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Characterization of Volatiles in Guava (*Psidium guajava* L. cv. Chung-Shan-Yueh-Pa) Fruit from Taiwan

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ABSTRACT

Guava (*Psidium guajava* L. cv. Chung-Shan-Yueh-Pa) is a cultivar used for juice processing in Taiwan because of its aroma. Volatile compounds were isolated from guava fruit by simultaneous steam distillation and solvent extraction according to the Likens-Nickerson method. Compounds were identified by capillary GC-MS and sensorily characterized by GC-sniffing. A total of sixty five compounds were identified. The major constituents identified in the guava fruits were: α-pinene, 1,8-cineole, β-caryophyllene, nerolidol, globulol, C_6 aldehydes, C_6 alcohols, ethyl hexanoate and (Z)-3-hexenyl acetate. The presence of C6 aldehydes, C_6 alcohols, ethyl hexanoate, (Z)-3-hexenyl acetate, terpenes and 1,8-cineole is thought to contribute to the unique flavor of the guava fruit.

Key words: Taiwan, guava, volatile compounds, flavor, GC-sniffing, simultaneous steam distillation and solvent extraction

INTRODUCTION

Guava (*Psidium guajava* L.) is the most widely cultivated *Psidium* species belonging to the family Myrtaceae, which is native to Central America. It is distributed worldwide in the tropical and subtropical areas since early 17th century. The round-oval shaped fruit is green-yellow in color and has a light yellow or pink pulp. The aroma impression of the fruit is often described as "quince banana"-like.

Guava is a popular fruit crop in Taiwan. They are grown commercially for the fruits, and the leaves are used in a traditional therapy for dysentery or diabetes. Quite a few reports have been published covering the volatile compounds of guava fruits[(1-16)]. However, there are few studies on the sensory significance of volatile constituents in guava fruits. One of early reports concerning the volatile components in guava was given by MacLeod and Troconis[(3)], who described that the mixture of 2-methylpropyl acetate, hexyl acetate, benzaldehyde, ethyl decanoate, β-caryophyllene and α-selinene had a guava-like aroma among 40 volatile compounds identified in guavas from Venezuela. Pino *et al*.[(14)] identified 204 compounds out of the aroma concentrate of strawberry guava fruits, of which ethanol, α-pinene, (Z)-3-hexenol, (E)-β-caryophyllene, and hexadecanoic acid were the major constituents. The presence of many aliphatic esters and terpenic compounds is thought to contribute to the unique flavor of the guava fruits. Pino *et al*.[(15)] also characterized 173 volatile components in Costa Rican guava and sensorily characterized them by GC-sniffing. The major constituents were β-caryophyllene, α-terpineol, α-pinene, α-selinene, β-selinene, δ-cadinene, 4,11-selinadiene and α-copaene. Again the amounts of aliphatic esters and terpenic compounds were thought to contribute to the unique flavor of this fruit. Jordán *et al*.[(16)] studied the aromatic profile in commercial guavas and identified a total of 51 components as the principal components in guava essence and fresh fruit puree by GC-MS. In the olfactometric analyses, totals of 43 and 48 aroma active components were detected by the panelists in commercial essence and fruit puree, respectively. Principal differences between the aromas of the commercial guava essence and the fresh fruit puree could be related to the presence of acetic acid, 3-hydroxy-2-butanone, 3-methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, (Z)-3-hexen-1-ol, 6-methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate, 3-phenylpropanol, cinnamyl alcohol, α-copaene, and an unknown component. (E)-2-hexenal seems more important to the aroma of the commercial essence than that of the fresh fruit puree.

Psidium guajava L. cv. Chung-Shan-Yueh-Pa (guava) is a cultivar used for juice processing in Taiwan because of its aroma. The aim of the present work is to determine the volatile constituents of its fruits and their sensory significance.

MATERIALS AND METHODS

I. *Plant Material*

Fresh ripe and aromatic Taiwanese guava (*Psidium guajava* L. cv. Chung-Shan-Yueh-Pa) fruits were collected from a guava farm in Linnei, Yun-Lin County, Taiwan. The fruits were sent to the laboratory and processed within 2 hr.

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II. *Simultaneous Steam Distillation and Solvent Extraction*

The guava fruits (1 kg) were homogenized for 3 min to a slurry with 1 L of deionized water, and then placed into a 5 L round-bottom flask. The slurry was distilled and extracted for 2 hr in a Likens-Nickerson apparatus with 50 mL of pentane/ether (n-pentane:ether = 1:1, previously redistilled and checked as to the purity). One gram of menthyl acetate solution (200 μg/kg) was added to each sample as an internal standard. The volatile concentrate was dried over anhydrous sodium sulfate, preconcentrated with a spinning and distillation apparatus at 40°C, and then reconcentrated to about 50 μL using a 10 cm Vigreux column at 40°C. 1.0 μL of volatile concentrate was injected into the injection unit of GC system.

III. *GC Analyses*

Qualitative and quantitative analyses of the volatile compounds were carried out using an Agilent 6890 GC equipped with a 60 m × 0.25 mm i.d. DB-1 fused-silica capillary column, film thickness 0.25 μm, and a flame ionization detector. Injector and detector temperatures were maintanined at 250°C and 300°C, respectively. Oven temperature was held at 40°C for 1 min and then raised to 200°C at 2°C/min and held for 9 min. Carrier gas (nitrogen) flow rate was 1 mL/min. Kovats indices were calculated for separated components relative to a C_5-C_{25} n-alkanes mixture[17].

IV. *GC-sniffing Analyses*

Same types of GC, column and operation conditions as described above were employed. An SGE ODO-1 Olfactory Detector (Australia) was used for the olfactory detection. A fused silica T-junction was connected at the end of the capillary column. Fifty percent of the eluate was sent to the FID while the rest was diverted to the olfactory detector port crossed by a flow of humidified air.

V. *GC-MS Analyses*

The identification of the volatile compounds was carried out using an Agilent 6890 GC equipped with a 60 m × 0.25 mm i. d. DB-1 fused-silica capillary column, film thickness 0.25 μm, and an Agilent model 5973 N MSD mass spectrometer (MS). Injector was at 250°C. The GC conditions in GC-MS analysis were the same as GC analysis. Carrier gas (helium) flow rate was 1 mL/min. The electron energy was 70 eV, at 230°C. The constituents were identified by matching their spectra with those recorded in the MS library (Wiley 7n).

RESULTS AND DISCUSSION

Volatile compounds in *Psidium guajava* L. cv. Chung-Shan-Yueh-Pa were isolated by simultaneous steam distillation and solvent extraction and analyzed by GC and GC-MS. The identified compounds and the sensory description of the corresponding peaks at the sniffing port of the GC are shown in Figure 1 and Table 1. Sixty five compounds were identified in a total amount of 29,100 ± 1790 μg/kg of guava fruits. The major compounds were α-pinene, 1,8-cineole, (Z)-β-ocimene, β-caryophyllene, nerolidol, globulol, C_6 aldehydes, C_6 alcohols, ethyl hexanoate and (Z)-3-hexenyl acetate.

In comparison with guava volatiles published by others, α-pinene was reported as the major volatile constituent for the first time in this study. According to our previous study[6], (Z)-β-ocimene existed in the outer-flesh peel, but not in the inner flesh, whereas 1,8-cineole existed both in the outer and inner flesh of guava fruits. Chyau *et al.*[11] investigated the differences of volatile constituents between mature and ripe guava fruits and found that the major components in mature fruits were 1,8-cineole, (E)-2-hexenal and (E)-3-hexenal. Others[6,8,10-12,14-16] also reported the detection of α-pinene, nevertheless not a major component.

β-Caryophyllene was present in the highest content among the hydrocarbons. Wilson and Shaw[2] identified terpene hydrocarbons in guava puree and reported that β-caryophyllene played an important role in the aroma. The hydrocarbons were also dominated by β-caryophyllene in guavas from United States[1-2], Taiwan[6], Nigeria[9], Reunion[13] or Cuba[14- 15]. On the other hand, γ-bisabolene and zingiberene identified herein are tentatively reported for the first time in the guava fruit. These compounds had been reported in guava leaf oil[18], but not in the fruit.

Sesquiterpene alcohols have not been characterized as volatiles of guava fruits previously[9]. In the present analysis, sesquiterpene alcohols amounted to 6250 ± 433 μg/kg, nearly 21% of the total volatiles, and included nerolidol, globulol, veridiflorol, ledol, t-cadinol and α-cadinol. Paniandy *et al.*[13] reported nerolidol as the major volatile constituents in the guava fruit. Globulol was only reported in Cuba guava[15].

It is interesting to note the presence of C_6 alcohols, C_6 aldehydes, ethyl hexanoate and (Z)-3-hexenyl acetate in our samples. All of them have been identified in earlier studies[4,7] as important aroma compounds of guava fruits. Esters with unsaturated C_6 residues could originate from side reaction of β-oxidation of fatty acids, but due to the presence of high amounts of C_6 aldehydes and C_6 alcohols, a biogenetic pathway involving enzymatic oxidation of fatty acids and reduction of C_6 compounds[4] were also postulated.

The qualitative and quantitative variations of present results from those from other parts of the world may be attributable to the analytical methods and also to the ecological and climatic conditions.

The importance of the olfactometric analysis by GC-sniffing in the study of the aromatic profile was evident, because this technique allowed the detection of

Table 1. Volatile constituents of Taiwanese guava (*Psidium guajava* L. cv. Chung-Shan-Yueh-Pa) fruit and their odor description

Peak No.[a]	compound	RI[b]	concn[c] (μg/kg)	odor description
1	ethyl acetate[d]	597	300 ± 25.3	fruity, sour
2	ethyl propanoate	683	tr[e]	fruity, floral
3	methyl butanoate	698	109 ± 16.7	fruity, floral, burnt, sour
4	acetal	719	36.4 ± 5.54	tropical fruity, sweet
5	hexanal[d]	777	1700 ± 19	strong, green, grassy
6	ethyl butyrate[d]	783	212 ± 35.7	fruity, sweet, strawberry-candy
7	unknown 1	798	nd[f]	green, pungent, burnt
8	ethyl (E)-2-butenoate	808	tr	fruity, green
9	(E)-2-hexenal[d]	826	740 ± 54.3	strong green, grass, leaf
10	(Z)-3-hexenol[d]	840	1020 ± 16.7	cheese, nut cereal, weak green
11	hexanol[d]	854	486 ± 18.1	moldy, green, banana, herbal, soybean-cheese
12	methyl hexanoate	883	25.3 ± 5.12	fruity, green grass
13	α-pinene[d]	931	2260 ± 104	spicy, fruity, pine, green
14	benzaldehyde[d]	935	404 ± 17.3	almond
15	camphene	943	18.5 ± 4.25	green, camphor
16	6-methyl-5-hepten-2-one	963	47.9 ± 4.2	metallic, wet rubber, mushroom
17	β-pinene	969	54.4 ± 9.8	citrus, green
18	2-pentyl furan	973	tr	metallic, vegetable
19	ethyl hexanoate[d]	981	1650 ± 207	fruity, floral, over ripe, apple-like, sweet
20	(Z)-3-hexenyl acetate[d]	986	587 ± 18.6	fruity, candy, banana, green
21	hexyl acetate[d]	993	70.4 ± 7.28	ripe banana, sweet
22	hexanoic acid	1006	5.8 ± 0.98	sweaty
23	α-terpinene	1008	8.74 ± 1.02	pepper, citrus
24	ρ-cymene	1010	69.2 ± 10.9	fruity, sweet, green
25	1,8-cineole[d]	1019	2030 ± 182	pungent, spicy, minty, fruity, eucalyptus
26	limonene[d]	1021	827 ± 82.1	fruity, lemon, herbal, peppermint
27	(Z)-β-ocimene[d]	1027	1360 ± 85.8	wet cloth, fruit, floral, rose, spicy, citrus-like
28	γ-terpinene	1049	37 ± 1.03	herbaceous, fruity, sweet
29	methyl benzoate[d]	1067	tr	sweet, fruity
30	α-terpinolene[d]	1077	24.4 ± 0.31	fruity, oily, green,
31	linaloold	1089	19.8 ± 4.43	floral, fruity, sweet, floral
32	borneol	1110	3.05 ± 0.32	sweet herbaceous
33	allo-ocimene	1116	13.1 ± 0.82	green, fresh green with somewhat stinky undertone
34	(E)-2-nonenal	1129	10.2 ± 1.17	sweet, melon
35	ethyl benzoate[d]	1141	67.3 ± 1.55	honey, caramel, floral, balsamic, preserved fruity
36	4-terpinenol	1158	68.1 ± 7.92	sweet, citrus flower, floral
37	α-terpineol	1169	444 ± 8.53	oily, fruity, fragrant
38	ethyl octanoate[d]	1176	159 ± 41.2	floral, green leafy, anise
39	octyl acetate[d]	1188	75.1 ± 3.39	fruity, floral
40	ethyl phenylacetate	1193	53.8 ± 6.82	honey, floral, fruity
41	unknown 2	1203	nd	strong medicinal
42	bornyl acetate	1206	12.5 ± 1.58	earthy, pine, flora
43	β-ionone	1293	39.4 ± 4.31	rose, floral
44	3-phenyl propanylacetate	1335	104 ± 19.1	sweet, floral
45	α-copaene	1371	145 ± 15.5	citrus oil, sweet
46	ethyl decanoate	1390	9.47 ± 1.2	melon, sweet, fresh

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Table 1. Continued

Peak No.[a]	compound	RI[b]	concn[c] (μg/kg)	odor description
47	α-gurjunene	1403	88.1 ± 1.56	nd
48	β-caryophyllene[d]	1412	2730 ± 94.5	woody, fruity, sweet
49	aromadendrene	1432	426 ± 39.1	burnt citrus oil, sweet
50	α-humulene	1445	308 ± 6.43	fruity, fresh green
51	allo-aromadendrene	1452	91.2 ± 9.84	burnt citrus oil, sweet
52	β-selinene	1476	24 ± 1.38	fruity, floral
53	α-bisabolene	1487	250 ± 6.97	woody
54	β-bisabolene	1495	161 ± 8.58	fruity ,woody, fragrant
55	δ-cadinene	1508	176 ± 1.51	sweet, citrus oil
56	γ-bisabolene	1518	37.5 ± 6.72	sweet, orange juice, grassy
57	α-calacorene	1523	15.4 ± 3.26	nd
58	nerolidol	1548	1420 ± 137	waxy, oily, floral
59	unknown 3	1554	406 ± 38.6	orange juice
60	caryophyllene oxide	1569	964 ± 2.46	sweet, fruity, carrot
61	globulol	1576	2690 ± 176	fragrant, sweet
62	veridiflorol	1582	407 ± 34.1	sweet, green
63	ledol	1593	370 ± 34.5	sweet, juice
64	unknown 4	1604	187 ± 18.3	floral, sweet
65	zingiberene	1610	373 ± 18.5	fragrant, woody
66	unknown 5	1615	424 ± 19.6	orange, fragrant, woody
67	t-cadinol	1620	505 ± 18.5	woody
68	α-cubebene	1626	840 ± 31.6	fragrant, woody
69	α-cadinol	1637	855 ± 33.5	green, waxy, woody, fruity
70	ethyl hexadecanoate	1935	24.7 ± 0.42	nd

[a]Number refers to Figure1.
[b]Retention indices, using paraffin (C_5-C_{25}) as references.
[c]± standard deviation with three replication.
[d]Mass spectra and retention indices are consistent with those of authentic compounds.
[e]tr, trace.
[f]nd, not detected.

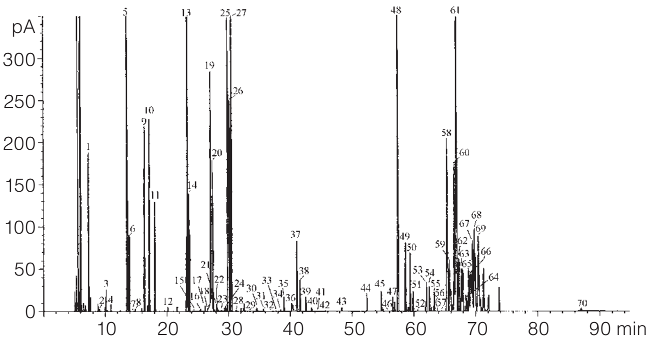

Figure 1. A typical capillary gas chromatogram of volatile constituents of Taiwanese guava (*Psedium guajava* L. cv. Chung-Shan-Yueh-Pa) fruit.

components that could not be achieved by GC-MS. The following compounds are thought to contribute to the complexity of the guava flavor. In green notes, major constituents were C_6 aldehydes, C_6 alcohols and (Z)-3-hexenyl acetate, especially hexanal and 2-hexenal which provide strong green aroma.

Fruity notes are due to the presence of many aliphatic esters, especially ethyl hexanoate, (Z)-3-hexenyl acetate and hexyl acetate. These esters provide pleasant odor. Linalool, β-ionone, nerolidol, and β-selinene provide floral note. Steves *et al.*[(1)] reported β-ionone as one of the most important contributors to the floral flavor of the fruit. The odor of 1,8-cineole was fresh, pungent. spicy, minty, fruity and eucalyptus, which can be detected easily in our samples. Although we detected α-pinene as the major compound in the fruit, but it has a weak note. All sesquiterpenes and alcohols provide the same odor as woody, sweet, and citrus-like odor. It can thus be concluded that C_6 aldehydes, C_6 alcohols, ethyl hexanoate, (Z)-3-hexenyl acetate, terpenes and 1,8-cineole contribute most to the typical guava flavor.

CONCLUSIONS

The volatile compounds of *Psidium guajava* L. cv. Chung-Shan-Yueh-Pa were isolated, identified and sensorily characterized in this study. The major constituents identified in the guava fruits were: α-pinene, 1,8-cineole, β-caryophyllene, nerolidol, globulol, C_6 aldehydes, C_6 alcohols, ethyl hexanoate and (Z)-3-hexenyl acetate. The presence of C_6 aldehydes, C_6 alcohols, ethyl hexanoate, (Z)-3-hexenyl acetate, terpenes and 1,8-cineole is thought to contribute to the unique flavor of the guava fruits.

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