



## Open Archive TOULOUSE Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author-deposited version published in : <http://oatao.univ-toulouse.fr/>  
Eprints ID : 11300

**To cite this version** : Leclair, Clotilde and Plotto, Anne and McCollum, Greg and Bai, Jinhe and Biotteau, Alice and Baldwin, Elizabeth A. and Reyes-De-Corcuera, José and Chervin, Christian *Gas Chromatography–Olfactometry Analyses of Volatiles Produced by ‘Fallglo’ and ‘US Early Pride’ Tangerines*. (2012) Proceedings of the Florida State Horticultural Society, vol. 125 . pp. 264-271.  
ISSN [0097-1219](http://www.issn.org/0097-1219)

Any correspondence concerning this service should be sent to the repository administrator: [staff-oatao@listes-diff.inp-toulouse.fr](mailto:staff-oatao@listes-diff.inp-toulouse.fr)

# Gas Chromatography–Olfactometry Analyses of Volatiles Produced by ‘Fallglo’ and ‘US Early Pride’ Tangerines

CLOTILDE LECLAIR<sup>1</sup>, ANNE PLOTTO\*<sup>1</sup>, GREG MCCOLLUM<sup>1</sup>, JINHE BAI<sup>1</sup>,  
ALICE BIOTTEAU<sup>1</sup>, ELIZABETH A. BALDWIN<sup>1</sup>, JOSÉ REYES-DE-CORCUERA<sup>2</sup>,  
AND CHRISTIAN CHERVIN<sup>3</sup>

<sup>1</sup>USDA–ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL 34945

<sup>2</sup>University of Florida–IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

<sup>3</sup>Université de Toulouse, ENSAT–INPT, 31326 Castanet, France

ADDITIONAL INDEX WORDS. *Citrus reticulata*, flavor, aroma volatiles, fruit quality, tangerine

‘Fallglo’ is a popular tangerine (*Citrus reticulata* Blanco) cultivar with high eating quality. However, ‘Fallglo’ may contain as many as 30–40 seeds per fruit. ‘US Early Pride’ is a seedless mutation of ‘Fallglo’ with similar quality attributes. The objective of the current study was to determine if ‘Fallglo’ and ‘US Early Pride’ fruit differed in the composition or quantity of aroma-active volatiles produced over time. Fruit were harvested bi-weekly from October to December. Juice was carefully extracted from 50 fruit, and volatiles were analyzed using gas chromatography (GC). Two subjects evaluated the GC effluents by olfactometry in triplicate runs for each sample. Volatile identification was done by GC-mass spectrometry and confirmed by sniffing of authentic standards. The same 32 aroma-active compounds were perceived in ‘Fallglo’ and ‘US Early Pride’, of which 25 were identified. Compounds were classified in odor descriptor groups: fatty (10 compounds), plastic or rubber (seven compounds), fruity or citrus (four compounds), floral (four compounds), mushroom (two compounds), green (two compounds) and other (one compound). ‘Fallglo’ and ‘US Early Pride’ had similar aroma intensities for the three first harvests (26 Oct., 3 Nov., 17 Nov.), but ‘Fallglo’ had higher levels of two fruity odorous peaks (*E*-2-pentenal and the coeluting compounds *E*-2-hexenal and ethyl 2 methyl butanoate) than did ‘US Early Pride’ at the December harvest. The last harvest showed significantly higher aroma intensity for six peaks in each cultivar, with only two peaks in common in both cultivars.

Tangerine (or mandarin) fruit are well known for their pleasant aroma and flavor, desirable combination of sugars and acids, and ease of peeling (Miyazaki et al., 2012). All these reasons are possible explanations for the increase in tangerine consumption that occurred during the last decade (House et al., 2011). Most literature on citrus volatiles comes from studies of orange and grapefruit juices, the major products of processed citrus (Tietel et al., 2011a). Over 300 aroma volatiles have been reported from gas chromatography (GC) and GC-mass spectrometry (GC-MS) analyses of fresh orange juices, but fewer than 25 compounds are believed to be aroma-active (Perez-Cacho and Rousseff, 2008). At least 100 volatiles have been identified in the juices of different tangerines such as terpene hydrocarbons, aldehydes, esters, alcohols and ketones (Kerbiriou et al., 2007; Miyazaki et al., 2011; Schieberle et al., 2003). A recent study (Tietel et al., 2011a), gathering the results from 8 reports on fresh tangerine aroma, showed that the most common compounds, found in at least 7 out of the 8 studies are linalool,  $\alpha$ -terpineol, terpinen-4-

ol, nonanal, carvone, decanal, limonene  $\alpha$ -pinene and myrcene. Moshonas and co-workers detected 27 volatiles components in ‘Fallglo’ tangerine juice (Moshonas et al., 1997), but only some of the detected volatiles are likely to have actual aroma activity. While several studies have reported on the aroma-active components in tangerines, there are none that compare ‘Fallglo’ with the newly developed ‘US Early Pride’ cultivar. By having a human subject smelling the effluents of a GC, gas-chromatography-olfactometry (GC-O), aroma-active compounds can be determined. In a recent study, about one third of volatiles detected by GC-MS in tangerine hybrids were perceived in a consensus of three panelists by GC-O (Miyazaki et al., 2012). Most compounds with aroma activity were terpene hydrocarbons, aldehydes and esters. In other studies involving GC-O, 38 aroma-active compounds were detected and identified in ‘Clementine’ (Schieberle et al., 2003), 25 in ‘Kinokuni’ and ‘Satsuma’ mandarins (Miyazawa et al., 2010) and 17 in ‘Mor’ mandarins (Tietel et al., 2011b).

‘Fallglo’ is an early-season tangerine hybrid released by the USDA in 1987 having the following pedigree: [*Citrus reticulata* Blanco  $\times$  (*C. paradisi* Macf.  $\times$  *Citrus reticulata*)  $\times$  ‘Temple’] (Hearn, 1987). ‘Fallglo’ produces early-maturing, attractive, intense orange colored fruit with a peel that is relatively easy to remove along with abundant, colorful, and flavorful juice. ‘Fallglo’ is the earliest maturing tangerine hybrid produced in Florida, typically coming into the market in early October (McCollum and Hearn, 2011). In 2009–2010, it represented 35% of the “early tangerine” class of fresh fruit in Florida (Putnam, 2011). However, the fruit typically contains 30 to 40 seeds. ‘US Early Pride’ is an

This research was supported by Research Grant No. IS-4368-10 from BARD, The United States–Israel Binational Agricultural Research and Development Fund, and grants from the Florida New Varieties Development and Management Corporation. Mention of a trademark or proprietary product is for identification only and does not imply a guarantee or warranty of the product by the U.S. Department of Agriculture. The U.S. Department of Agriculture prohibits discrimination in all its programs and activities on the basis of race, color, national origin, gender, religion, age, disability, political beliefs, sexual orientation, and marital or family status.  
\*Corresponding author; phone: (772) 462-5844; email: anne.plotto@ars.usda.gov

irradiation-induced mutant of 'Fallglo' (McCollum and Hearn, 2011). 'US Early Pride' exhibits the attributes of 'Fallglo', but is distinguished by being low-seeded, with an average of 1.8 seeds per fruit. 'US Early Pride' trees were evaluated for 3 years at the USDA farm in St. Lucie County, FL, and tree size and growth habits of 'US Early Pride' have been similar to 'Fallglo' throughout the evaluation (McCollum and Hearn, 2011).

There are no reports in the literature on which volatiles produced by 'Fallglo' have high aroma activity. Moreover, results of consumer panel evaluations (see companion study in this issue: Biotteau et al., 2012) indicate that 'Fallglo' was perceived to have greater tangerine flavor than did 'US Early Pride' in at least one session. Therefore, the objectives of this study were:

- 1) to identify those volatile compounds that potentially contribute to aroma activity in 'Fallglo' by using GC-O;
- 2) to determine if there are differences in aroma-active compounds between 'Fallglo' and its mutation 'US Early Pride'; and
- 3) to determine if changes of aroma-active volatiles occur over the harvest season.

## Materials and Methods

### Plant material

'Fallglo' and 'US Early Pride' were harvested from mature trees grown at the USDA, ARS, Whitmore farm (Leesburg, FL) on 26 Oct., 3 Nov., Nov. 17, and 1 Dec. of 2011, labeled respectively as H1, H2, H3 and H4. Trees were grown under the same environmental conditions of soil, irrigation and illumination. Each sample was a juice composite from approximately 50 fruit, which was then sub-sampled for the replicated GC-MS and GC-O runs.

### Sample preparation

Fruit were washed with a commercial fruit cleaner (Fruit Cleaner 395, JBT Food Tech, Lakeland, FL, or Sooty Mold Kleen 278, DECCO, Monrovia, CA) and rinsed. Fruit were then sanitized in a stabilized mixture of hydrogen peroxide and peroxy acetic acid (PAA) (Peraclean®, Degussa Canada Inc., Brampton, ON, Canada) for 3 min at 30 to 35 °C and then were dried for 30 min prior to processing in the lab. Individual fruit were manually peeled and halved longitudinally so that one half of each fruit was evaluated by a taste panel (Biotteau et al., 2012), and the other half was juiced manually for quality parameters using a commercial table-top juicer (Model 932, Hamilton-Beach, Washington, NC). Fruit were carefully peeled before juicing to avoid the presence of tangerine peel oil in the samples. This was done because it has been shown that the aroma profile of tangerine peel oil is distinctly different from the tangerine juice (Buettner et al., 2003; Schieberle et al., 2003; Umamo et al., 2002). Therefore the method of sampling chosen in this study represents the volatile profile perceived by consumers when they eat the fruit, rather than when they peel it. For volatile analysis by GC-MS and GC-O, 40 mL of tangerine juice was mixed with 40 mL of a saturated NaCl solution (359 g·L<sup>-1</sup>; 25 °C) to help drive the volatiles into the headspace, to inactivate enzyme activity and to inhibit microbial growth. Six mL of the mixture were placed into 20 mL glass vials capped with magnetic crimp caps containing Teflon-coated septa (Gerstel, Inc., Baltimore, MD) and stored at -20 °C until analyzed.

### Headspace sampling and GC-MS analysis

Extraction of aroma volatiles was performed by the SPME method with an MPS-2 autosampler (Gerstel Inc., Linthicum,

MD). Vials containing juice were incubated at 40 °C for 30 min and then a 2-cm SPME fiber (50/30 µm DVB/CAR/PDMS) was inserted into the headspace of the sample vial and exposed for 60 min. Preliminary experiments with 5-, 15-, 30-, and 60-min exposure times showed that 60-min exposure time generated the most compounds in the terpene and sesquiterpene group, as well as carotenoid-derived volatiles, that were shown in previous studies to be important aroma volatiles compounds in tangerine (Miyazaki et al., 2012). The analytes were thermally desorbed from the SPME fiber in the GC injector (splitless mode) port for 10 min at 250 °C. Separation of volatile compounds was accomplished using an Agilent 6890 GC (Agilent Technologies, Santa Clara, CA) equipped with a DB-5 (60-m length, 0.25-mm i.d., 1.00-µm film thickness; J&W Scientific, Folsom, CA) column, coupled with a 5973N MS detector (Agilent Technologies). The column oven was programmed to increase at 4 °C·min<sup>-1</sup> from the initial 40 °C to 230 °C, then ramped at 100 °C·min<sup>-1</sup> to 260 °C and held for 11.7 min for a total run time of 60 min. Helium was used as carrier gas at flow rate of 1.5 mL·min<sup>-1</sup>. Inlet, ionizing source, and transfer line were kept at 250, 230, and 280 °C, respectively. Mass units were monitored from 40 to 250 m/z and ionized at 70 eV. Data were collected using the ChemStation G1701 AA data system (Hewlett-Packard, Palo Alto, CA). Samples were run in triplicate on a DB-5 column (J&W, Agilent Technologies). A mixture of C-5 to C-15 n-alkanes was run each day under the same conditions to calculate retention indices (RIs).

### Gas chromatography-olfactometry

Extraction and desorption of aroma volatiles into the GC injector was performed by the SPME method using the same parameters as described above for the GC-MS analysis, except that the juice samples were equilibrated in a water bath instead of the autosampler, and a magnetic stir bar was used with constant stirring of the samples. After exposure, the SPME fiber was manually inserted into the injector of a gas chromatograph (Model 7890A GC, Agilent Technologies) to desorb the extract for 10 min at 250 °C. The GC was equipped with an HP-5 (Agilent Technologies) capillary column (30 m length, 0.32 mm i.d., 0.25 µm film thickness) and the column effluent was split (1:2) to a flame ionization detector (FID) and an olfactory detection port (Model ODP-2, Gerstel). The olfactory port was connected to a humidified air make-up (8.9 mL·min<sup>-1</sup>). The oven temperature program was 40 °C for 2 min, then increased to 180 °C at 6 °C·min<sup>-1</sup>, then to 250 °C at 10 °C·min<sup>-1</sup> and then held for 7 min for a total run time of 39.3 min. The carrier gas (He) flow rate was 1.75 mL·min<sup>-1</sup>. RIs of volatile compounds and olfactometry peaks were calculated using a series of n-alkanes (C-5 to C-15) that was run every day under the same chromatographic conditions.

**OSME ANALYSIS.** The time-intensity method Osme was used for olfactory analysis (Le Guen et al., 2000). Training sessions were conducted using tangerine oil to familiarize the two female panelists with the optimum positioning, time intensity device and verbal descriptors. Test samples were analyzed after panelists demonstrated reliable consistency and reproducibility, that was considered reached when the panelists detected at least 80% of the aroma-active compounds in three out of the four last runs of the training. Panelists adjusted their seating positions to evaluate comfortably the GC effluents during the first 30 min of the GC-O runs.

GC-O data were recorded using a computer program written in LabVIEW 8.5 (National Instruments, Austin, TX). Panelists actuated a large slide bar on the computer screen with a mouse to

conveniently report aroma intensity using an 11-points scale (0 = no aroma perceived, 10 = extremely strong). Every 100 ms, the program simultaneously collects graphs and saves voltage data from aroma intensity. This data acquisition rate was sufficiently fast to collect data for the aromagrams. Aroma descriptors were manually written in a notebook. FID output was simultaneously recorded using EZChrom Elite (v. 3.3.2 SP2, Agilent Technologies).

Each sample was evaluated three times per panelist, each replicate representing a different GC run. The samples were run in a random order of presentation to avoid introducing bias into the results. Data processing was as follows:

- 1) For each panelist, select the aroma-active peaks that were detected in at least two out of three replications. To consider the differences of perception thresholds among the panelists, and possible partial anosmia to specific compounds (Plotto et al., 2006), peaks that were detected by only one panelist were kept in consideration.

- 2) Align the selected peaks for the two panelists.

- 3) Calculate the average retention times and RIs for the selected peaks.

- 4) Perform analysis of variance (ANOVA) for maximum odor intensities ( $I_{max}$ ), and area under the curve ( $A_c$ ). Obtain  $I_{max}$  and  $A_c$  averages.

Volatile compounds were identified by their RIs from FID and GC-MS analysis, and confirmed by running authentic standards (Sigma-Aldrich, Milwaukee, WI) on the GC-O and GC-MS. When there was an aroma-active peak detected by panelists, but no compound was detected by the FID or MS detectors, published aroma descriptors (Acree and Arn, 2004; Rychlik et al., 1998) and RIs were used to tentatively identify such compounds, and confirmed by running standards.

Finally, a blank run was performed under the same conditions to assure there was no odorous contaminant from the vial, septum or NaCl solution.

### Statistical analysis

GC-O data were analyzed using XLSTAT, version 2011.1.05 (Addinsoft, Paris, France). Two analyses of variances (ANOVA) were performed for each olfactory peak and for both  $I_{max}$  and  $A_c$ . The first ANOVA was to determine the effect of cultivar on data from each harvest analyzed separately, and the second ANOVA was to determine harvest date effect on each cultivar analyzed separately. The model used was:  $X_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + \epsilon_{ijk}$ , where  $X_{ijk}$  is the perceived intensity of a compound of the  $j$ th cultivar (or harvest) evaluated by the  $i$ th panelist in the  $k$ th replicate,  $\mu$  is the overall mean intensity,  $\alpha_i$  is the effect of the  $i$ th panelist,  $\beta_j$  is the effect of the  $j$ th cultivar (or harvest),  $\alpha_i\beta_j$  is the effect of the interaction between the  $i$ th panelist and the  $j$ th cultivar (or harvest) and  $\epsilon_{ijk}$  is the random residual.

Separation of means was performed with the Tukey's honestly significant difference (HSD) test with  $\alpha < 0.05$ .

A pairwise multiple comparison procedure (Holm-Sidak method) was performed using Sigmaplot, v.11 (Systat Software Inc, Chicago, IL) to analyze the effects of the harvest and the cultivar on the total number of peaks detected in each run.

## Results and Discussion

### Determination of aroma-active peaks

For each harvest of the two cultivars studied, three replicates were done by each panelist in a total of 48 sniffing sessions. The two panelists varied in their perception of volatiles, one panelist

could detect as few as ten, and as many as 21 volatiles per run, with an average of 17 volatiles, while the other panelist detected from 15 to 35 volatiles, with an average of 22 volatiles. A total of 52 aroma-active peaks were detected, though some of them had low intensity and inconsistent detection among the replications per sample. Therefore a consensus among all the replications was built with 32 aroma-active volatiles that were detected at least by one panelist in at least two out of the three replications. Each of the 32 aroma-active volatiles was present in both cultivars. Ten volatiles were described as fatty, seven as plastic or rubber, four as fruity or citrus, four as floral, two as mushroom, two as green and ethanol kept in its own group (Table 1).

Moreover, some volatiles had a relatively high aroma intensity rating that was greater than four in all replications of at least one harvest. These were, as identified by their GC-O retention time in minutes (min) and descriptor: 8.6 min-fatty (Average  $I_{max}$  = 5.38), 11.4 min-mushroom (Average  $I_{max}$  = 5.59) and 11.6 min-metallic, plastic (Average  $I_{max}$  = 7.66) for both cultivars. In addition, the following peaks: 8.1 min-fruity (Average  $I_{max}$  = 3.68), 9.4 min-fatty, cooked vegetables (Average  $I_{max}$  = 4.59) and 17.4 min-fatty, putty (Average  $I_{max}$  = 4.85), were perceived with high intensity in 'Fallglo'.

### Volatiles identification

Out of the 32 aroma-active volatiles, 25 were identified using their RI, GC-MS report and their aroma descriptor (Table 1).

Volatiles identified by GC-MS were *E*-2-pentenal, *E*-2-hexenal and octanal in the fruity group; hexanal,  $\alpha$ -phellandrene and nonanal in the green and floral groups; heptanal,  $\alpha$ -pinene, terpinolene, 1,3,8-p-menthatriene and  $\beta$ -cyclocitral in the fatty group, pentanal,  $\beta$ -myrcene, *p*-cymene and *E*-2-nonanal in the plastic, rubber group (Table 1). They were all confirmed by sniffing standards by GC-O, except 1,3,8-p-menthatriene, not available commercially.

Eight volatiles (ethyl-2-methyl butanoate,  $\beta$ -damascenone, ethyl butanoate,  $\beta$ -ionone, methional, 2,4-nonadienal, 1-octen-3-one and 1-nonen-3-one) were not detected by the GC-MS, but were identified by comparing their RI, the characteristic descriptor from previous publications (Acree et al., 2004; Miyazaki et al., 2012; Rychlik et al., 1998) and sniffing of standards by GC-O (except for  $\beta$ -damascenone and 1-nonen-3-one). The detection thresholds of four of these volatiles,  $\beta$ -damascenone, 2,4-nonadienal, methional, and  $\beta$ -ionone (thresholds of the remaining four volatiles are discussed below) are low (respectively,  $2 \cdot 10^{-6}$ ,  $1 \cdot 10^{-5}$ ,  $2 \cdot 10^{-4}$ , and  $0.205 \mu\text{g}\cdot\text{L}^{-1}$  in water) (van Gemert, 2003). Therefore, even though they were present in the fruit at very low concentrations and they were not detected by the GC-MS, they are still aroma-active. This shows that, in some cases, the human nose can detect aroma-active volatiles at much lower concentrations than an instrument detector.

Two of the volatiles identified by GC-MS had descriptors different than published, and therefore, co-elution with potent aroma-active compounds was suspected. The 11.4 min – mushroom peak had RI of 978. The compound identified by GC-MS with a similar RI was *E*-2-heptenal, but this compound's odor was described as fatty, almond (Rychlik et al., 1998), green, grassy (Miyazaki et al., 2012), and green, vegetable, plant by GC-O of a chemical standard. However, 1-octen-3-one, described as mushroom (Miyazaki et al., 2012; Rychlik et al., 1998) has a RI similar to *E*-2-heptenal, and a lower threshold:  $5 \cdot 10^{-6}$ – $0.004 \mu\text{g}\cdot\text{L}^{-1}$  for 1-octen-3-one vs.  $0.013$ – $0.051 \mu\text{g}\cdot\text{L}^{-1}$  for *E*-2-heptenal (van Gemert, 2003). Therefore, 1-octen-3-one was suspected to

Table 1. Aroma-active volatiles in 'Fallglo' and 'US Early Pride', retention indices (RI) by GC-O, GC-MS and published values, compound identification and references.

Aroma-active peak (Retention time-descriptor)	RI (GC-O)	RI (GC-MS)	RI published (8)	Compound	References
<b>fruity, citrus</b>					
6.0 min - fruity	748	736	754	<i>E</i> -2-pentenal (st)	2,3
8.1 min - fruity, green	840	849	845	<i>E</i> -2-hexenal (st)	1,2,4
	840	-	852 (3)	ethyl 2-methylbutanoate (*st)	2,3,6
12.1 min - citrus	1003	1018	1006	octanal (st)	1,2,3,4
21.5 min - apple, honey	1407	-	1386	$\beta$ -damascenone (*)	3,7
<b>green</b>					
6.8 min - grassy, green, fruity	782	786	801	hexanal (st)	1,2,3,4,6
	782	-	803 (3)	ethyl butanoate (*st)	2,3
14.5 min - apple, green	1110	1122	1105 (3)	dehydro- <i>p</i> -cymene	2,3
<b>floral</b>					
10.2 min - fresh,floral, citrus	927	-	-	unk	3
12.8 min - fresh, floral, minty	1037	1032	1007	$\alpha$ -phellandrene (st)	2,4,5
14.8 min - perfume, sweet, plastic	1122	1127	1104	nonanal (st)	2,4,5
24.0 min - violet	1514	-	1493	$\beta$ -ionone (*st)	2,3
<b>fatty</b>					
4.3 min - chocolate, fatty	677	-	-	unk	
7.3 min - fatty, toast, skunk	805	-	-	unk	
8.6 min - fatty, rubber	859	-	-	unk	
9.4 min - fatty, cooked potatoes, green	893	-	909	methional (*st)	
9.6 min - garbage, cooked vegetables	901	903	903	heptanal (st)	1,2,3,4
10.6 min - fatty, rubber, pungent	944	952	939	$\alpha$ -pinene (st)	1,2,4,5,6
13.5 min - fatty	1067	1054	1115	1,3,8- <i>p</i> -menthatriene	2
14.2 min - toast, fatty	1097	1120	1091 (3)	terpinolene (st)	1,2,3,5
17.4 min - fatty, putty	1233	-	1217	2,4-nonadienal (*st)	3
18.1 min - fatty, putty, rubber	1263	1258	1250	$\beta$ -cyclocitral (st)	2
<b>plastic, rubber</b>					
4.6 min - green, plastic	689	674	732	pentanal (st)	2
6.4 min -green, plastic	766	-	766	unk	
11.6 min - metallic, plastic	986	1003	992	$\beta$ -myrcene (st)	1,2,3,4,5,6
13.1 min - rubber, green	1046	1052	1027	<i>p</i> -cymene (st)	2,3
15.6 min - plastic, green, plant	1165	-	-	unk	3
15.8 min - plastic, pungent	1156	-	-	unk	3
16.3 min - plastic, woody	1186	1185	1162	<i>E</i> -2-nonenal (st)	2,3,6
<b>mushroom</b>					
11.4 min - mushroom	978	-	980	1-octen-3-one (*st)	3,7
14.0 min - mushroom	1088	-	1076	1-nonen-3-one (*)	
<b>other</b>					
2.8 min - ethanol, fresh	614	510	668	ethanol	1,2,3,4,5,6

References: 1) Tietel et al., 2011a; 2) Miyazaki et al., 2011; 3) Miyazaki et al., 2012; 4) Moshonas and Shaw, 1997; 5) Dharmawan et al., 2007; 6) Tietel et al., 2011b; 7) Miyazawa et al., 2010; 8) Acree and Arn, 2004.

(\*), Volatiles not detected on the GC-MS, identified according to RI and aroma descriptor.

(st), Volatiles confirmed with standards

unk, volatiles not identified (unknown).

be the aroma-active compound, even though it was not detected by GC-MS, and it was confirmed by running a chemical standard in a GC-O session. Similarly, the 14.0 min-mushroom peak with RI 1088 was identified by GC-MS as *E*-2-octenal (RI = 1080). But the GC-O of an *E*-2-octenal chemical standard resulted in a fatty odorous peak, not mushroom. At the same RI, 1-nonen-3-one is described as mushroom (Acree and Arn, 2004) and it has a threshold of  $8 \cdot 10^{-9} \mu\text{g}\cdot\text{L}^{-1}$ , much lower than that of *E*-2-octenal ( $3.4 \cdot 10^{-4} - 0.02 \mu\text{g}\cdot\text{L}^{-1}$ ) (van Gemert, 2003). Therefore it is suspected that 1-nonen-3-one is responsible for the mushroom odor, rather than *E*-2-octenal.

Two GC-O peaks were described as “green, grassy” and “fruity”: they were at 6.8 min and 8.1 min (Table 1). The GC-MS identified hexanal and *E*-2-hexenal at these retention times, respectively. However, both of these C-6 aldehydes have only “green,” “grassy” or even “tallow” descriptors (Acree and Arn, 2004; Rychlik et al., 1998). It was suspected that both aldehydes co-eluted with an ester: hexanal with ethyl butanoate and *E*-2-hexenal with ethyl-2-methyl butanoate (Table 1). GC-O of standards confirmed the identities of both esters. The lower thresholds for these esters than for the co-eluting aldehydes explain why they were not detected by GC-MS, but they were perceived at the same time as the aldehydes. The published threshold range of hexanal is  $0.0045 - 0.75 \mu\text{g}\cdot\text{L}^{-1}$  while that of ethyl butanoate is  $10^{-6} - 0.018 \mu\text{g}\cdot\text{L}^{-1}$  (van Gemert, 2003). Likewise, thresholds of the co-eluting volatiles *E*-2-hexenal and ethyl-2-methyl butanoate are  $0.01 - 0.5 \mu\text{g}\cdot\text{L}^{-1}$  and  $8.10^{-5} - 3.10^{-4} \mu\text{g}\cdot\text{L}^{-1}$ , respectively (van Gemert, 2003). These results confirm the power of GC-O when combined with GC-MS to identify important aroma-active volatiles in foods.

Ethanol, hexanal, *E*-2-hexenal,  $\alpha$ -pinene, heptanal, myrcene, octanal,  $\alpha$ -phellandrene, *p*-cymene, nonanal and decanal were reported in previous GC-MS studies concerning ‘Fallglo’ (Moshonas, 1997; Bai, 2011). Moreover, eight other volatiles found in those studies (limonene, copaene, caryophyllene, valencene, ethyl acetate, 1-penten-3-one,  $\beta$ -pinene and 3-carene) were detected by GC-MS in the present study but not detected by GC-O as aroma-active. Furthermore, our results also show the challenges in determining the identities of aroma-active compounds that are not detected by GC-MS. Seven aroma-active volatiles remained unidentified, mostly having “fatty” or “plastic” descriptors.

### Comparison between ‘Fallglo’ and ‘US Early Pride’

Considering the number of peaks detected by GC-O, there were no significant differences between the two cultivars within any harvest, except for the last harvest (Table 2). For H4, the number of peaks detected in ‘Fallglo’ was higher than in ‘US Early Pride’.

The differences between ‘Fallglo’ and ‘US Early Pride’ for each aroma-active compound for all harvests were analyzed (data not shown). The sums of the squared peak height (*I*<sub>max</sub>) differences for each compound between ‘Fallglo’ and ‘US Early Pride’ were respectively 33, 27, 25, and 42 for H1, H2, H3, and H4. The number of significant differences ( $\alpha = 0.05$ ) were three, one, one and four peaks for the harvests in the same order, respectively. Therefore, most differences were in the last harvest, H4 (Table 3).

For H4, out of the 30 aroma-active volatiles identified, 21 volatiles had higher intensities for ‘Fallglo’ and nine had higher intensities for ‘US Early Pride’, but these differences were not all significant (Table 3). Most volatiles of the fruity, fatty and green groups tended to have higher intensity in ‘Fallglo’, while most volatiles of the floral and mushroom groups were perceived with greater intensity in ‘US Early Pride’. Only four volatiles showed significant differences ( $\alpha = 0.05$ ) between the two cultivars. They

Table 2. Average number of aroma-active peaks detected in tangerine samples by GC-O by two panelists in three replicated runs.<sup>z</sup>

Harvest <sup>y</sup>	Fallglo	US Early Pride
H1	18.00 b A	16.67 a A
H2	17.33 b A	18.17 a A
H3	18.83 ab A	19.83 a A
H4	24.67 a A	21.33 a B

<sup>z</sup>Means followed by the same small letter within a column and a capital letter within a row are not significantly different by the Tukey test ( $\alpha < 0.05$ ).

<sup>y</sup>Harvest dates were: H1 = 26 Oct., H2 = 3 Nov., H3 = 17 Nov., H4 = 1 Dec., 2011.

Table 3. Average GC-O peak height (or *I*<sub>max</sub>, mean of two panelists with 3 replications each) for ‘Fallglo’ and ‘US Early Pride’ harvested 1 Dec. 2011 (H4). *P*-value lower than 0.05 indicates significant difference between the two samples.

Aroma-active peak (Retention time-descriptor)	<i>P</i> -value	Fallglo	US Early Pride
<b>fruity, citrus</b>			
6.0 min - fruity	0.022	3.71	1.27
8.1 min - fruity	0.037	6.12	3.89
12.1 min - citrus	0.597	3.68	2.83
21.5 min - apple, honey	0.848	3.26	3.08
<b>green</b>			
6.8 min - grassy, green, fresh	0.454	5.52	4.55
14.5 min - apple, green	0.504	2.90	2.18
<b>floral</b>			
10.2 min - fresh, floral, citrus	0.741	1.82	1.34
12.8 min - fresh, floral, minty*	0.685	1.89	2.39
14.8 min - perfume, sweet, plastic	0.849	2.91	3.17
24.0 min - violet*	0.320	0.73	1.71
<b>fatty</b>			
4.3 min - chocolate, fatty	0.271	2.19	1.29
7.3 min - fatty, toast, skunk	0.470	2.41	1.51
8.6 min - fatty, rubber	0.501	7.76	7.39
9.4 min - fatty, cooked vegetables, green	0.271	6.94	6.07
9.6 min - garbage, cooked vegetables	0.208	5.70	3.17
10.6 min - fatty, rubber, pungent*	0.484	2.78	1.93
13.5 min - fatty	0.243	3.41	1.86
14.2 min - toast, fatty	0.023	3.80	3.20
17.4 min - fatty, putty	0.211	4.39	2.90
18.1 min - fatty, putty, rubber*	0.778	0.92	1.17
<b>plastic, rubber</b>			
4.6 min - green, plastic	0.746	3.74	3.41
6.4 min - green, plastic	0.697	1.30	0.81
11.6 min - metallic, plastic	0.178	8.52	8.91
13.1 min - rubber, green*	0.927	3.92	3.99
15.8 min - plastic, pungent	0.241	3.47	1.37
15.6 min - plastic, green, plant	0.319	2.74	1.44
16.3 min - plastic, woody	0.675	2.89	2.50
<b>mushroom</b>			
11.4 min - mushroom	0.470	4.61	5.57
14.0 min - mushroom	0.000	2.59	4.60
<b>other</b>			
2.8 min - ethanol, fresh	0.447	0.93	1.95
<b>TOTAL Peaks height</b>	0.111	107.55	91.44

\*Detected by only one of the two panelists.

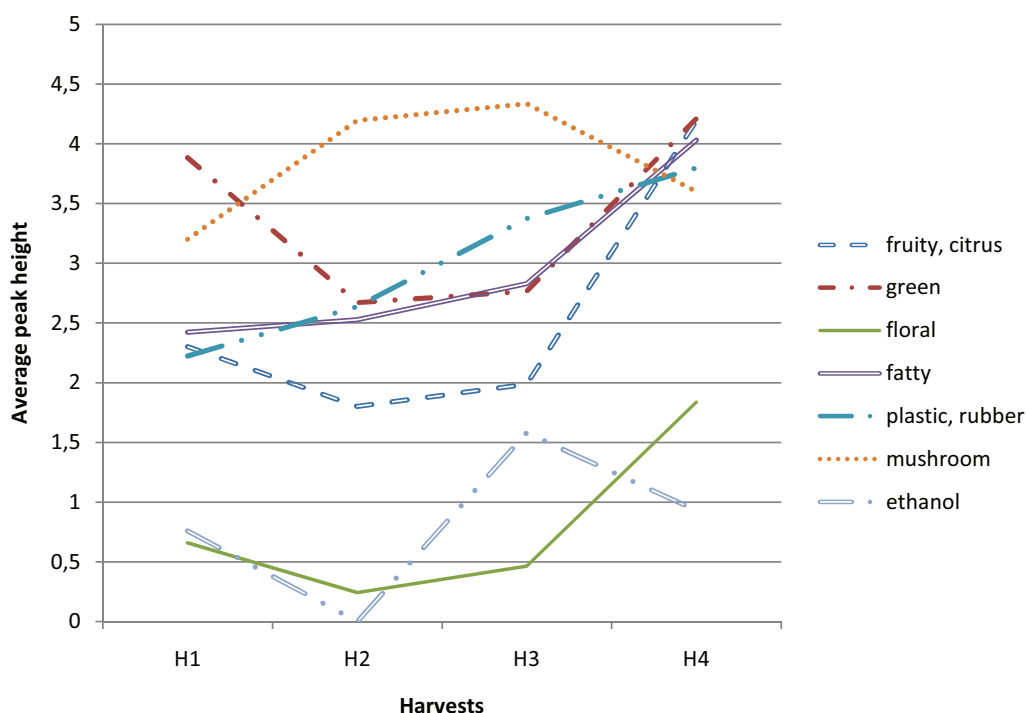


Fig. 1. Average GC-O peaks height for 'Fallglo', over four harvests, the volatiles being organized in groups according to their aroma descriptor. H1 = 26 Oct., H2 = 3 Nov., H3 = 17 Nov., H4 = 1 Dec., 2011.

were: 6.0 min - fruity, 8.1 min - fruity, and 14.2 min - toast, and were higher in 'Fallglo' than in 'US Early Pride', whereas the peak at 14.0 min - mushroom was greater in 'US Early Pride' (Table 3). Those differences were similar for the area under the curve (data not shown) for 6.0 min - fruity, 8.1 min - fruity, and 14.0 min - mushroom.

Differences in aroma-active volatiles found by GC-O could explain the results from a sensory evaluation that was done to compare the preference between 'Fallglo' and 'US Early Pride' for each one of the four harvests (see companion study in this issue - Biotteau et al., 2012). Panelists were asked to choose which of the two samples, 'Fallglo' or 'US Early Pride', they preferred. To explain their choice, one of the options was that the chosen cultivar had a greater tangerine aroma. The answers to this question were not significantly different between 'Fallglo' and 'US Early Pride' for the three first harvests, but for the last harvest, 'Fallglo' was perceived to have a greater tangerine aroma than 'US Early Pride'.

### Changes of Volatiles over Harvest Maturity

'FALLGLO'. The number of peaks in H4 was significantly higher than in H1 and H2 (Table 2). When considering the seven aroma groups (volatiles classified according to their descriptors), we saw that for the fruity-citrus, the floral, the plastic-rubber and the fatty groups, there was a general increasing trend of peak intensity over the harvest season, especially for the last harvest (Fig. 1). Looking at the GC-O peak heights of each compound, there was a trend for 20 volatiles to be higher at the last harvest than at the three earlier harvests (data not shown). The following peaks were significantly higher in H4 than in all of the other harvests: 6.0 min - fruity, 8.1 min - fruity, 9.4 min - fatty, and 4.6 min - green-plastic (Table 4). Furthermore, the 4.3 min - chocolate

Table 4. Average peak height (or I<sub>max</sub>) detected by GC-O by two panelists in three replicated runs<sup>z</sup>, for the four 'Fallglo' harvests.<sup>y</sup>

Aroma-active peak (Retention time-descriptor)	H1	H2	H3	H4
<b>fruity, citrus</b>				
6.0 min - fruity	1.15 b	0.77 b	1.33 b	3.71 a
8.1 min - fruity	2.69 b	2.50 b	3.28 b	6.12 a
<b>fatty</b>				
4.3 min - chocolate, fatty	0.00 b	1.00 ab	1.49 ab	2.19 a
9.4 min - fatty, cooked vegetables, green	2.93 b	3.83 b	3.35 b	6.94 a
10.6 min -fatty, rubber, pungent	1.50 ab	0.00 b	1.16 ab	2.78 a
13.5 min - fatty	0.00 b	2.46 ab	1.94 ab	3.41 a
<b>plastic, rubber</b>				
4.6 min - green, plastic	0.57 b	1.44 b	1.85 b	3.74 a

<sup>z</sup>Means followed by the same small letter within a row are not significantly different by the Tukey test ( $\alpha < 0.05$ ).

<sup>y</sup>Harvest dates were: H1 = 26 Oct., H2 = 3 Nov., H3 = 17 Nov., H4 = 1 Dec., 2011.

and 13.5 min - fatty peaks were significantly higher in H4 than in H1, and the 10.6 min - rubber peak was significantly higher in H4 than in H2. There were no significant differences in any compound between the three first harvests.

'US EARLY PRIDE'. Considering the seven aroma groups (volatiles classified according to their descriptor), there was a general increasing trend of peak intensities over the harvest season, except for the green group and the ethanol, which decreased first then increased back to the initial level (Fig. 2). For

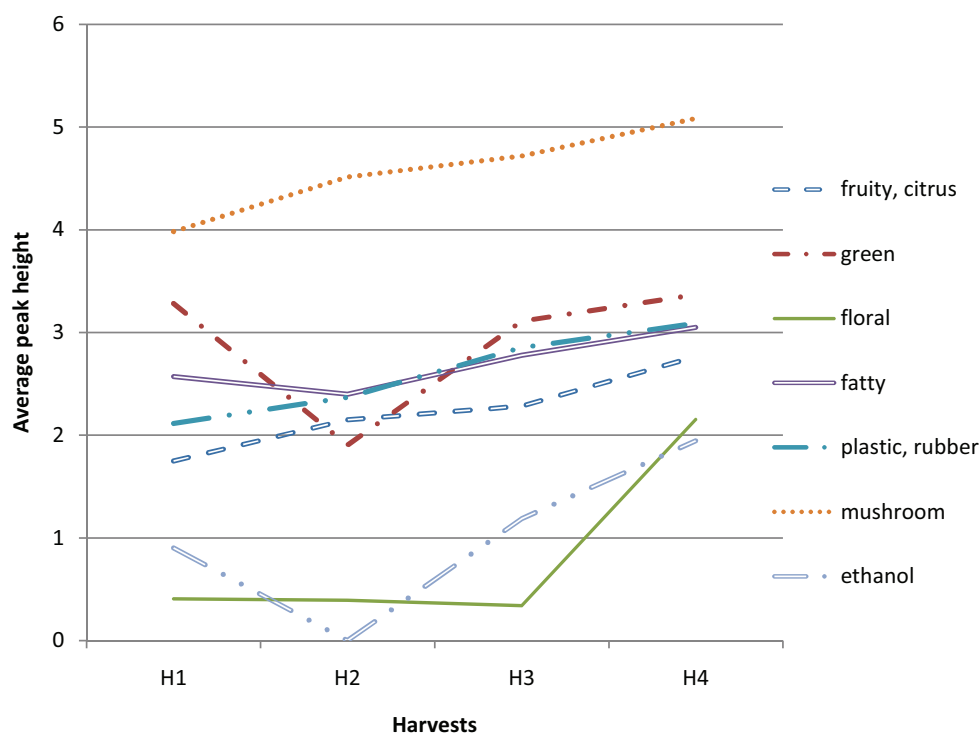


Fig. 2. Average GC-O peaks height for 'US Early Pride', over four harvests, the volatiles being organized in groups according to their aroma descriptor. H1 = 26 Oct., H2 = 3 Nov., H3 = 17 Nov., H4 = 1 Dec., 2011.

Table 5. Average peak height (or I<sub>max</sub>) detected by GC-O by two panelists in three replicated runs, for the four 'US Early Pride' harvests.<sup>y</sup>

Aroma-active peak (Retention time-descriptor)	H1	H2	H3	H4
<b>floral</b>				
12.8 min - fresh, floral, minty	0.00 b	0.45 b	0.00 b	2.39 a
14.8 min - perfume, sweet, plastic	0.31 b	0.93 b	0.48 b	3.17 a
24.0 min - violet	0.42 ab	0.00 b	0.28 ab	1.71 a
<b>fatty</b>				
8.6 min - fatty, rubber	3.60 b	6.04 ab	4.19 b	7.39 a
9.4 min - fatty, cooked vegetables, green	5.80 a	3.05 b	4.33 ab	6.07 a
<b>plastic, rubber</b>				
4.6 min - green, plastic	0.40 b	1.94 ab	2.58 ab	3.41 a
13.1 min - rubber, green	0.85 b	2.41 ab	3.52 ab	3.99 a

<sup>z</sup>Means followed by the same small letter within a row are not significantly different by the Tukey Test ( $\alpha < 0.05$ ).

<sup>y</sup>Harvest dates were: H1 = 26 Oct., H2 = 3 Nov., H3 = 17 Nov., H4 = 1 Dec., 2011.

compound-by-compound GC-O peak height (I<sub>max</sub>), there was a trend for 18 volatiles to be higher for the last harvest than for the three others (data not shown). Twenty-three volatiles did not differ significantly among the harvests concerning the maximum intensity. Two peaks, one at 12.8 min - fresh, floral and one at 14.8 min - perfume, sweet, were significantly higher in H4 than in all the other harvests (Table 5). Furthermore, the two "plastic, rubber" peaks: 4.6 min - green, plastic and 13.1 min - rubber,

green, were higher in H4 than in H1; the 8.6 min - fatty peak was higher in H4 than in H1 and H3; the 24.0 min - violet was significantly higher in H4 than in H2; and the 9.4 min - fatty, cooked vegetables peak was significantly higher in H1 and H4 than in H2 (Table 5).

Comparing the two cultivars, 'Fallglo' had two more fatty and fruity peaks with greater intensities at H4 than 'US Early Pride', and 'US Early Pride' had three more floral and one more plastic/rubber peaks with greater intensities at H4 (Tables 4 and 5). The two fruity peaks might be of particularly importance to explain the differences in tangerine flavor perceived by consumer panelists (Biotteau et al., 2012). Indeed, they have high (3.71) and very high (6.12) intensities at the last 'Fallglo' harvest; and they were significantly higher in 'Fallglo' than in 'US Early Pride' at H4 (Table 3) but not in the previous harvests (data not shown). Even though 'Fallglo' was perceived to have "more tangerine flavor" at H4, it could also be a result of slightly over mature fruit, as expressed by higher BrimA index (Biotteau et al., companion study). It was found that descriptors such as "sulfury" and "pumpkin/vegetable" were more pronounced when tangerines passed their optimum maturity (Plotto et al., 2011). 'US Early Pride' meets minimum maturity requirements two weeks later than 'Fallglo', usually by the fourth week of October in Florida, and can be harvested as late as the end of November (McCollum and Hearn, 2011). The differences observed for the last harvest (1 Dec.) might be a consequence of over ripeness for 'Fallglo' fruit.

## Conclusion

This study identified 30 aroma-active GC-O peaks in two cultivars, 'Fallglo' and its seedless mutation 'US Early Pride'.



All of them were present in both cultivars, and at all harvests. Most of those peaks were described as fatty, plastic or metallic, green, floral, fruity and mushroom. GC-MS combined with RIs, aroma descriptors and standards identified 17 volatiles, whereas eight volatiles, having very low thresholds and low concentrations in the cultivars studied were identified using their RIs, aroma descriptor and standards. Seven volatiles remain unknown. All the volatiles identified, except two (methional, and 1-nonen-3-one) were found in previous tangerine volatiles studies. The results for the first three harvests were consistent, showing no significant differences within one cultivar (except one fatty peak for 'US Early Pride'). Further, there was no compound that had consistently greater or lesser intensity in 'Fallglo' or in 'US Early Pride' throughout the study. Therefore, we can conclude that, at their optimum of maturity, the two cultivars are not significantly different concerning their aroma-active compounds.

### Literature Cited

- Acree, T. and H. Arn. 2004. Flavornet and human odor space. <<http://www.flavornet.org/index.html>>. Accessed 13 June 2012.
- Bai, J., E.A. Baldwin, and A. Plotto. 2011. Stability of headspace volatiles in a 'Fallglo' tangerine juice–NaCl matrix system at room temperature. *Proc. Fla. State Hort. Soc.* 124:207–212.
- Biotteau, A., C. Leclair, A. Plotto, and G.T. McCollum. 2012. A comparative study of 'Fallglo' and its seedless mutation 'US Early Pride' tangerines by sensory evaluation. *Proc. Fla. State Hort. Soc.* 125:272–275.
- Buettner, A., M. Mestres, A. Fischer, J. Guasch, and P. Schieberle. 2003. Evaluation of the most odour-active compounds in the peel oil of Clementines (*Citrus reticulata* Blanco cv. Clementine). *Eur. Food Res. Technol.* 216:11–14.
- Dharmawan, J., S. Kasapis, P. Curran, and J. Jonhson. 2007. Characterization of volatile compounds in selected citrus fruits from Asia. Part I: freshly squeezed juice. *Flavour Fragr. J.* 22:228–232.
- Hearn, C.J. 1987. The 'Fallglo' citrus hybrid in Florida. *Proc. Fla. State Hort. Soc.* 100:119–121.
- House, L.A., Z. Gao, T.H. Spreen, F.G. Gmitter Jr., M.F. Valim, A. Plotto, and E.A. Baldwin. 2011. Consumer preference for mandarins: Implications of a sensory analysis. *Agribusiness: An Intl. J.* 27:450–464.
- Kerbiriou, P., A. Plotto, K. Goodner, E. Baldwin, and F. Gmitter. 2007. Distribution of aroma volatiles in a population of tangerine hybrids. *Proc. Fla. State Hort. Soc.* 120:267–275.
- Le Guen, C., C. Prost, and M. Demaimay. 2000. Critical comparison on three olfactometric methods for the identification of the most potent odorants in cooked mussels (*Mytilus edulis*). *J. Agr. Food Chem.* 48:1307–1314.
- McCollum, T. and J. Hearn. 2011. 'US Early Pride', a very low-seeded, early-maturing mandarin hybrid. *HortScience* 46(12):1695–1697.
- Miyazaki, T., A. Plotto, K. Goodner, and F. Gmitter. 2011. Distribution of aroma volatile compounds in tangerine hybrids and proposed inheritance. *J. Sci. Food Agr.* 91:449–460.
- Miyazaki, T., A. Plotto, E. Baldwin, J. Reyes-de-Corcuera, and F. Gmitter. 2012. Aroma characterization of tangerine hybrids by gas-chromatography–olfactometry and sensory evaluation. *J. Sci. Food Agr.* 92:727–735.
- Miyazawa, N., A. Fujita, and K. Kubota. 2010. Aroma character impact compounds in Kinokuni mandarin orange (*Citrus kinokuni*) compared with Satsuma mandarin orange (*Citrus unshiu*). *Biosci. Biotechnol. Biochem.* 74:835–842.
- Moshonas, M. and P. Shaw 1997. Quantification of volatile constituents in Mandarin juices and its use for comparison with orange juices by multivariate analysis. *J. Agr. Food Chem.* 45:3968–3972.
- Perez-Cacho, P. and R. Rousseff. 2008. Fresh squeezed orange juice odor: A review. *Crit. Rev. Food Sci. Nutr.* 48:681–695.
- Plotto, A., E. Baldwin, G.T. McCollum, and F.G. Gmitter, Jr. 2011. Sensory evaluation of tangerine hybrids at multiple harvests. *Proc. Fla. State Hort. Soc.* 124:260–263.
- Plotto, A., K.W. Barnes, and K.L. Goodner. 2006. Specific anosmia observed for  $\beta$ -ionone, but not for  $\alpha$ -ionone: Significance for flavor research. *J. Food Sci.* 71(4):S1–S6.
- Putnam, A.H. 2011. Florida Agricultural Statistics Service, Florida Citrus statistics. 2009–2010.
- Rychlik, M., P. Schieberle, and W. Grosch. 1998. Compilation of odor thresholds, odor qualities and retention indices of key food odorants. Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München.
- Schieberle, P., M. Mestres, and A. Buettner. 2003. Characterization of aroma compounds in fresh and processed mandarin oranges, p. 162–174. In: K.R. Cadwallader and H. Weenen (eds.). *Freshness and shelf life of foods*. Vol. 863. Amer. Chem. Soc., Washington, DC.
- Tietel, Z., A. Plotto, E. Fallik, E. Lewinsohn, and R. Porat. 2011a. Taste and aroma of fresh and stored mandarins. *J. Sci. Food Agr.* 91:14–23.
- Tietel, Z., R. Porat, K. Weiss, and D. Ulrich. 2011b. Identification of aroma-active compounds in fresh and stored 'Mor' mandarins. *Intl. J. Food Sci. Tech.* 46:2225–2231.
- Umamo, K., Y. Hagi, and T. Shibamoto. 2002. Volatile chemicals identified in extracts from newly hybrid citrus Dekopon (Shiranhui mandarin). *J. Agr. Food Chem.* 50:5355–5359.
- Van Gemert, L.J. 2003. Odour thresholds. Compilation of odour threshold values in air, water and other media. Oliemans Punter and Partners, Utrecht, The Netherlands.