bar. At the completion of trapping the SPME fiber was removed from the vial and desorbed for 2 min at  $280^\circ\text{C}$  in the splitless inlet of an Agilent (Agilent, Palo Alto, CA) 6890 gas chromatograph equipped with a DB-5 column (30 m  $\times$  0.32 mm ID,  $1\ \mu\text{m}$  film thickness; J&W Scientific, Folsom, CA). The oven was programmed to hold at  $32^\circ\text{C}$  for 3 min, then ramp up to  $200^\circ\text{C}$  at  $0.1^\circ\text{C s}^{-1}$ . Helium carrier gas flow was  $0.03\ \text{mL s}^{-1}$ . Hydrogen, air and nitrogen make-up gas flows were 0.7, 7.5 and  $0.8\ \text{mL s}^{-1}$ , respectively. The effluent was split equally between a flame ionization detector (FID,  $250^\circ\text{C}$ ) and a SGE ODO II sniffer port (Austin, TX, USA). Peaks of interest from the FID detector were quantified by the use of standard curves generated from purchased standards. Identification of the peaks utilized retention times of the standards, retention index values and aroma of the peak. As an additional aid to identification, an identical sample was trapped in the same manner using SPME as previously described and analyzed using GCMS. The analysis system in this case was a Agilent 6890 paired with a 5973 mass selective detector. The column used was a DB-5 (30 m  $\times$  0.32 mm ID,  $1\ \mu\text{m}$  film thickness; J&W Scientific, Folsom, CA) and carrier flow was  $0.02\ \text{mL s}^{-1}$ . The inlet and column temperatures were as previously described for the GCO system. Identifications were made by comparison to Wiley/NBS library spectra and retention index values of standards.

Three panelists that had been extensively trained on use of the GCO system and on the recognition of citrus juice aromas sniffed the humidified effluent that came from the olfactory port. Upon sensing an odor the panelist would slide the control on a self-made variable potentiometer, the amount of movement related to the intensity of the odor. Both FID and sniffer responses were outputted into separate channels of the Agilent ChemStation software. Using this software it was possible to overlay the two datasets and determine which of the FID peaks were aroma-active and possibly contributing to the flavor of the juice. Each treatment was run and evaluated a total of six times. To be deemed aroma-active the compound had to be detected by at least two of the three panelists and in three out of six runs. Peak areas from the olfactory potentiometer were normalized by setting the peak with the highest intensity to 100 in order to adjust for differences between panelists. Averages of the normalized intensities were taken across all three panelists.

### 2.6. Statistics

The quality and sensory data were analyzed using a factorial design with test, storage duration and packing line step as fixed

effects and lot, nested within test, as a random effect using the PROC Mixed procedure in SAS (SAS Institute, Cary, NC). The volatiles data consisted of samples pooled across lot and were analyzed using the General Linear Model (GLM, SAS) with treatment and storage as main effects. Transformations of the data were performed when appropriate for data analysis. All mean comparisons were made at the 5% level of significance using the Bonferroni test.

### 3. Results

#### 3.1. Effect on quality and sensory attributes (Tests 1 and 2)

Quality and sensory data from Tests 1 and 2 were combined since there were little or no differences in response of the quality parameters to storage or packing line treatment between the two tests (Table 1). Percent juice was not altered by storage, while fruit that had gone through the washer stage or beyond had slightly less juice than fruit taken from the bin. SSC was increased by storage, while TA was decreased, leading to a progressive increase in the SSC/TA ratio as storage time advanced. SSC, TA, and SSC/TA were not changed by packing line treatment. Sensory panel evaluations indicated that the freshness of the orange flavor decreased progressively as a result of storage. The packing line also caused the freshness rating of the fruit to decline, with the fruit taken after the waxing and packing steps being significantly less fresh in flavor than fruit taken from the bin. Fruit were judged to be tarter after 3 weeks of storage than after 0 or 6 weeks, and sweetness declined in fruit stored for longer than 3 weeks. The packing line had no influence on either tartness or sweetness. Hedonic ratings of the fruit indicated that fruit were liked less by the panelists following storage. A packing

line effect on the hedonic score was significant, but the change amounted to only 0.4 units.

Juice ethanol content increased as a result of the packing line and also due to storage (Fig. 1). At the initial storage time waxed and packed fruit had ethanol levels over twice those of either the bin or washed fruit for Test 1. Similar results were obtained for Test 2. Ethanol amounts were higher after 3 weeks for all of the packing line steps, although the waxed fruit (waxed and packed) accumulated greater amounts than unwaxed fruit. In Test 1, following 3 weeks of storage, fruit had progressively more ethanol following each step, unlike in Test 2 where bin and washed fruit had similar amounts of ethanol. This same pattern held also following 6 weeks of storage for Test 1. Although ethanol amounts tended to increase from 3 to 6 weeks the amount of change was much smaller than that observed in the initial 3 weeks of storage.

#### 3.2. Effect on quality, sensory attributes and aroma volatiles (Test 3)

Quality parameters were not affected by the packing or by storage in Test 3, while sensory evaluations indicated that the flavor of the fruit changed as a result of these treatments (Table 2). Panel ratings of the freshness character of the packed fruit declined by 23% during the initial 3 weeks, corresponding to a significant decline in the hedonic score. There was no further decrease in either of these ratings as the storage was increased to 6 weeks and no change occurred throughout the entire storage period in the fruit that was not packed. The tartness rating was not altered as a result of storage or packing line treatment and there was no consistent trend for change in sweetness.

Table 1  
Quality and sensory attribute means from navel oranges subjected to different steps on the packing line (Tests 1 and 2)

		Juice (%)	SSC	TA	SSC/TA	Fresh	Tart	Sweet	Hedonic
Storage <sup>a</sup>									
	0	40.1 <sup>b</sup>	12.4a	0.83a	15.7a	98.7a	94.8a	111.2a	6.5a
	3	40.4	12.8b	0.73b	18.4b	89.0b	101.5b	111.4a	6.2b
	6	39.6	12.8b	0.70b	19.2b	80.5c	96.5a	105.2b	5.7c
Step <sup>c</sup>									
	Bin	41.4a	12.6	0.72	18.1	94.1a	100.2	110.9	6.4a
	Washed	40.0b	12.6	0.78	16.9	90.9ab	95.6	108.4	6.1ab
	Waxed	39.1b	12.7	0.74	17.8	86.2b	96.6	108.2	6.1ab
	Packed	40.0b	12.9	0.75	18.2	85.8b	98.6	109.7	6.0b
Effect	d.f.	Juice (%)	SSC	TA	SSC/TA	Fresh	Tart	Sweet	Hedonic
Test (T)	1	NS <sup>d</sup>	NS	NS	NS	NS	NS	NS	NS
Storage (S)	2	NS	*	**	**	**	**	**	**
T × S	2	**	NS	NS	NS	**	NS	NS	NS
Step (St)	3	**	NS	NS	NS	**	NS	NS	*
T × St	3	*	NS	NS	NS	NS	NS	NS	NS
S × St	6	NS	NS	NS	NS	NS	NS	NS	NS
T × S × St	6	NS	NS	NS	NS	NS	NS	NS	NS

Fruit were evaluated after 0, 3 and 6 weeks of storage at 5 °C, followed by 4 d at 13 °C and 3 d at 20 °C. Data were combined for Tests 1 and 2.

<sup>a</sup> Storage time in weeks. Means for storage times represent 144 fruit per value.

<sup>b</sup> Values with a different letter are significantly different ( $P \leq 0.05$ ).

<sup>c</sup> Means for step represent 108 fruit per value.

<sup>d</sup> Main effects or interactions are indicated as non-significant (NS) or significant at either the  $*P \leq 0.05$  or  $**P \leq 0.01$  level.

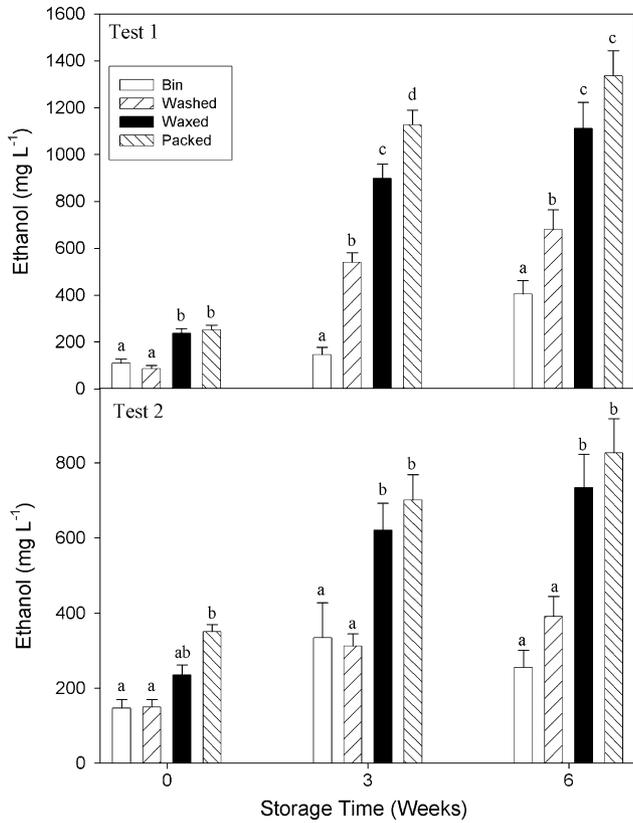


Fig. 1. Ethanol concentrations present in navel oranges taken from either the harvest bin (Bin) or off the packing line after the washer (Washed), waxer (Waxed), or after packing (Packed) following 0, 3 and 6 weeks of storage at 5 °C, followed by 4 d at 13 °C and 3 d at 20 °C for two separate tests (Tests 1 and 2). Means were taken across the three grower lots and represent the average of 18 fruit. Bars with different letters are significantly different from each other ( $P \leq 0.05$ ) within a storage time.

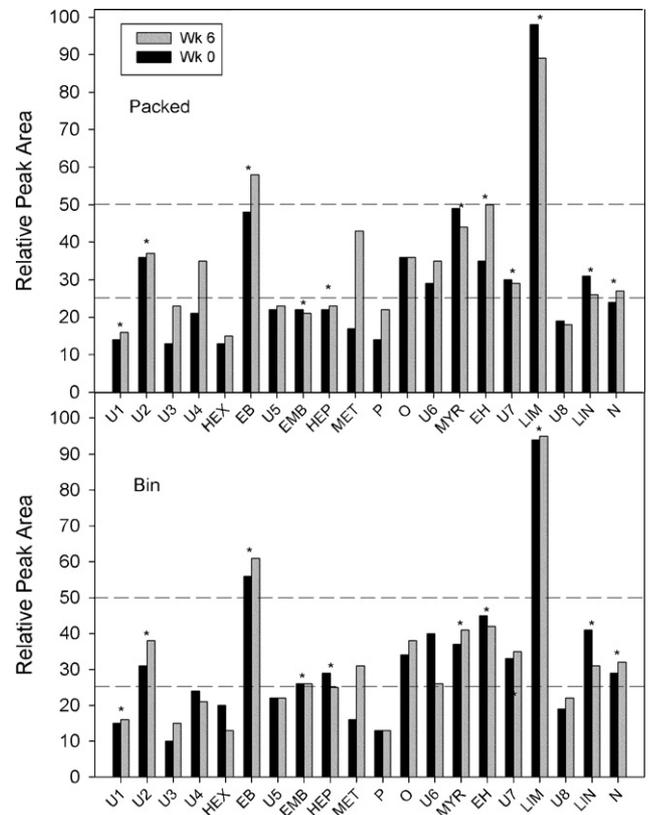


Fig. 2. Peak areas of aroma-active compounds from aromagrams generated in tandem with GC-FID data from waxed and unwaxed oranges stored for 0 and 6 weeks. Areas were normalized by expressing them as a percentage of the largest peak present in each aromagram. Each bar represents a mean of 18 separate determinations with there being 6 determinations per panelist. Stars above the bars indicate that a GC-FID peak was associated with the aromagram peak. Dashed lines are provided to compare the relative responses of the compounds. Compounds and abbreviations were: unknown (U1–8), hexenal (HEX), ethyl butanoate (EB), ethyl 2-methyl butanoate (EMB), heptanal (HEP), methional (MET),  $\alpha$ -pinene (P), 1-octen-3-one (O),  $\beta$ -myrcene (MYR), ethyl hexanoate (EH), limonene (LIM), linalool (LIN), (*E*)-3-nonenal (N). The compounds HEX, MET, P and O are tentative identifications based upon standard retention index values and odor.

Table 2

Quality and sensory attribute means from navel oranges with and without packing line treatment from Test 3, following 0, 3 and 6 weeks of storage at 5 °C, followed by 4 d at 13 °C and 3 d at 20 °C

Treatment <sup>a</sup>	Storage <sup>b</sup>	Juice (%)	SSC	TA	SSC/TA	Fresh	Tart	Sweet	Hedonic
Bin	0	37.4 <sup>c</sup>	14.4	0.83	19.8	100.8a	93.3	119.4a	6.3a
	3	33.1	13.3	0.79	19.1	94.1a	103.8	110.1b	6.0a
	6	37.7	14.2	0.98	17.9	95.4a	96.9	113.8ab	6.0a
Packed	0	38.7	14.1	0.82	18.8	93.4a	93.8	117.1a	6.2a
	3	34.5	13.2	0.78	19.7	72.3b	101.7	109.5a	5.3b
	6	35.1	14.1	0.98	17.9	71.6b	102.0	113.3a	5.2b
Effect	d.f.	Juice (%)	SSC	TA	SSC/TA	Fresh	Tart	Sweet	Hedonic
Storage (S)	2	NS <sup>d</sup>	NS	NS	NS	*	NS	**	NS
Treatment (T)	1	NS	NS	NS	NS	**	NS	NS	**
S × T	2	NS	NS	NS	NS	*	NS	NS	*

<sup>a</sup> Fruit were taken directly from the harvest bins prior to the packing line (bin) or after running on the packing line (packed).

<sup>b</sup> Storage time in weeks.

<sup>c</sup> Means are taken across all three grower lots and represent a mean of 36 fruit, values with a different letter are significantly different ( $P \leq 0.05$ ) within a treatment.

<sup>d</sup> Main effects or interactions are indicated as non-significant (NS) or significant at either the \* $P \leq 0.05$  or \*\* $P \leq 0.01$  level.

Table 3  
Aroma active compounds present in navel oranges from Test 3 with and without packing line treatment following 0, 3 and 6 weeks of storage at 5 °C followed by 4 d at 13 °C and 3 d at 20 °C as determined by GC–olfactometry

Compound	Aroma Descriptor	Bin <sup>a</sup>			Packed <sup>a</sup>			Significance <sup>b</sup>		
		0 <sup>c</sup>	3	6	0	3	6	Storage (S)	Treatment (T)	T*S
Unknown 1 (U1)	Sweet	2.6a	3.5ab	3.8b	3.1a	4.0a	3.2a	NS	NS	*
Unknown 2 (U2)	Metallic, unpleasant	3.8a	4.0a	4.8a	4.1a	6.8b	3.9a	**	NS	**
Ethyl butanoate (EB)	Fruity, sweet	85.1a	104.4a	80.1a	96.5a	132.4b	167.2c	**	**	**
Ethyl 2-methyl butanoate (EMB)	Fruity	ND	0.47a	0.21b	ND	0.94a	0.58b	**	**	**
Heptanal (HEP)	Oily, fatty	5.0a	3.0b	3.6b	4.6a	3.1b	2.1c	**	**	**
β-Myrcene (MYR)	Musty, balsamic	53.7a	59.2a	51.1a	69.4a	29.2b	52.6c	**	**	**
Ethyl hexanoate (EH)	Fruity	5.8a	6.9a	4.6b	5.6a	10.1b	14.5c	**	**	**
Unknown 7 (U7)	Fatty, lemony	4.0a	3.0b	3.5ab	4.1a	2.9b	3.4b	**	NS	NS
Limonene (LIM)	Citrus, fresh, minty	2263a	2099a	2163a	2648a	1208b	1838b	**	NS	**
Linalool (LIN)	Floral, green, citrus	32.0	30.3	29.4	33.1	26.1	25.5	NS	NS	NS
(E)-2-Nonenal (N)	Fatty, tallowy	3.7a	3.3a	6.1b	3.4a	4.0a	5.0a	**	NS	NS

All of these components had measurable FID detector peaks that were associated with aromas identified by the panel. Values are presented in  $\mu\text{g L}^{-1}$ .

<sup>a</sup> Fruit were taken either from the harvest bin (bin) or after packing (packed). Values represent the mean of six determinations from a pooled juice sample of six fruit (two fruit from each grower lot).

<sup>b</sup> Main effects or interactions are indicated as non-significant (NS) or significant at either the  $*P \leq 0.05$  or  $**P \leq 0.01$  level. Values with a different letter are significantly different ( $P \leq 0.05$ ) within a treatment (bin or packed).

<sup>c</sup> Storage times in weeks.

A total of 20 odorants were consistently noted to be present by the panelists in the GCO analysis of the juice samples from each of the treatments (Fig. 2). Eleven of these odorants produced FID peaks that clearly corresponded to the accompanying aromagram peaks and provided a quantification of the actual amount of the compound present (Table 3). Two compounds, methional and  $\alpha$ -pinene, were tentatively identified by odor and comparison of retention time with retention index values from standards, but were not detectable by FID (Fig. 2). Data obtained from the olfactory portion of the volatile measurements were found to be very useful in the recognition of compounds that did have odor and potential flavor impact and secondarily of use in providing an estimate of the relative odor impact of each compound. Most of the compounds had relative peak areas on the aromagram that ranged from 10 to 40% of the largest peak area. Compounds with relative aromagram peak areas greater than 40% and with the highest potential impact on flavor were ethyl butanoate, methional,  $\beta$ -myrcene, ethyl hexanoate, limonene, and linalool. Correspondingly, these strongly aroma-active compounds were present in the juice in the greatest amounts (Table 3). The compounds unknown 3, unknown 4, methioninal,  $\alpha$ -pinene, and ethyl hexanoate all had greater relative odor intensities in packed versus bin fruit, especially following 6 weeks of storage. The change in odor intensity of ethyl hexanoate was reflected in the significant increases in actual amount of the compound in the packed but not in bin fruit (Table 3). Ethyl butanoate increased in concentration by 73% over the course of the 6-week storage period but there was no significant change in this compound in the bin fruit (Table 3). The relative aroma contribution of this compound, however, did not appear to be greatly different between packed and bin fruit (Fig. 2). The aroma impact of limonene appeared to lessen during storage in the packed but not bin fruit (Fig. 2), matched by a decline of limonene amount in the packed fruit (Table 3). Storage enhanced the amount of ethyl

2-methyl butanoate and (E)-2-nonenal in both packed and bin fruit over the initial (no storage) concentration but decreased the amount of heptanal (Table 3). Due to the presence of sometimes overlapping peaks in the chromatogram, ethanol was difficult to accurately quantify using the available chromatographic system, and as a result it was decided to assay ethanol by enzymatic means. Ethanol, while it strongly increased during storage and was present in very large amounts in all of the fruit samples (Fig. 3), was not identified by the panelists as an odor-active compound even though it was one of the largest peaks visible in the FID chromatogram.

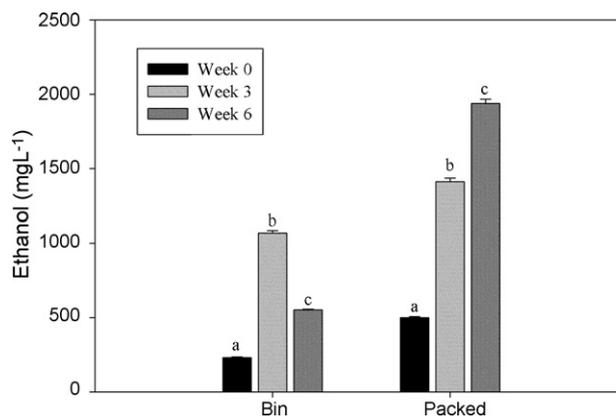


Fig. 3. Ethanol concentrations present in navel oranges with (Packed) and without (Bin) packing line treatment from Test 3. Measurements were made after 0, 3 and 6 weeks of storage at 5 °C, followed by 4 d at 13 °C and 3 d at 20 °C. Bars represent means of four determinations of pooled juice samples from six fruit (two fruit per grower lot). Bars with different letters are significantly different from each other ( $P \leq 0.05$ ) within a treatment (bin or packed).

#### 4. Discussion

Previous research has reported the decline in flavor that takes place in commercially packed oranges during storage (Biale, 1961). Prior to storage and subsequent marketing, oranges are commonly packed using a mechanized packing line to wash, wax, sort and finally pack the fruit into boxes. This process normally involves subjecting the fruit to numerous drops, impacts, high- or low-pressure wash sprays and the application of a coating to the fruit to impede water loss during storage. In this experiment we evaluated fruit that had gone through different stages of the packing process to better understand the contribution to flavor change that each of the stages might play in a commercial packing line.

In the first test we observed large incremental increases in the juice ethanol level in oranges as they moved through the packing line (Table 1). The difference was particularly pronounced following 3 weeks of storage. This indicated that not only did waxing increase ethanol as has been often reported (Davis and Hoffman, 1973; Hagenmaier and Goodner, 2002; Hagenmaier and Baker, 1994), but also that ethanol accumulation was altered as well by passage through other parts of the line. Fruit from the second test, however, responded in terms of enhanced ethanol primarily as a result of the waxing step and, with the exception of time 0, there were no significant differences in ethanol specifically due to the washing or packing step. It is not possible to say whether this was a result of differences in the fruit due to time of season or some other factor, or in how the packing line was run on that day. In the case of the packinghouses used in this test, introduction of fruit onto the packing line occurred by a dry dump process rather than dumping into a soak tank as is also commonly practiced in California. Dry dumping is not as gentle and can impose considerable mechanical stress on the individual fruit, including impact from drops, squeezes and scrapes (Grierson et al., 1986). Following dumping, the fruit were passed through a series of rotating brushes with overhead sprayers. Washing, at either low (Vines et al., 1968) or high pressure (Petracek et al., 1998), has been previously shown to influence internal CO<sub>2</sub> concentrations and may be indicative of a wounding response. Since our second sampling point took place after the washer and includes both dumping and washing, it is not possible to say which of the two steps was responsible for the increase in ethanol, and in fact both steps may have acted in a cumulative manner. Our final sampling point was from packed boxes and included grading, sizing and movement into accumulation bins, all potential sources for additional drops and impacts. Also, these fruit did pass through a heated (43–49 °C) tunnel in order to dry the wax that was applied, but internal pulp temperatures are not altered during the 2–2.5 min that the fruit reside in the tunnel (data not shown).

Ethanol has been noted as an indicator of damage in fruit and has been reported to be produced in response to heat injury (Song et al., 2001), freezing (Forney et al., 2000; Obenland et al., 2003; Tan et al., 2005) and mechanical injury (Norman et al., 1967). Eaks (1961) found that internal CO<sub>2</sub> levels in oranges and lemons increased when fruit were dropped or given pressure bruises, both events that can often occur on packing lines.

Increases in internal CO<sub>2</sub>, along with enhanced ethanol, are both indicative of the presence of fermentative metabolism caused by disruption of normal aerobic respiration. In Test 1 of this study ethanol levels in the fruit were increased by the washing, waxing and packing steps following 3 weeks of storage, but only the washing and waxing steps caused changes after 6 weeks (Fig. 1). While the waxing step would be expected to cause increased ethanol primarily due to the inhibitory effect of the coating on gas exchange, the enhancement of ethanol levels by washing and packing indicates that the metabolism of the fruit was also altered by passage through the other portions of the packing line, presumably due to mechanical injury to the fruit.

In Tests 1 and 2 the effect of both the packing line and storage in reducing the likeability (hedonic score) of the fruit were fairly modest with storage being the more dominant factor (Table 1). The impact on fruit flavor was more strongly noticed in the influence on the freshness of the flavor where the freshness rating declined substantially due to both storage and packing line effects. In the case of the packing line effect the differences were not significant until comparisons were made using fruit that had passed through the waxer, indicating that even though ethanol readings indicated a physiological effect of the washer on the fruit in Test 1 (Fig. 1), this effect was not sufficient to affect the freshness rating. Waxing has been reported to cause off-flavor in citrus (Cohen et al., 1990; Hagenmaier, 2002) and is thought of as a key aspect in the any loss of flavor quality that may occur due to the packing process. It was noticed that while five of the six lots of fruit responded fairly similarly to packing and storage in terms of hedonic and freshness ratings there was one lot that was more strongly influenced by these treatments (data not shown). Given that the packing house in this case was the same as that for all but one of the other lots and that waxing and packing line conditions were believed to be the same, this highlights the likely fact that oranges vary in their physiological response to the packing process and it is possible that flavor changes could occur that are even more pronounced than what were observed in Tests 1 and 2 of this experiment.

The quality parameters consisting of percent juice, SSC, and TA did not appear to be directly involved in the flavor changes that were observed (Table 1). Percent juice was altered only slightly as a result of treatment and seems unlikely to have had any influence. As was observed in this study and as has been previously reported (Cohen et al., 1990), storage can act to raise SSC/TA, which would generally be beneficial to flavor. Although SSC/TA was increased by storage in this study, both the hedonic and sweetness rating decreased over the same time period, leading one to believe that this was not the predominant change affecting flavor in this instance.

Ethanol has been the volatile of predominant concern in packed citrus given the strong stimulatory effect on ethanol accumulation by fruit coatings applied during packing (Davis and Hoffman, 1973; Hagenmaier and Baker, 1994) and studies that have shown correlations between ethanol levels in the fruit and off-flavor (Cohen et al., 1990; Ke and Kader, 1990). It has also been shown, though, that fruit coatings and storage can influence additional key flavor-related volatiles in citrus fruit (Baldwin et al., 1995). In light of this, an additional experi-

ment was conducted (Test 3) to identify and quantify changes in aroma-active volatiles following commercial packing and storage and relate these changes to sensory panel results from the same fruit. Sensory evaluations using the same fruit indicated that both freshness and hedonic ratings declined during storage, but only in the packed fruit (Table 2). Percent juice, SSC and TA were nearly unchanged in any of the treatments and provide evidence that other factors, such as aroma volatiles, are likely responsible for the observed decline in flavor ratings. Of the aroma-active compounds that were identified and could be quantified, ethyl butanoate and ethyl hexanoate both strongly increased, while limonene decreased during storage only in the packed fruit (Table 3). Both ethyl butanoate and ethyl hexanoate have a sweet, fruity essence and are believed to contribute to orange juice flavor (Ahmed et al., 1978; Buettner and Schieberle, 2001). Increases in these compounds, even though both are considered positive effectors of flavor, could lead to an altered balance in the fruit aroma away from what is considered “fresh” or desirable. Limonene, with a fresh, minty, citrusy aroma, is the most abundant volatile present in orange juice and is also thought to have an influence in determining flavor (Moshonas and Shaw, 1994; Tønder et al., 1998). It is possible that change in this volatile could also play a role in the perceived flavor changes that occurred in the packed fruit, but this is unclear given the large abundance of limonene in both packed and unpacked fruit. There were also four other components noted by sniffing alone with no quantifiable FID peaks (unknown 3, unknown 4, methional and  $\alpha$ -pinene) that appeared to increase in amount to a greater degree in the packed fruit. Given that the mode of quantifying differences by sniffing is subjective and much less precise, interpretation of the potential consequences of these changes must be done with some caution. Methional (tentative identification) has an aroma like cooked potato and has been reported to be a contributor to off-flavor that develops during orange juice storage (Bezman et al., 2001). The other compound tentatively identified was  $\alpha$ -pinene, which has a pine-like, citrusy odor, and has been reported to contribute to orange flavor (Moshonas and Shaw, 1994), although this seems not to be totally clear (Plotto et al., 2004). The aroma of unknown 3 was fruity, while that of unknown 4 was unpleasant and described by one panelist as like dry cereal grain.

Ethanol was by far the most abundant volatile quantified in both packed and unpacked fruit (Fig. 3) and was the second-largest peak in the FID chromatogram. Regardless of this, the panelists evaluating the effluent from the olfactory port of the GC were not readily able to detect the ethanol peak. For this reason ethanol was not included in Fig. 2 or Table 3. Ethanol has a relatively weak odor and a correspondingly high aroma threshold of  $100 \mu\text{L L}^{-1}$  in water (Fazzalari, 1978). Very high levels of ethanol in oranges would be expected to impart a fermented taste and strongly reduce the organoleptic properties of the fruit, although the level that this occurs at has not been defined. However, these high concentrations of ethanol and other fermentative metabolites, such as acetaldehyde may not be amounts that are commonly reached in commercially packed navels. Obenland (unpublished data) assayed navel oranges from eleven different packing houses in California after 4 weeks of storage and

found an ethanol range of 560 to  $1488 \mu\text{L L}^{-1}$ , with the average being  $849 \mu\text{L L}^{-1}$ . Although navel oranges may have different flavor responses in relation to increases in ethanol, Hagenmaier (2002) reported that a level greater than  $1500 \mu\text{L L}^{-1}$  or more was related to reduced flavor in mandarin oranges while a similar level only slightly reduced the flavor quality of Valencia oranges (Ke and Kader, 1990). Ethanol cannot be singled out as being solely responsible for these changes in flavor, however, as other flavor-related volatiles are also changing in amounts at the same time. It is also believed that ethanol at moderate levels may act as an accentuator of other aromas and be beneficial to orange juice flavor (Nisperos-Carriedo and Shaw, 1990). It seems likely that in this study where flavor changes began to be noted at ethanol concentrations less than  $1500 \text{ mg L}^{-1}$  (Fig. 3) that ethanol may play some role in modulating flavor, but clearly other changes, such as alterations in flavor volatiles were involved.

In conclusion, both commercial packing and storage of navel oranges caused reductions in the flavor quality of navel oranges. Percent juice, SSC and TA either did not change or did not change in a manner that would explain the loss in flavor quality. Large changes in the amount of certain flavor-related volatiles, however, did occur. These changes involved increases in both volatiles thought to positively and negatively influence flavor. Taken together the changes may be responsible for the perceived loss of fresh flavor following packing and/or storage that were the primary sensory difference noted by panelists tasting the fruit. There was evidence in the form of differences in the amount of ethanol accumulation that the packing line itself has a physiological effect on the fruit, but this was not translated into significant differences in flavor. Whether this is true with fruit more prone to physical damage or with fruit run over packing lines that impart greater amounts of physical impacts to the fruit is unknown. This study suggests that both minimizing storage time and application of coatings that do not influence the volatile flavor profile in the fruit would be appropriate goals in trying to maintain the flavor quality of navel oranges following harvest.

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