Differences of Volatile and Nonvolatile Constituents between Mature and Ripe Guava (*Psidium guajava* Linn) Fruits

Charng-Cherng Chyau,* Shu-Yueh Chen, and Chung-May Wu

Food Industry Research and Development Institute (FIRDI), P.O. Box 246, Hsinchu 300, Taiwan, Republic of China

During the ripening of guava (*Psidium guajava* L.) fruits, the contents of total pectin, total sugars, reducing sugars, and acidity dropped obviously from the mature to the ripe stage, but the Brix-acid ratio increased inversely. Volatile constituents of mature and ripe guava fruits were identified by GC, GC/MS, and GC/FT-IR. A total of 34 components were identified, in which 17 components were further identified by authentic compounds. In quantitative distribution, total amounts of 134 mg/kg of mature fruit and 93 mg/kg of ripe fruit were determined. The major constituents in mature fruit were 1,8-cineole, (*E*)-2-hexenal, and (*E*)-3-hexenal. Ethyl hexanoate and (*Z*)-3-hexenyl acetate were the major volatile components of ripe fruit.

INTRODUCTION

Guava (*Psidium guajava* L.) is an important cultivated species of the Myrtle family. In Taiwan, the round-oval fruit with white flesh was harvested for processing about 3 months after blooming. During ripening, the color of the peel changed from green during the maturing stage to light yellow during the ripening stage, and the characteristic flavors were formed gradually. Although some studies about guava volatiles have been published (Stevens et al., 1970; Torline and Ballschmieter, 1973; Wilson and Shaw, 1978; Idstein and Schrier, 1985; Nishimura et al., 1989; Vernin et al., 1991; Ekundayo and Ajani, 1991), most were undertaken to analyze the volatiles and to evaluate the characteristic compounds only in the pink and white flesh fruit. Stevens et al. (1970) characterized C_6 alcohols and aldehydes as the major volatiles in guava puree. Wilson and Shaw (1978) reported β-caryophyllene as the highest single component in the hydrocarbon fraction. Recently, Vernin et al. (1991) indicated that (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, pentan-2-one, cinnamyl alcohol, 3-phenylpropyl acetate, and 3-phenylpropyl alcohol were the major constituents in guava aroma of fruits from Egypt. The objective of this study was to further evaluate the volatile constituents and the relationship of the proximate composition between the mature and ripe guava fruits.

EXPERIMENTAL PROCEDURES

Fruits. Fresh mature and ripe guava fruits (white flesh) were picked from the same bushes grown south of Taiwan. The fruits were stored at −30 °C before quality analysis. Otherwise, the aroma isolation was carried out within 24 h after harvest.

Sample Preparation. Guava puree was prepared by blending guava fruit with distilled water (1:4 w/w) in a Waring blender and was homogenized for 3 min.

Quality Analysis. Total acidity was determined according to the AOAC (1984) method. Five grams of guava puree was put into a 250-mL Erlenmeyer flask, into which 125 mL of distilled water and three drops of 1% phenolphthalein (E. Merck) solution were further added. This was titrated with 0.1 N sodium hydroxide until the mixture changed to a pink color (the endpoint). Total acidity was determined as anhydrous citric acid and was expressed as percent by weight. Soluble solids were determined by hand refractometer at 25 °C and were expressed as Brix. Isolation of total pectin was according to the method of Ishii and Yokotsuka (1972). Four grams of guava puree and 16 mL of 99.9% ethanol were added, and the mixture was stirred for 3 min. The jellified materials were collected by centrifugation at 10000*g* for 10 min, washed twice with 75% (v/v) ethanol, and then dissolved in 100 mL of distilled water. Five milliliters of 0.1 N sodium hydroxide was added to 0.5 mL of this solution and allowed to stand for 1 h; 0.5 mL of this mixture was taken for pectin content assay according to the *m*-hydroxydiphenyl (*m*-phenylphenol, Tokyo Kasei) method (Blumenkrantz and Asboe-Hanson, 1973). The isolation of sugars was carried out according to an AOAC (1984) method, and the reducing sugars were determined according to the DNS (dinitrosalicylic acid, Sigma) method (Rick and Stegbauer, 1974). Fifty grams of the above guava puree was added to 150 mL of 80% ethanol and blended for 1 min, and then 80% ethanol was added to make 250 mL and allowed to stand for 1 h. After this solution was filtered through Whatman No. 4 filter paper, neutral lead acetate solution was added to 20 mL of this filtrate until no more precipitation was found again. The suspension of sugar solution was collected by centrifugation at 3000*g* for 20 min and was made up to 50 mL by adding distilled water. Two milliliters of DNS solution was added to 2 mL of this solution for the determination of reducing sugar. Absorption measurements were taken at 545 nm by using a Perkin-Elmer Junior III spectrometer. The reducing sugar content was calculated by using glucose (0–1 mg) as standard.

Isolation of Volatiles. A total of 400 g fruit and 400 mL of distilled water was blended in a Waring blender for 1 min. After the internal standard (dimethyl octanyl acetate, 1.147 mg) was added to this aliquot, the pulps were immediately subjected into a rotary evaporator for vacuum distillation (45 °C, 20 mbar). Approximately 400 mL of distillate was collected by passing a −10 °C condenser and three liquid nitrogen traps. The distillates were combined and extracted with 40 mL of *n*-pentane (99%, E. Merck, glass distilled)/methylene chloride (99.5%, E. Merck, glass distilled) (2:1 v/v) once and 15 mL for another four times (Idstein and Schreier, 1985; Leahy and Reineccius, 1984). The extract was preconcentrated with a distillate apparatus at 40 °C (Fischer Co., type MRHA 500C) and carefully reconcentrated to approximately 50 μL by using a 10 cm × 0.2 cm i.d. Vigreux column at 40 °C.

Table I. Quality Measurement of Mature and Ripe Fruits

measure items	mature fruits	ripe fruits
diameter of fruits, cm	5.0–5.4	5.9–6.1
average weight of fruits, g	91.97	123.33
total pectin,[a] %	3.40	0.67
reducing sugars,[a] %	3.66	2.90
total sugars,[a] %	5.62	4.68
acidity,[a] % (as citric acid)	0.48	0.31
Brix-acid ratio[a]	14.20	20.00
pH[a]	4.33	4.48

[a] Average of twice experiments.

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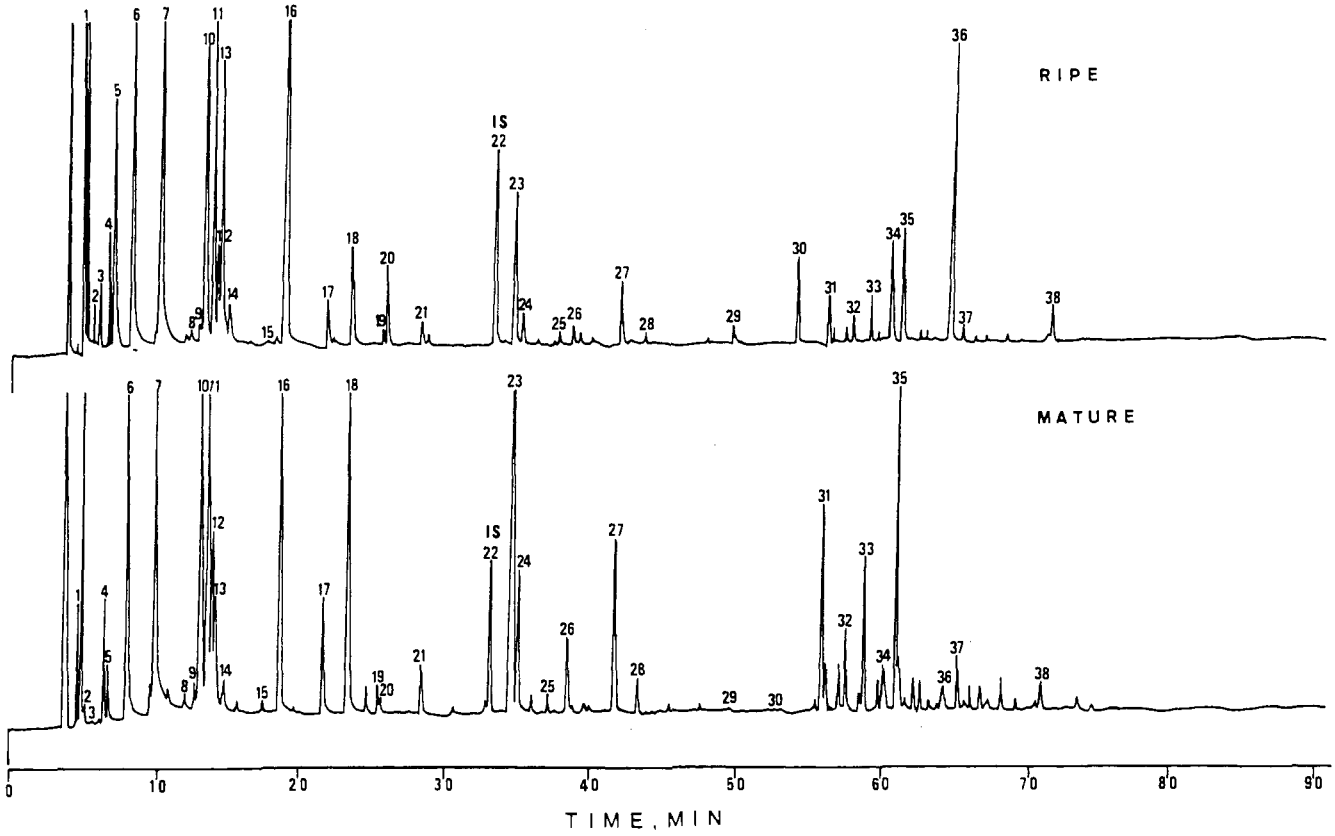

Figure 1. Capillary gas chromatograms of mature and ripe guava volatile components.

Capillary Gas Chromatography (HRGC). A CP-Wax 52 CB Chrompack column (50 m × 0.32 mm i.d., df = 0.22 μm, bonded polyethylene glycol phase) was connected to a flame ionization detector (FID) in a Hewlett-Packard 5890A gas chromatograph system. A Hewlett-Packard Model 3392-A integrator was used to determine the GC peak area. The amount of each component was determined by using an internal standard method. The column temperature was programmed as follows: 50 °C held 5 min, raised to 200 °C at 2 °C/min. The injector and detector temperatures both were 250 °C. Hydrogen carrier gas was used at a linear velocity of 24.87 cm/s. Sample injection volume was 0.3 μL with a split ratio of 1/100. Kovats indices were calculated for separated components relative to a C_8–C_{25} *n*-alkanes (Alltech Associates, Inc.) mixture (Schomburg and Dielmann, 1973). Authentic compounds for gas chromatographic analysis were obtained commercially.

Gas Chromatography/Fourier Transform-Infrared Spectrometry (GC/FT-IR). GC/FT-IR analysis was carried out with a Hewlett-Packard 5965A IRD system interfaced to a Hewlett-Packard 5890A GC equipped with an FID. The system used a liquid nitrogen cooled narrow band Hg–Cd–Te detector (750–4000 cm^{-1}) and a 120 mm × 1 mm i.d. light pipe. Light pipe and transfer lines were held at 220 and 240 °C, respectively. The gas chromatographic column was the same as for the GC analysis except for the df = 1.2 μm. Helium (31.1 cm/s) was employed as carrier gas. The temperature program was 60 °C (2 min isothermal) raised to 160 °C at 3 °C/min, held at 160 °C 1 min, and then heated to 200 °C at 5 °C/min. A spectra resolution of 8 cm^{-1} at a scan rate of 3 spectra/s was chosen.

Gas Chromatograph/Mass Spectrometry (GC/MS). A Hewlett-Packard 5840A gas chromatograph equipped with the same capillary column as in the GC analysis was connected directly into a quadrupole mass spectrometer (Hewlett-Packard 5985B MS system). The column temperature was programmed from 50 (10-min isothermal) to 200 °C at 2 °C/min. The carrier gas (He) was at the flow velocity of 31.1 cm/s. The temperatures of the ion source and the connection parts were 200 °C. The electron energy was 70 eV. The electron multiplier voltage was 2600 V.

RESULTS AND DISCUSSION

Quality and Maturity. The maturity of guava fruit is related to changes in carbohydrates, nonvolatile organic acid, volatile flavor constituent, and pectin content (Salunkhe and Desai, 1984). Growers depend on these factors to harvest fruits from orchards. From this standpoint, the mature and ripe fruits were selected for analysis of volatile constituents and proximate composition. Table I shows the quality measurements of fruits from mature and ripe stages. The pectin content of guava was obviously higher in the mature stage than in the ripe stage. In Wilson's data, which was quoted by Salunkhe and Desai (1984), the total pectin (0.99%) was close to that in the ripe guava (0.67%). Roe and Brummer (1981) indicated that the ripening of fruits was accompanied by the solubilization of pectin. It was proposed that the texture of the fruit might also be affected. In acidity, the mature fruits were higher than the ripe fruits. This phenomenon was due to the catabolic reactions and the decreasing of acids (Tressl and Albrecht, 1985). This factor might affect the flavor acceptance of the fruits and its processed products (Luh, 1980). The acidities of mature and ripe fruits are greater reported in Wilson's data (0.80%). In sugars, similar catabolic reactions were observed. The contents of sugars decreased from the mature stage to the ripe stage. Salunkhe and Desai (1984) reported that the level of fructose increased during ripening and then decreased in the overripe fruit; this might be relative to the changes of sugar contents. The increasing Brix–acid ratio from the mature to the ripe stage also might affect the sweetness and flavor of the fruit.

Volatile Components. The volatile compounds from two different mature fruits were isolated by vacuum distillation–solvent extraction, and subsequently, the volatiles were identified by the comparison of GC retention indices, MS and/or IR spectra, and authentic compounds.

Table II. Quantitative Comparison of Volatile Components from Mature and Ripe Guava Fruits

peak[a]	component	Kovats index CP-Wax[b]	MW	amounts, μg/kg mature[c]	amounts, μg/kg overripe[c]	ID
	esters					
1	ethyl acetate	872.7	88	614.1	8884.2	*e*
2	ethyl propionate	940.0	102	57.4	84.5	*e*
3	propyl acetate	968.6	102	tr[d]	163.5	*g*
5	ethyl 1-butyrate	1030.9	116	1158.6	2725.2	*e*
8	ethyl 2-butenoate	1179.9	114	408.3	290.0	*g*
13	ethyl hexanoate	1231.3	144	5403.8	12975.1	*e*
16	(*Z*)-3-hexenyl acetate	1313.1	142	16510.8	11797.7	*e*
20	ethyl octanoate	1430.2	172	1181.9	2387.1	*e*
21	1-octyl acetate	1472.3	142	835.6	733.4	*e*
22	dimethyl octanyl acetate (I.S.)[d]	1558.1	198			*e*
26	ethyl benzoate	1643.0	150	1193.9	403.5	*f*
30	3-phenylpropyl acetate	1947.4	178	tr	2028.3	*e*
36	cinnamyl acetate	2156.6	176	557.0	6688.1	*e*
	alcohols					
17	1-hexanol	1365.4	102	3019.8	2378.7	*e*
18	(*Z*)-3-hexen-1-ol	1392.5	100	8736.9	2630.6	*e*
19	(*E*)-2-hexen-1-ol	1418.1	100	931.5	226.2	*f*
38	cinnamyl alcohol	2311.9	134	379.2	1204.0	*g*
	carbonyl compounds (aldehydes and ketones)					
6	1-hexanal	1080.3	100	13183.4	8398.0	*e*
7	(*E*)-3-hexenal	1130.7	98	19103.4	5268.6	*f*
10	(*E*)-2-hexenal	1202.1	98	21858.5	4724.2	*e*
15	3-hydroxy-2-butanone	1280.9	88	369.6	156.6	*g*
32	cinnamic aldehyde	2013.2	132	1359.1	462.3	*g*
	monoterpene and sesquiterpene derivatives					
4	α-pinene	1016.1	136	1470.6	640.8	*e*
9	limonene	1192.4	136	755.8	213.6	*e*
14	(*E*)-ocimene	1238.4	136	1199.0	590.3	*e*
23	β-caryophyllene	1577.0	204	11352.6	2771.6	*f*
24	γ-carylphyllene	1585.8	204	2136.3	468.5	*f*
25	alloaromadendrene	1618.6	204	310.9	180.4	*g*
27	α-terpineol	1710.0	154	4790.6	1357.2	*f*
28	δ-cadinene	1731.2	204	367.8	243.1	*g*
33	elemol	2038.3	222	2258.0	764.7	*g*
35	veridiflorol	2083.3	222	5650.9	1928.9	*g*
37	α-cadinol	2175.3	222	782.4	356.9	*g*
	miscellaneous and unidentified compounds					
11	1,8-cineole	1205.0	154	30954.0	7238.0	*g*
12	unknown	1207.1	?	3892.1	1424.0	*g*
29	hexanoic acid	1871.6	130	tr	982.0	*e*
31	sesquiterpene	1978.6	204	2400.8	1121.9	*g*
34	unknown	2073.0	?	797.4	2430.3	*g*

[a] Number refers to Figure 1. [b] Retention indices, using paraffin (C_8–C_{25}) as references. [c] Average of duplicate experiments (by wet basis). [d] I.S., internal standard. tr, amounts less than 10 μg/kg. [e] Mass spectrum and Kovats index are consistent with those of authentic compounds. [f] Identified by GC/MS and GC/FT-IR. [g] Tentatively identified by mass spectra data only.

Figure 1 shows the capillary gas chromatograms of volatiles from mature and ripe guava fruits. It was found that the difference between these two samples were obvious in quantitative analysis but not in qualitative analysis. The identified compounds and quantitative distribution are represented in Table II. In total, 12 esters, 8 alcohols, 7 hydrocarbons, 5 carbonyls, 1 acid, and 1 component with miscellaneous structure were identified, from which 17 compounds were further identified by authentic compounds. In quantitative distribution, total amounts of 134 mg/kg of mature fruit and 93 mg/kg of ripe fruit were determined. Carbonyls (55.9 mg/kg) were present in the highest concentrations in mature fruit, whereas esters (49.2 mg/kg) were the highest in ripe fruit. The major volatile constituents in mature fruit were 1,8-cineole (31 mg/kg), (*E*)-2-hexenal (21.9 mg/kg), and (*E*)-3-hexenal (19.1 mg/kg). The 1,8-cineole was reported as the major volatile constituent for the first time. Vernin et al. (1991) detected this compound in guava fruit from Egypt but did not record the contents. Hashinaga et al. (1987) used the Tenax GC trap method to analyze the volatiles of leaves and fruits of guava. They found that 1,8-cineole was identified in the volatiles of guava leaves but was not detected in the fruits. According our previous paper (Chyau and Wu, 1989), the 1,8-cineole existed in the outer-flesh peel and inner flesh of guava fruits. The odor of 1,8-cineole was fresh, diffusive, and camphoraceously cool (Arctander, 1964). The formation of 1,8-cineole was not understood in the present study. Because of the green-note characteristics, (*E*)-2-hexenal and (*E*)-3-hexenal were supposed to give the impact in the flavor of mature fruits. On the other hand, the major constituents in ripe fruit are ethyl hexanoate (13 mg/kg) and (*Z*)-3-hexenyl acetate (11.8 mg/kg). They all had been previously reported in guava fruit (MacLeod and Troconis, 1982; Idstein and Schreier, 1985; Nishimura et al., 1989; Vernin et al., 1991) and considered important flavor compounds (MacLeod and Troconis, 1982). However, the esters might have considerable effect on ripe fruit flavor; a significant group of the ethyl esters existed in large amount (28.9% in total). It was presumed that these constituents might impart the character of ripe fruit flavor.

The other group of C_6 compounds, including 1-hexanal, (*E*)-2-hexenal, (*E*)-3-hexenal, 1-hexanol, (*Z*)-3-hexen-1-

ol, (*E*)-2-hexen-1-ol, and hexanoic acid, were also related to the maturity of fruit. The presence of C_6 aldehydes and alcohols was largely responsible for the characteristic odor of green leaves of vegetables and trees, and these compounds were also the constituents of aroma in various fruits. It had been reported that many higher plants had the ability to produce these C_6 compounds (Hatanaka et al., 1986). Tressel and Drawert (1973) reported that the 1-hexanal and (*E*)-2-hexenal were formed by enzymatic formation from linoleic and linolenic acid, respectively. The reduction of aldehydes to alcohols was also involved in the biosynthetic pathway. In the identification of (*E*)-2-hexen-1-ol and (*E*)-3-hexenal, the FT-IR data were used to compensate the insufficient MS spectra. In these constituents, (*E*)-3-hexenal was not identified by Vernin et al. (1991) or MacLeod and Troconis (1982). Also, the (*E*)-2-hexen-1-ol was not identified. The typical aldehyde absorptions at 2809 and 2717 cm^{-1} and the *E* configuration of the double bond could be deduced from the peaks at 997 cm^{-1}. In combination with MS spectra information, peak 7 was characterized as (*E*)-3-hexenal. In the other compound, the OH band was present at 3662 cm^{-1} and the *E*-configured band also occurred at 997 cm^{-1}. From this result and the MS spectra, peak 19 was considered to be (*E*)-2-hexen-1-ol. Furthermore, comparison of the spectroscopic data with the commercially available IR vapor-phase library (EPA library) proved the identity of the compounds to agree with the above results.

Another important class of compounds was hydrocarbons. Among this group, β-caryophyllene was the major sesquiterpene hydrocarbon in the volatiles of mature and ripe fruits. It was also identified as the major sesquiterpene hydrocarbon in previous papers (Stevens et al., 1970; Wilson and Shaw, 1978). But MacLeod and Troconis (1982) found slightly more α-humulene than β-caryophyllene, and the former was not identified in this study. Table I showed clearly that the hydrocarbons decreased from mature stage to ripe stage. This result was similar to the previous results of Hashinaga et al. (1987).

3-Phenylpropyl acetate was found to be one of the trace compounds in mature fruits, but it was found clearly increasing in ripe fruits. It was reported as one of the minor constituents in guava puree from Taiwan and was considered a characteristic sweet flavor of ripening guavas from Amami Island (Nishimura et al., 1989).

A further important group of guava aroma substances was represented by the cinnamyl derivatives, including cinnamic aldehyde, cinnamyl alcohol, and cinnamyl acetate. Cinnamyl alcohol was considered a reduction product of cinnamic acids (Idstein and Schreier, 1985), and the production of cinnamyl acetate was assumed to have relationship with cinnamyl alcohol.

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Registry No. Pectin, 9000-69-5; ethyl acetate, 141-78-6; ethyl propionate, 105-37-3; propyl acetate, 109-60-4; ethyl 1-butyrate, 105-54-4; ethyl 2-butenoate, 10544-63-5; ethyl hexanoate, 123-66-0; (*Z*)-3-hexenyl acetate, 3681-71-8; ethyl octanoate, 106-32-1; 1-octyl acetate, 112-14-1; ethyl benzoate, 93-89-0; 3-phenylpropyl acetate, 122-72-5; cinnamyl acetate, 103-54-8; *n*-hexanol, 111-27-3; (*Z*)-3-hexen-1-ol, 928-96-1; (*E*)-2-hexen-1-ol, 928-95-0; cinnamyl alcohol, 104-54-1; 1-hexanal, 66-25-1; (*E*)-3-hexenal, 69112-21-6; (*E*)-2-hexenal, 6728-26-3; 3-hydroxy-2-butanone, 513-86-0; cinnamaldehyde, 104-55-2; α-pinene, 80-56-8; limonene, 138-86-3; (*E*)-ocimene, 3779-61-1; β-caryophyllene, 87-44-5; γ-caryophyllene, 123938-16-9; alloaromaldendrene, 25246-27-9; α-terpineol, 98-55-5; δ-cadinene, 483-76-1; elemol, 639-99-6; α-cadinol, 481-34-5; 1,8-cineole, 470-82-6; hexanoic acid, 142-62-1; viridiflorol, 552-02-3.