

# Characterization of the Most Odor-Active Volatiles in Fresh, Hand-Squeezed Juice of Grapefruit (*Citrus paradisi* Macfayden)

Andrea Buettner and Peter Schieberle\*

Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München, Lichtenbergstrasse 4, D-85748 Garching, Germany

By application of the aroma extract dilution analysis on an extract prepared from fresh grapefruit juice, 37 odor-active compounds were detected in the flavor dilution (FD) factor range of 4–256 and subsequently identified. Among them the highest odor activities (FD factors) were determined for ethyl butanoate, *p*-1-menthene-8-thiol, (*Z*)-3-hexenal, 4,5-epoxy-(*E*)-2-decenal, 4-mercapto-4-methylpentane-2-one, 1-heptene-3-one, and wine lactone. Besides the 5 last mentioned compounds, a total of 13 further odorants were identified for the first time as flavor constituents of grapefruit. The data confirmed results of the literature on the significant contribution of 1-*p*-menthene-8-thiol in grapefruit aroma but clearly showed that a certain number of further odorants are necessary to elicit the typical grapefruit flavor.

**Keywords:** Grapefruit; flavor; 4-mercapto-4-methylpentane-2-one; 1-heptene-3-one; wine lactone; grapefruit; aroma extract dilution analysis

## INTRODUCTION

The characteristic flavors of fresh citrus juices attract consumers all over the world. Consumer demand for the availability of citrus juices throughout the year has led to the development of sophisticated technologies for juice processing. However, although much care is taken by the citrus industry to use more careful techniques, the flavor of a manufactured juice generally differs from that of a freshly hand-squeezed juice.

Orange juice is the major citrus product, but grapefruit juice is becoming more and more popular. Due to its bitterness and higher acidity, grapefruit juice has a harsher flavor than orange juice and, furthermore, the fruity note is less pronounced (Shaw, 1991).

The first studies on the volatile constituents of grapefruit were performed before gas chromatography was introduced into flavor analysis (Kirchner and Miller, 1953; Kirchner et al., 1953). In an extract from 2760 gal of fresh grapefruit, these authors identified limonene and  $\alpha$ -pinene as the main volatile constituents followed by  $\beta$ -caryophyllene and its oxide. In lower quantities, citral, carvone, linalol, carveol,  $\alpha$ -terpineol, geraniol, and hex-3-en-1-ol were detected.

Further comprehensive qualitative and quantitative studies on the volatile constituents of grapefruit juice volatiles or essential oil compounds, respectively, have been performed by Shaw and his group (Moshonas and Shaw, 1971, 1984; Coleman et al., 1972; Wilson and Shaw, 1978, 1980), and to date >260 volatile constituents have been identified as recently summarized by Nijssen et al. (1996).

It is well accepted that, in general, only a limited number of volatiles contribute to the overall food aroma [cf. review by Schieberle (1995)], and it has always been a challenge for flavor chemists to identify character

impact odorants of a given food. In grapefruit juice two compounds, namely, nootkatone (MacLeod et al., 1964) and *p*-1-menthene-8-thiol (Demole et al., 1982), have been identified and proposed to make significant contributions to the juice aroma. The thiol has a very low odor threshold of  $0.1 \times 10^{-9}$  g/L (Demole et al., 1982), and, because it occurs in a 200-fold higher concentration in the juice, a significant aroma contribution is very probable. Nootkatone, however, was shown to be present in grapefruit juice at only 8-fold above its odor threshold in water (Pino et al., 1986). The latter authors also calculated the odor activity values (OAV; ratio of concentration to odor threshold) for 32 other known volatile constituents of grapefruit juice. They reported that among them, ethyl butanoate, ethyl acetate, limonene, methyl butanoate, and acetaldehyde showed the highest OAVs. *p*-1-Menthene-8-thiol was, however, not taken into account in their studies. Shaw et al. (1980) found that in a fresh grapefruit juice, hydrogen sulfide is another potent flavor contributor, because it was present in concentrations >1000-fold above its odor threshold in air. In addition, methyl sulfide was suggested as a further probable odor contributor.

In many studies performed in the literature steam distillation of the juice was used to isolate the volatile fraction, and it has been claimed that this technique might have led to artifact formation (Cadwallader and Xu, 1994). Therefore, these authors isolated the volatiles from a fresh grapefruit juice by purging the headspace above the juice onto a cryotrap. Twenty-three volatiles were identified, of which 2 components, namely, methyl acetate and propyl acetate, were newly identified in grapefruit. Both, however, were excluded from contribution to the grapefruit aroma because their concentrations did not exceed their odor thresholds. Furthermore, neither nootkatone nor *p*-1-menthene-8-thiol could be detected using this technique (Cadwallader and Xu, 1994).

\* Author to whom correspondence should be addressed (telephone +49-89-289 132 65; fax +49-89-289 141 83; e-mail Peter.Schieberle@lrz.tu-muenchen.de).

**Table 1. Most Odor-Active Volatiles (FD  $\geq$  4) in Freshly Squeezed Grapefruit Juice (White Marsh Seedless)**

no. <sup>a</sup>	odorant <sup>b</sup>	odor quality <sup>c</sup>	CC fraction <sup>d</sup>	RI on			FD factor <sup>e</sup>	earlier identified as volatile in grapefruit <sup>f</sup>
				FFAP	OV-1701	SE-54		
1	ethyl acetate	fruity	2	888	<600	<600	8	[1]
2	ethyl propanoate	fruity	2	951	765	714	8	[2]
3	ethyl 2-methylpropanoate	fruity	2	955	812	751	64	
4	butane-2,3-dione	buttery	3	970	692	<600	8	
5	( <i>R</i> )- $\alpha$ -pinene	pine tree	1	1010	945	929	32	[3]
6	1-penten-3-one <sup>h</sup>	etheral, pungent	3	1026	765	683	32	[2]
7	ethyl butanoate	fruity	2	1028	856	802	256	[1]
8	( <i>S</i> )-ethyl 2-methylbutanoate	fruity	2	1041	907	845	32	[4]
9	hexanal	green	3	1072	882	800	16	[1]
10	( <i>Z</i> )-3-hexenal	green, leaf-like	3	1135	882	798	256	
11	myrcene	moss-like	1	1154	1020	989	32	[5]
12	( <i>R</i> )-limonene	citrus-like	1	1188	1054	1025	32	[3]
13	1-hepten-3-one <sup>h</sup>	geranium-like	2	1190	954	886	256	
14	2- <i>s</i> and 3-methylbutanol	malty	5	1211	835	739	32	[1]
15	ethyl hexanoate	fruity	2	1226	1058	1002	16	
16	octanal	green, citrus-like	3	1279	1087	1000	32	[1]
17	1-octen-3-one <sup>h</sup>	mushroom-like	3	1292	1067	986	64	
18	1,5( <i>Z</i> )-octadien-3-one <sup>h</sup>	geranium-like	3	1367	1081	982	64	
19	4-mercapto-4-methylpentan-2-one <sup>h</sup>	black currant-like		1377	1053	937	128	
20	nonanal	soapy, citrus-like	3	1383	1193	1102	32	[2]
21	3-isopropyl-2-methoxypyrazine <sup>h</sup>	earthy, beany	3	1427	1143	1092	64	
22	methional <sup>h</sup>	cooked potato	3	1449	1040	900	32	
23	acetic acid	sour, pungent	AF	1450	781	610	16	
24	decanal	green, soapy	3	1493	1290	1204	4	[2]
25	( <i>Z</i> )-2-nonenal <sup>h</sup>	fatty, green	3	1500	1252	1143	32	
26	( <i>E</i> )-2-nonenal	fatty, tallowy	3	1530	1278	1157	32	
27	linalol <sup>g</sup>	flowery	4	1537	1194	1100	32	[3]
28	<i>p</i> -1-menthene-8-thiol <sup>g,h</sup>	grapefruit-like	4	1598	1348	1283	128	[6]
29	butanoic acid	sweaty, rancid	AF	1622	996	821	8	
30	phenylacetaldehyde	honey-like	3	1639	1175	1050	4	[4]
31	2- <i>s</i> and 3-methylbutanoic acid	sweaty	AF	1660	1030	875	16	
32	ethyl 3-hydroxyhexanoate <sup>g</sup>	fruity, sweet	5	1674	1245	1134	32	[1]
33	4,5-epoxy-( <i>E</i> )-2-decenal <sup>h</sup>	metallic	3	2000	1552	1380	128	
34	4-hydroxy-2,5-dimethyl-3( <i>2H</i> )-furanone <sup>g</sup>	caramel-like	AF	2024	1242	1062	16	
35	3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3 <i>H</i> )-benzofuranone (wine lactone) <sup>g,h</sup>	sweet, spicy	3	2220	1687	1456	128	
36	nootkatone <sup>g</sup>	grapefruit-like	3	2515	2006	1814	8	[7]
37	vanillin <sup>h</sup>	vanilla-like	5	2567	1638	1397	8	

<sup>a</sup> Compound numbers correspond with peak numbering in Figure 1. <sup>b</sup> The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on three stationary phases given in the table (FFAP, OV-1701, SE-54), mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port. <sup>c</sup> Odor quality perceived at the sniffing port. <sup>d</sup> Fraction in which most of the compound appeared after separation in an acidic fraction and fractions 1–5, respectively, after additional column chromatography of the neutral/basic fraction on silica gel. <sup>e</sup> Flavor dilution (FD) factor determined in extracts containing the juice volatiles. Analyses were performed by two assessors in duplicates. The data differed to not more than two FD factors. <sup>f</sup> Reported in the literature as volatile compound in grapefruit juice: [1] Moshonas and Shaw (1971); [2] Coleman et al. (1972); [3] Kirchner and Miller (1953); [4] Nunez et al. (1986); [5] Hunter and Brogden (1965); [6] Demole et al. (1982); [7] MacLeod and Buigues (1964). <sup>g</sup> The stereochemistry was not determined. <sup>h</sup> The MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote *b*.

By sniffing of serial dilutions of flavor extracts using techniques such as CHARM analysis, the aroma extract dilution analysis (AEDA), or static headspace olfactometry [SHO; cf. review by Schieberle (1995)], single volatiles in food extracts can be ranked according to their odor potencies. By application of the AEDA on a fresh, hand-squeezed orange juice, we could recently identify the most-odor active compounds in the juice (Hinterholzer and Schieberle, 1998). However, to date, such methods have not been systematically applied to fresh, hand-squeezed grapefruit juice. Using AEDA and SHO, the purpose of the following investigation was, therefore, to characterize the most odor-active compounds in a fresh grapefruit juice.

#### EXPERIMENTAL PROCEDURES

Fresh grapefruit (*Citrus paradisi* Macf. cv. White Marsh seedless) grown in the United States were purchased at a local market.

**Chemicals.** 1-Hepten-3-ol was obtained from Aldrich (Steinheim, Germany), (*Z*)-3-hexenol was from Fluka (Buchs, Switzerland). The reference compounds of the odorants listed in Table 1 were obtained from the suppliers given in parentheses: **1–3, 5, 9, 11, 12, 14–16, 21–24, 26, 31, 32,** and **34** (Aldrich, Steinheim, Germany); **4, 29,** and **37** (Merck, Darmstadt, Germany); **7** (Fluka, Neu-Ulm, Germany); **17** and **36** (Lancaster, Mühlheim, Germany); **20** and **36** (Roth, Karlsruhe, Germany); **27** (EGA, Steinheim, Germany); **30** (Sigma, München, Germany). For chiral analysis the following reference compounds were used: (*R*)-limonene, (*S*)-limonene, (*R*)-( $\alpha$ )-pinene, (*S*)-( $\alpha$ )-pinene (Aldrich, Steinheim, Germany). The enantiomerically pure (*S*)-ethyl 2-methylbutanoate was synthesized according to the procedure of Fuhrmann and Grosch (unpublished methods).

**Syntheses.** 1-Hepten-3-one and (*Z*)-3-hexenal were prepared by oxidation of 1-hepten-3-ol and (*Z*)-3-hexen-1-ol using Dess–Martin periodinane following the general procedure reported by Fielder and Rowan (1995).

The following compounds were synthesized according to the literature given in parentheses: *p*-1-menthene-8-thiol (Hinter-

holzer and Schieberle, 1998); 1,5-(*Z*)-octadien-3-one (Ullrich and Grosch, 1988a); 4-mercapto-4-methylpentan-2-one (Guth, 1997); (*Z*)-2-nonenal (Ullrich and Grosch, 1988b); 4,5-epoxy-(*E*)-dec-2-enal (Schieberle and Grosch, 1991); and 3a,4,5,7-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (Guth, 1996).

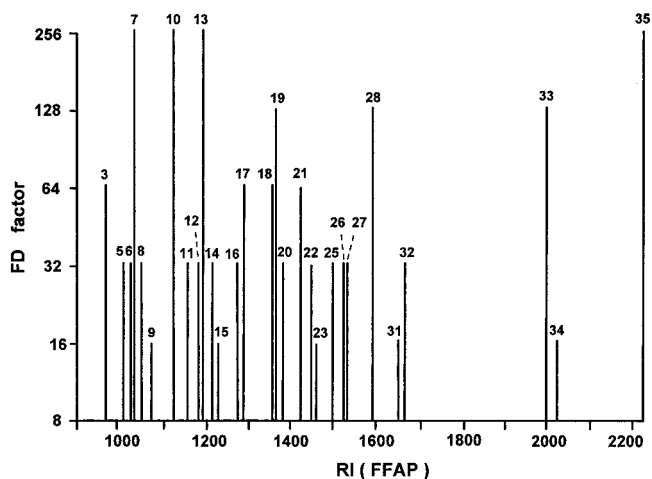
**Isolation of the Juice Volatiles.** Fresh grapefruit juice (600 mL) obtained by careful hand-squeezing of six fruits in a kitchen juicer was immediately poured into an aqueous saturated CaCl<sub>2</sub> solution (600 mL) to inhibit enzymic reactions (Buttery et al., 1987) and finally adjusted to the original pH of the juice (pH 3.5). The aqueous mixture was extracted with diethyl ether (500 mL) for 6 h in a liquid-liquid extractor (500 mL; Normag, Hofheim, Germany); the extract was dried for 12 h over Na<sub>2</sub>SO<sub>4</sub> and finally concentrated to 100 mL by distilling off the solvent on a Vigreux column (50 × 1 cm) at 38 °C. The volatile fraction was isolated by high-vacuum distillation using the apparatus described previously (Sen et al., 1991). For AEDA, the distillate was concentrated at 38 °C to 400 μL by using a Vigreux column and by microdistillation (Schieberle, 1991a,b). One microliter of this extract was used for HRGC/olfactometry.

**Enrichment of Odorants for Identification.** For the identification experiments the juice volatiles were isolated from 3 L of juice as described above. Acidic volatiles were isolated by treatment of the aroma distillate with aqueous sodium bicarbonate (Schieberle, 1991a,b). The organic phase containing the neutral and basic compounds was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to 1 mL. The solution was applied onto a water-cooled (10–12 °C) brown glass column (20 × 1 cm i.d.) filled with a slurry of silica gel 60 [7% water, purified according to the method of Esterbauer (1968)] in *n*-pentane and was separated into five fractions (fraction 1–5) using the solvent mixtures described recently (Hinterholzer and Schieberle, 1998). Each fraction was concentrated at 38 °C to ~100 μL and analyzed by HRGC/O and HRGC/MS.

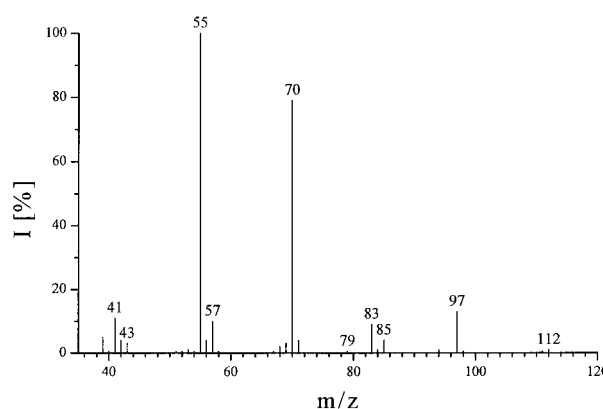
**High-Resolution Gas Chromatography (HRGC)/Olfactometry (O) and HRGC/Mass Spectrometry (MS).** HRGC was performed with a type 8000 gas chromatograph (Fisons Instruments, Mainz, Germany), using the following fused silica capillaries: free fatty acid phase (FFAP; 30 m × 0.32 mm i.d., 0.25 μm df; Chrompack, Mülheim, Germany), SE-54 (30 m × 0.32 mm i.d., 0.25 μm df; J&W Scientific, Fisons Instruments), and OV-1701 (30 m × 0.32 mm i.d., 0.25 μm df; Chrompack). The samples were applied by the cold on-column injection technique at 35 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (SE-54, OV-1701) or 60 °C (FFAP), respectively, held for 2 min isothermally, then raised at 6 °C/min to 180 °C, and finally raised at 10 °C/min to 230 °C and held for 10 min. The flow rate of the carrier gas helium was 2.5 mL/min. At the end of the capillary, the effluent was split 1:1 (by volume) into an FID and a sniffing port using two deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). The FID and the sniffing port were held at 220 and 240 °C, respectively. Linear retention indices (RI) of the compounds were calculated using a series of *n*-alkanes (Halang et al., 1978). MS analysis was performed with an MS 8230 (Finnigan MAT, Bremen, Germany) in tandem with the capillaries described above. Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV with isobutane as the reactant gas.

**AEDA.** The FD factors of the odor-active compounds were determined by AEDA (Schieberle, 1995) of the following dilution series: The original extract (400 μL) from 600 mL of fresh juice was stepwise diluted with diethyl ether (1 + 1) until no odorant was detectable by sniffing of the highest dilution. HRGC/O was performed with aliquots (0.5 μL) using capillary FFAP. A total number of three experienced sniffers was used to perform the AEDA experiments. Their response (sensitivity) to the individual compounds did not differ by >2 FD factors. In Figure 1 the averaged data are displayed.

**Chiral Analysis.** The chiral analysis of limonene, α-pinene, and ethyl 2-methylbutanoate was performed by HRGC/FID and HRGC/MS using the cyclodextrine capillaries described recently (Hinterholzer and Schieberle, 1998). The compounds



**Figure 1.** Flavor dilution chromatogram obtained by applying the AEDA on an extract prepared from freshly squeezed grapefruit juice. Numbers correspond to Table 1.



**Figure 2.** Mass spectrum of 1-hepten-3-one.

were separated into their enantiomers without derivatization, and the order of elution was assigned by using optically pure reference compounds.

## RESULTS AND DISCUSSION

The volatiles from 600 mL of a freshly hand-squeezed grapefruit juice were isolated by solvent extraction at room temperature followed by high-vacuum distillation of the extract at 25 °C and 6 mPa. Sniffing of an aliquot of the extract on a strip of filter paper evoked the characteristic odor of grapefruit.

By application of HRGC/O on an aliquot of the flavor extract equal to ~2 mL of fresh juice (600 mL of juice → 300 μL of distillate → 1 μL for HRGC/O), 29 odor-active regions were detectable in the FD factor range of 16–256 (Figure 1). A great variety of odor qualities, such as fruity, green, or citrus-like, were perceived; however, with the elution of compound **28**, a clear grapefruit-like odor was detected. To rank the odorants according to their odor potencies, the AEDA was applied. Sniffing of serial dilutions of the extract revealed the highest FD factors for compounds **7** (Figure 1; fruity), **10** (green), and **13** (geranium-like). Somewhat lower FD factors were found for the black currant-like smelling compound **19**, the grapefruit-like smelling compound **28**, the metallic smelling odorant **33**, and the sweet spicy smelling compound **35**.

To isolate enough material for the identification experiments, the volatile fraction from 3 L of juice was isolated and the compounds were enriched by column

chromatography. Of the seven primary odorants showing FD factors of 256 and 128, respectively, compounds **7** and **10** could readily be identified by MS-EI and MS-CI as ethyl butanoate (**7**, Table 1) and (*Z*)-3-hexenal (**10**), the latter being identified for the first time as a grapefruit constituent. The geranium-like smelling compound **13** could be enriched by silica chromatography in a fraction containing long-chain aldehydes and ketones. However, it was not possible to obtain an unequivocal mass spectrum. Because its retention index on the SE-54 column was by 100 units lower than that of 1-octen-3-one (**17**; Table 1) and by 200 units higher than that of 1-pentene-3-one (**6**), we proposed the structure of compound **13** as 1-hepten-3-one. This assumption was confirmed by comparing the retention indices on the three different stationary HRGC phases and the odor quality and odor intensity at the sniffing port with that of the synthesized reference compound. This procedure allowed the identification of **13** as 1-hepten-3-one (Table 1). By the same approach, compound **33** was identified as 4,5-epoxy-(*E*)-2-decenal. The series of homologous epoxyalkenals needed for this approach had been synthesized previously by us (Schieberle and Grosch, 1991).

On the basis of its retention index and odor quality, compound **35** was proposed to be identical with 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (wine lactone), previously identified by us among the most important odorants of freshly squeezed orange juice (Hinterholzer and Schieberle, 1998). By comparing the sensory properties and the retention data of compound **35** on three columns with those of the reference compound, the structure could be unambiguously assigned.

The intense grapefruit-like odor of compound **28** suggested its structure as 1-*p*-menthene-8-thiol, previously reported by Demole et al. (1982) as a character impact odorant in grapefruit juice. Synthesis of the reference compound, which is commercially not available, and comparison of its odor quality and odor threshold in the sniffing port as well as the retention indices on three columns confirmed the structure given for **28** in Table 1.

The intensely black currant-like smelling compound **19** was not present among the key odorants of orange juice (Hinterholzer and Schieberle, 1998), and there was no hint in the literature that this odorant had previously been identified in grapefruit juice.

Several thiols have been reported to elicit black currant-like odors at the sniffing port, such as 3-mercapto-3-methylbutyl formate (Schieberle, 1991a,b), 4-methoxy-2-mercapto-2-methylpentane (Guth and Grosch, 1991), or 4-mercapto-4-methylpentan-2-one (Guth, 1997).

Again, comparison of the odor quality and odor threshold in the sniffing port as well as the retention indices on three stationary phases with those of reference odorants led to confident identification of compound **19** as 4-mercapto-4-methylpentan-2-one (Table 1).

The data clearly show that retention indices are a very helpful tool to identify, in particular, flavor compounds with extremely low odor thresholds. However, it has to be pointed out that in the identification experiments the reference compound always has to be used to confirm, especially, the sensory properties of the odorant present in the food extract. For example,

if a food flavor compound will give a very intense odor without any peak monitored by the FID, the reference compound has to show the same low odor threshold in air.

In total, all 37 odorants detected by AEDA in the FD factor range 4–256 could be identified. The results of the identification experiments are summarized in Table 1. Besides the very potent aroma compounds (*Z*)-3-hexenal, 1-hepten-3-one, 4-mercapto-4-methylpentan-2-one, 4,5-epoxy-(*E*)-2-decenal, and the wine lactone, a total of 13 further odorants are reported for the first time as grapefruit constituents (Table 1). Among them, 1-octen-3-one, 1,5(*Z*)-octadien-3-one, 3-isopropyl-2-methoxy-pyrazine, methional, (*Z*)-2-nonenal, and vanillin are further odorants showing high odor activities, although no signal was displayed by the FID (data not shown). This is probably why these compounds have not been reported in previous investigations on grapefruit volatiles performed without the application of GC/O.

## CONCLUSIONS

Results of the application of the AEDA on an extract prepared from freshly squeezed grapefruit juice corroborated the significance of *p*-1-menthene-8-thiol and ethyl butanoate for the grapefruit aroma. However, our data suggest that, in particular, (*Z*)-3-hexenal, 1-hepten-3-one, 4-mercapto-4-methylpentan-2-one, and the wine lactone are further, but previously unknown, contributors to the grapefruit flavor. A comparison with recent data on freshly squeezed orange juice (Hinterholzer and Schieberle, 1998) reveals that the major parts of the key odorants in both juices are identical, but some show significant differences in their FD factors. It is worthwhile to mention that the key grapefruit odorants 1-hepten-3-one and 4-mercapto-4-methylpentan-2-one were not present among the orange juice odorants [cf. Hinterholzer and Schieberle (1998)]. On the other hand, each key odorant (FD  $\geq$  64) in oranges was also present in grapefruit.

To elucidate which compounds are in fact responsible for the flavor differences between fresh orange juice and fresh grapefruit juice, quantitative studies and flavor recombination experiments are necessary further steps.

## LITERATURE CITED

- Cadwallader, K. R.; Xu, Y. Analysis of volatile compounds in fresh grapefruit juice by purge and trap/gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 782–784.
- Coleman, R. L.; Lund, E. D.; Shaw, P. E. Analysis of Grapefruit Essence and Aroma Oils. *J. Agric. Food Chem.* **1972**, *20*, 100–103.
- Demole, E.; Enggist, P.; Ohloff, G. 1-*p*-Menthene-8-thiol: a powerful flavor impact constituent of grapefruit juice (*Citrus paradisi* Macfayden). *Helv. Chim. Acta* **1982**, *65*, 1785–1794.
- Esterbauer, H. On the autoxidation of methyl linoleate in water. III. Chromatographic separation of water-soluble reaction products. *Fette Seifen Anstrichm.* **1968**, *70*, 1–8.
- Fielder, S.; Rowan, D. D. The Synthesis of 3,4-<sup>2</sup>H<sub>2</sub>-3*Z*-Hexenal and 6,6,6-<sup>2</sup>H<sub>3</sub>-3*Z*-Hexenal. *J. Labelled Compd. Radiopharm.* **1995**, *36*, 465–470.
- Guth, H. Determination of the configuration of wine lactone. *Helv. Chim. Acta* **1996**, *79*, 1559–1571.
- Guth, H. Identification of character impact odorants in different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022–3026.

- Guth, H.; Grosch, W. A comparative study of the potent odorants of different virgin olive oils. *Fat Sci. Technol.* **1991**, *93*, 335–339.
- Halang, W. A.; Langlais, R.; Kugler, E. Cubic spline interpolation for the calculation of retention indices in temperature-programmed gas–liquid chromatography. *Anal. Chem.* **1978**, *50*, 1809–1832.
- Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J.* **1998**, *13*, 49–55.
- Hunter, G. L. K.; Brogden, W. B. Analysis of the terpene and sesquiterpene hydrocarbons in some citrus oils. *J. Food Sci.* **1965**, *30*, 383–387.
- Kirchner, J. G.; Miller, J. M. Volatile Oil Constituents of Grapefruit Juice. *J. Agric. Food Chem.* **1953**, *1*, 512–518.
- Kirchner, J. G.; Miller, J. M.; Rice, R. G.; Keller, G. J.; Fox, N. M. Volatile water-soluble constituents of grapefruit juice. *J. Agric. Food Chem.* **1953**, *1*, 510–512.
- MacLeod, W. D.; Buigues, N. M. Sesquiterpenes. I. Nootkatone, a new grapefruit flavor constituent. *J. Food Sci.* **1964**, *29*, 565–568.
- Moshonas, M. G.; Shaw, P. E. Analysis of volatile flavor constituents from grapefruit essence. *J. Agric. Food Chem.* **1971**, *19*, 119–120.
- Moshonas, M. G.; Shaw, P. E. Direct gas chromatographic analysis of aqueous citrus and other fruit essences. *J. Agric. Food Chem.* **1984**, *32*, 526–530.
- Nijssen, L. M.; Visscher, C. A.; Maarse, H.; Willemsen, L. In *Volatile Compounds in Foods*; TNO Nutrition and Food Research Institute: Zeist, The Netherlands, 1996.
- Nunez, A. J.; Maarse, H. Headspace methods for volatile components of grapefruit juice. *Chromatographia* **1986**, *21*, 44–48.
- Pino, J.; Torricellea, R.; Örsi, F. Correlation between sensory and gas-chromatographic measurements on grapefruit juice volatiles. *Nahrung* **1986**, *30*, 783–790.
- Schieberle, P. Primary odorants in popcorn. *J. Agric. Food Chem.* **1991a**, *39*, 1141–1144.
- Schieberle, P. Primary odorants of pale Lager beer. *Z. Lebensm. Unters. Forsch.* **1991b**, *193*, 558–565.
- Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Gaonkar, A. G., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1995; pp 403–431.
- Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb—Differences to the crust and effect of a longer dough fermentation. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 130–135.
- Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of  $\beta$ -damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757–759.
- Shaw, P. E. Fruits II. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 305–327.
- Shaw, P. E.; Ammons, J. M.; Braman, R. S. Volatile sulfur compounds in fresh orange and grapefruit juices: identification, quantitation, and possible importance to juice flavor. *J. Agric. Food Chem.* **1980**, *28*, 778–781.
- Ullrich, F.; Grosch, W. Identification of the most intense odour compounds formed during autoxidation of methyl linolenate at room temperature. *J. Am. Oil Chem. Soc.* **1988a**, *65*, 1313–1317.
- Ullrich, F.; Grosch, W. Flavour deterioration of Soya-bean oil: Identification of intense odour compounds formed during flavour reversion. *Fat Sci. Technol.* **1988b**, *9*, 332–336.
- Wilson, C. W.; Shaw, P. E. Quantitative composition of cold-pressed grapefruit oil. *J. Agric. Food Chem.* **1978**, *26*, 1432–1434.
- Wilson, C. W.; Shaw, P. E. Glass capillary gas chromatography for quantitative determination of volatile constituents in cold-pressed grapefruit oil. *J. Agric. Food Chem.* **1980**, *28*, 919–922.

Received for review January 27, 1999. Accepted October 22, 1999.

JF990071L