Analysis of Volatile Components in Fresh Grapefruit Juice by Purge and Trap/Gas Chromatography†

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Qualitative and quantitative analyses of volatile flavor components in fresh grapefruit juice were performed by purge and trap/gas chromatography/mass spectrometry (P&T/GC/MS) and P&T/GC with flame-ionization detection (FID). Seventeen compounds were positively identified. Four additional compounds were tentatively identified on the basis of either MS or retention index (RI) data. Qauntification of positively identified components was done by P&T/GC/FID using butyl acetate as internal standard and calibration curves. Compounds not previously identified in grapefruit juice were methyl acetate, propyl acetate, and 2-methylethyl propionate (tentatively identified).

INTRODUCTION

Extensive research efforts have been directed toward determining identities and quantities of volatile components that are important contributors to natural grapefruit aroma (Moshonas and Shaw, 1971; Radford et al., 1974; Shaw et al., 1980; Wilson and Shaw, 1980; Nunez et al., 1984, 1985). In those studies, the analytical procedures involved distillation of the juice with subsequent organic solvent extraction and concentration of the extract prior to gas chromatographic (GC) analysis. These steps can introduce artifacts or otherwise alter the delicate volatile composition of the juice. Several alternative procedures have been employed for the analysis of orange juice in order to minimize compositional changes. Moshonas and Shaw (1987) analyzed orange juice by direct-injection gas chromatography but were unable to completely eliminate qualitative and quantitative flavor changes induced by heating of the juice during vacuum distillation. Equilibrium headspace methods, which are of low sensitivity and are generally limited to the analysis of highly volatile compounds, have been used with limited success for the analysis of orange juice flavor (Marsili, 1986; Lum et al., 1990; Nisperos-Carriedo and Shaw, 1990; Paik and Venables, 1991). Marsili et al. (1989) demonstrated that many important flavor-impacting components could be analyzed using a simple pentane extraction/gas chromatographic technique; however, this method was unsuitable for measuring compounds more volatile than octanal and α-pinene due to their loss during the pentane evaporation step. None of the above methods are applicable to the wide range of flavor components found in either fresh orange or grapefruit juice. Purge and trap/gas chromatography (P&T/GC) analysis offers the possibility of increased sensitivity, while at the same time allowing for analysis of components with widely varying volatilities. Furthermore, the likelihood of sample alteration is minimized when cryogenic trapping/flash heated injection is employed.

This paper describes the use of a P&T/GC method for the identification and quantification of volatile components in fresh grapefruit juice. In order to assure highest accuracy, an internal standard was added to fresh juice to correct for variations in analyte recovery. Sample temperature was maintained at 40 °C during purging to minimize artifact formation and other component changes.

MATERIALS AND METHODS

Materials. Fresh grapefruit (Marsh Ruby) were purchased from a local market. Authentic standard compounds were purchased from Aldrich Chemical Co. (St. Louis, MO).

Juice Extraction. Fresh juice was hand-extracted using a domestic reamer-type juicer (Proctor-Silex, Baltimore, MD). Seeds and coarse pulp were removed by filtration through two layers of cheesecloth. Fresh juice was immediately subjected to analysis as described below.

Purge and Trap/Gas Chromatography/Mass Spectrometry (P&T/GC/MS). A Chrompack purge and trap injector (Chrompack, Inc., Raritan, NJ) was used to purge volatile compounds from juice samples (7 mL each) for subsequent cryogenic trapping into a deactivated fused silica open tubular (FSOT) trap. Trapped volatiles were then thermally desorbed and analyzed by GC/MS. P&T conditions were as follows: purge time, 10 min; helium purge flow, 10 mL/min; precondenser temperature, −15 °C; cryogenic trap temperature, −120 °C (5-min precool time); trap material, deactivated 0.32-mm-i.d. × 11-cm FSOT column; sample temperature, 40 °C; desorption oven temperature, 50 °C; injection temperature, 200 °C; and injection time, 10 min. The −15 °C precondenser was used to trap the bulk of the liquid matrix, i.e., water, which could eventually block the cryogenic trap, reducing sampling efficiency. The GC/MS system consisted of an HP 5790 GC/HP 5970B mass-selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). Separations were performed on a Supelcowax 10 FSOT column (60-m length × 0.25-mm i.d. × 0.25-μm df; Supelco, Inc., Bellefonte, PA). Injector block temperature was maintained at 200 °C. Helium was used as carrier gas at 25 cm/s. Oven temperature was programmed from 40 to 80 °C at a rate of 6 °C/min with an initial hold time of 6 min; oven temperature was then further increased to 200 °C at a rate of 15 °C/min with a final hold time of 10 min. MSD conditions were as follows: capillary direct interface temperature, 200 °C; ion source temperature, 200 °C; ionization voltage, 70 eV; mass range 33–300 amu; electron multiplier voltage, 2200 V; and scan rate, 1.6 scans/s.

Compound Identification. Peak identifications were based on GC retention indices (RI) (van den Dool and Kratz, 1963) and mass spectra of unknowns compared with those of authentic standard compounds under identical conditions. Tentative identifications were based on standard MS library information (Hewlett-Packard Co., 1988) or by RI comparison with standard compounds.

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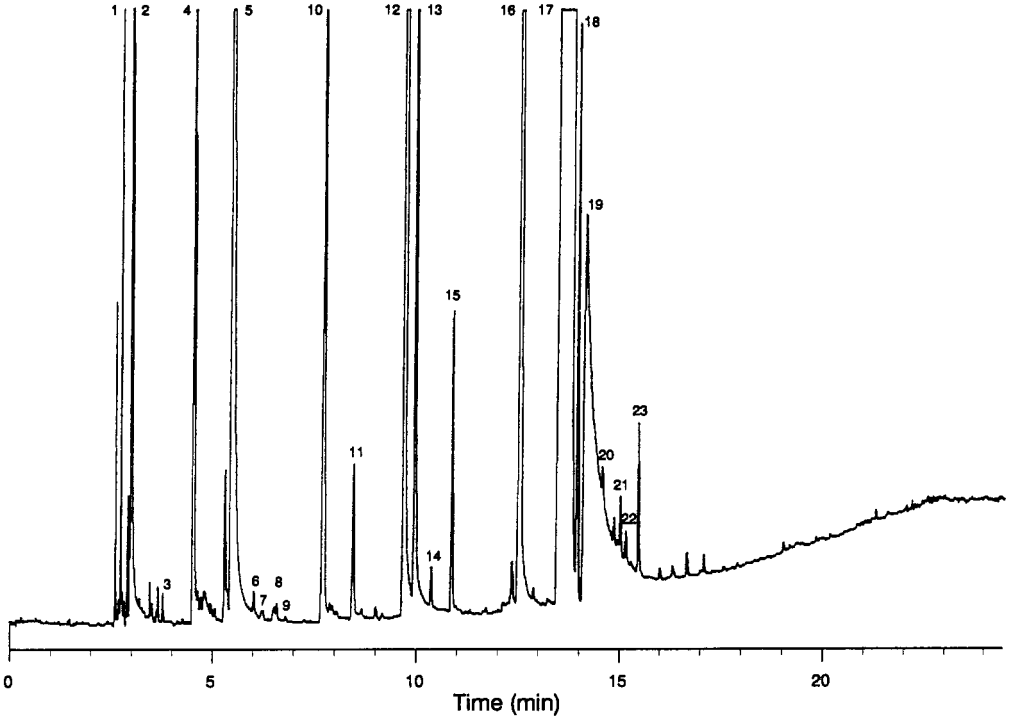

Figure 1. Typical purge and trap gas chromatogram of fresh grapefruit juice flavor volatiles purged at 40 °C.

Quantitative Analysis. Fresh juice was divided into three 100.0-mL aliquots. Each aliquot was spiked with 0.1 μL of butyl acetate as internal standard (IS) using a series 7101 syringe (Hamilton Co., Reno, NV) and stored at 0 °C until analyzed. Each juice sample was analyzed in triplicate, with all analyses being completed within 10 h of sample preparation. P&T/GC conditions were the same as given previously except for the following: GC system consisted of an HP 5790 GC (Hewlett-Packard) equipped with an FID and a 60-m × 0.32-mm-i.d. × 0.25-μm df Supelcowax 10 FSOT column; the sample consisted of 2 mL of juice plus 5 mL of deionized/distilled water; and helium linear velocity was 40 cm/s. The FID temperature was 250 °C, and the amplitude range was set for high sensitivity. Data were recorded and analyzed using an HP 3396A integrator (Hewlett-Packard). The concentration of each component was calculated using calibration curves of analyte/IS area (or peak height) ratio versus concentration of analyte. In order to achieve more accurate quantitative results, standard solutions were prepared in grapefruit juice matrix (GJM) which contained nonvolatile materials of fresh juice. GJM was prepared by reconstituting grapefruit juice concentrate previously prepared from fresh grapefruit juice by vacuum evaporation (55–60 °C, −100 kPa) from 750 to 150 mL using a Büchi Rotavapor (Switzerland). GJM was determined to be essentially volatile free by P&T/GC with only a trace of limonene being detected. Standard solutions were prepared by dilution of a standard stock solution in grapefruit juice matrix (GJM) as follows: 1:10, 1:25, 1:50, 1:100, and 1:200. Each standard solution was analyzed in duplicate following the addition of 0.1 μL of butyl acetate. Standard stock solution consisted of 100 mL of GJM spiked with the following standard amounts: acetaldehyde, 15.8 mg; methyl acetate, 9.3 μg; ethyl acetate, 90 μg; ethanol, 200 mg; ethyl propionate, 8.9 μg; propyl acetate, 8.4 μg; methyl butyrate, 9.0 μg; α-pinene, 43 μg; ethyl butyrate, 22 μg; hexanal, 83 μg; β-pinene, 8.6 μg; sabinene, 21 μg; β-myrcene, 40 μg; limonene, 8.4 mg; *trans*-2-hexenal, 85 μg; ethyl hexanoate, 87 μg; and τ-terpinene, 8.5 μg. All standard solutions were vigorously shaken to facilitate emulsion formation since some of the standard compounds were present at concentrations exceeding their solubility in GJM.

RESULTS AND DISCUSSION

A typical purge and trap/gas chromatogram of fresh grapefruit juice sample is presented in Figure 1. Of 21 components detected, 17 were positively identified and quantified. Most of the volatile components identified in the present study have been identified previously in citrus juice, essence oil, and aroma. Concentrations of the majority of the compounds identified in this study have not been previously reported for grapefruit juice; however, comparative data exists for fresh orange juice. Limonene, ethanol, acetaldehyde, and ethyl acetate were the major volatile components in fresh grapefruit juice (Table 1). The ethanol and acetaldehyde content was lower than previously reported for fresh orange juice (Lum et al., 1989; Nisperos-Carriedo and Shaw, 1990); however, their con-

Table 1. Volatile Flavor Compounds in Fresh Grapefruit Juice

peak no.	compd name	RI[a]	mean area ratio[b]	concn (ppm)	% RSD[c]
1	methanol[d]	514	0.085	ND[f]	79
2	acetaldehyde	541	1.05	5.4	7.2
3	methyl acetate	628	0.0048	0.026	6.1
4	ethyl acetate	709	0.678	1.65	6.6
5	ethanol	811	0.821	66.0	38
6	ethyl propionate	874	0.0036[e]	0.0068	8.6
7	2-methylethyl propionate[g]	896	0.0014[e]	ND	19
8	propyl acetate	939	0.0031[e]	0.0065	16
9	methyl butyrate	965	0.00091[e]	0.0019	16
10	α-pinene	1016	0.355	0.054	12
11	ethyl butyrate	1038	0.052	0.033	4.9
12	butyl acetate (IS)[h]	1073			
13	hexanal	1081	0.255	0.50	5.5
14	β-pinene	1093	0.013	0.0015	15
15	sabinene	1107	0.089	0.017	18
16	β-myrcene	1155	1.42	0.36	8.0
17	limonene	1190	58.9	9.9	8.6
18	β-phellandrene[g]	1195	0.149	ND	5.1
19	limonene (ghost peak)				
20	*trans*-2-hexenal	1217	0.0043[e]	0.033	36
21	ethyl hexanoate	1235	0.048	0.042	25
22	τ-terpinene	1242	0.022	0.0027	36
23	β-ocimene[g]	1254	0.046	ND	5.9

[a] RI, retention index. [b] Mean area ratio, compound peak area/IS peak area. [c] % RSD, percent relative standard deviation (n = 9). [d] Compound tentatively identified by RI only. [e] Mean height/area ratio, compound peak height/IS peak area. [f] ND, not determined. [g] Compound tentatively identified by MS only. [h] IS, internal standard.

centrations would be expected to vary with fruit maturity. Concentrations of limonene and other essential oil components, such as β-myrcene, sabinene, γ-terpinene, and α- and β-pinene, were in the range reported for hand-squeezed orange juice (Moshonas and Shaw, 1986, 1987; Nisperos-Carriedo and Shaw, 1990). Two additional essential oil components, tentatively identified as β-phellandrene and β-ocimene, also were detected. The concentration of ethyl acetate was similar to that previously reported for fresh orange juice (Lum et al., 1990; Nisperos-Carriedo and Shaw, 1990).

Three components, methyl acetate, 2-methylethyl proprionate (tentatively identified), and propyl acetate, have not been previously reported in fresh grapefruit juice. Methyl acetate and propyl acetate were present at concentrations below their aroma thresholds (Devos et al., 1990); therefore, they may not significantly impact the flavor of fresh grapefruit juice. The difficulty in earlier detection of these components may be due to their high volatility and low concentration, resulting in their loss during sample preparation. For the same reason, the percent relative standard deviations (%RSD) for most components were rather high, especially for the more polar components, such as methanol and ethanol. %RSD values for nonpolar components, such as esters and monoterpene hydrocarbons, were comparatively lower. Lower RSD values associated with the nonpolar constituents may be because they are more readily purged from the aqueous juice matrix, whereas polar components are less effectively purged possibly due to van der Waals forces. There are no previous reports regarding standard deviation ranges for the volatile components in grapefruit juice.

The experimental results indicated that the P&T technique is suitable for the qualitative and quantitative analyses of fresh grapefruit juice. The major advantage of the technique is its higher sensitivity, requiring only 2 mL of sample, and its ability to accurately measure highly volatile components, such as acetaldehyde. Since this technique did not require sample preparation strategies such as distillation and solvent extraction, volatile component changes or losses were minimized. Using an internal standard technique allowed for accurate quantification of volatile components. The method is versatile and may be applied to substances other than grapefruit juice, e.g., orange juice, milk, etc. One potential drawback to this method is that components having higher vapor pressures, such as the oxygenated mono- and sesquiterpenes were not effectively purged and thus could not be determined. This means that nootkatone, an important grapefruit flavor component (Boelens and Valverde, 1988), was not detected. Another limitation of the method is that volatile sulfur compounds, such as hydrogen sulfide, methyl sulfide, and 1-*p*-menthene-8-thiol which are thought to contribute to the flavor of fresh grapefruit juice (Shaw et al., 1980; Demole et al., 1982), were not detected. However, P&T/GC may be adapted for the analysis of such components if a sulfur-selective detector (e.g., flame photometric detector) is employed.

LITERATURE CITED

Boelens, M. H.; Valverde, F. Aroma chemicals and citrus oils. *Perfumer Flavorist* **1988**, *13*, 2–12.

Demole, E.; Enggist, P.; Ohloff, G. 1-p-Menthene-8-thiol: a powerful flavor-impact constituent of grapefruit juice (*Citrus paradisi* Macfadyen). *Helv. Chim. Acta* **1982**, *65*, 1785–1792.

Devos, M.; Patte, F.; Rouault, J.; Laffort, P.; Van Gemert, L. J. *Standardized Human Olfactory Thresholds*; Oxford University Press: New York, 1990; pp 104, 148.

Hewlett-Packard Co. Wiley/NBS Database (PBM-Format), Palo Alto, CA, 1988.

Lum, O. L.; Wong, M. K.; Lee, C. K. A simple headspace-gas chromatographic method for the quantitative determination of organic volatiles of fresh orange juice. *Food Chem.* **1990**, *37*, 313–317.

Marsili, R. Measuring volatiles and limonene-oxidation products in orange juice by capillary GC. *LC-GC* **1986**, *4*, 358–362.

Marsili, R.; Kilmer, G.; Miller, N. Quantitative analysis of orange-oil components in orange juice by a simple solvent extraction-gas chromatographic procedure. *LC-GC* **1989**, *7*, 778–783.

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. Quantities of volatile flavor components in aqueous orange essence and fresh orange juice. *Food Technol.* **1986**, *40* (11), 100–103.

Moshonas, M. G.; Shaw, P. E. Quantitative analysis of orange juice flavor volatiles by direct-injection gas chromatography. *J. Agric. Food Chem.* **1987**, *35*, 161–165.

Nisperos-Carriedo, M. O.; Shaw, P. E. Volatile flavor components of fresh and processed orange juices. *Food Technol.* **1990**, *44* (4), 134–138.

Nunez, A. J.; Bemelmans, J. M. H.; Maarse, H. Isolation methods for the volatile components of grapefruit juice. Distillation and solvent and extraction methods. *Chromatographia* **1984**, *18*, 153–158.

Nunez, A. J.; Maarse, H.; Bemelmans, J. M. H. Volatile flavour components of grapefruit juice (*Citrus paradisi* Macfadyen). *J. Sci. Food Agric.* **1985**, *36*, 757–763.

Paik, J. S.; Venables, A. C. Analysis of packaged orange juice volatiles using headspace gas chromatography. *J. Chromatogr.* **1991**, *540*, 456–463.

Radford, T.; Kawashima, K.; Friedel, P. K.; Pope, L. E.; Gianturco, M. A. Distribution of volatile compounds between the pulp and serum of some fruit juices. *J. Agric. Food Chem.* **1974**, *22*, 1066–1070.

Shaw, P. E.; Ammons, J. M.; Braman, R. S. Volatile sulfur compounds in fresh orange and grapefruit juices: identification, quantification, and possible importance to juice flavor. *J. Agric. Food Chem.* **1980**, *28*, 778–781.

van den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

Wilson, C. W. III; 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.

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