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Volatile Components and Aroma Active Compounds in Aqueous Essence and Fresh Pink Guava Fruit Puree (*Psidium guajava* L.) by GC-MS and Multidimensional GC/GC-O

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Characterization of the aromatic profile in commercial guava essence and fresh fruit puree by GC-MS yielded a total of 51 components quantified. Commercial essence was characterized to present a volatile profile rich in components with low molecular weight, especially alcohols, esters, and aldehydes, whereas in the fresh fruit puree terpenic hydrocarbons and 3-hydroxy-2-butanone were the most abundant components. In the olfactometric analyses totals of 43 and 48 aroma active components were detected by the panelists in commercial essence and fruit puree, respectively. New components were described for the first time as active aromatic constituents in pink guava fruit (3-penten-2-ol and 2-butenyl acetate). Principal differences between the aroma of the commercial guava essence and the fresh fruit puree could be related to 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 to be more significant to the aroma of the commercial essence than of the fresh fruit puree.

KEYWORDS: Volatile; aroma; essence; guava; olfactometry

INTRODUCTION

Aqueous essences of tropical fruits are being used by the flavor industry in a wide range of applications to enhance product acceptance. Therefore, it is important to characterize the aromatic profiles of these aqueous essences. All of the research conducted to date has been related to the volatile components found in the whole fruit, puree, juice, or essential oil. No research on the aromatic profile of the aqueous essence of guava fruit has been completed.

Guava aroma has been reported since the early 1960s as having a unique quince and banana-like aroma (*1*). One of the first reports concerning the volatile components in Hawaiian guava was made by Stevens et al. (*2*). These authors reported β-ionone as one of the most important contributors to the floral flavor of the fruit. Wilson and Shaw (*3*) identified 12 terpene hydrocarbons in guava puree and reported that β-caryophyllene plays an important role in the aroma. However, MacLeod and de Troconis (*4*) reported that 2-methylpropyl acetate, hexyl acetate, and benzaldehyde had a guava-like aroma among 40 volatile compounds identified in guava from Venezuela. Later, Zheng et al. (*5*) declared that the sharp characteristic aroma of guava fruit was attributed mainly to 1,1-diethoxymethane and 1,1-diethoxyethane.

Askar et al. (*6*) studied the aroma constituents in white and pink guava fruits from Egypt and identified and quantified a total of 88 volatile compounds, with hexanal as the major component. These authors concluded that C_6 aldehydes, alcohols, and esters are important aroma compounds in guava fruits, but the characteristic guava aroma is mainly due to cinnamyl derivatives, β-caryophyllene, and C_6 derivates. These results were similar to those of Vernin et al. (*7*), who studied the aromatic profile of guava from Egypt and identified a total of 132 components in it. Quantitatively, the major constituents were (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, 2-pentanone, cinnamyl alcohol, 3-phenylpropyl acetate, and 3-phenylpropanol. Nevertheless, 3-phenylpropyl acetate, cinnamyl alcohol γ- and δ-lactones, and ethyl esters may play an important role in the characteristic sweet, pleasant flavor of Egyptian guava.

Ekundayo et al. (*8*) analyzed volatile constituents of *Psidium guajava* L. fruit from trees grown in Nigeria, where they identified a total of 25 components. Free long-chain saturated fatty acids (mainly lauric and myristic acids) were the most abundant group of constituents. Large amounts of β-caryophyllene and oxygen-containing sesquiterpenes were typical for Nigerian guava.

Hashinaga et al. (*9*) reported that immature guava fruit contained high levels of isobutanol, butanol, and sesquiterpenes, which decreased during maturation. In mature and ripe fruits, levels of ethyl acetate, ethyl caproate, ethyl caprylate, and (*Z*)-

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hexenyl acetate were especially increased. These results were reproduced by Chyau et al. (*10*), who studied the differences of volatile constituents between mature and ripe guava fruits and found that the major components in mature fruit were 1,8-cineole, (*E*)-2 hexanal, and (*E*)-3-hexenal. Ethyl hexanoate and (*Z*)-3-hexenyl acetate were the major volatile components of ripe fruit.

In 1994, Bassols and Demole (*11*) found for the first time pentane-2-thiol as a naturally occurring component of guava flavor that contributed significantly to the strong and characteristic tropical fragrance of fresh guava fruits. A more complete study about the occurrence of this component was reported by Koening et al. (*12*). These authors studied the enantiomeric distribution of 2-pentanethiol in guava fruit stored under controlled conditions until fully ripe. The enantiomeric composition of these compounds was responsible for the characteristic aroma of guava fruit, and the result revealed the presence of the enantiomerically pure (*S*)-2-pentanethiol in guava fruit.

Ortega and Pino (*13*) reported quantities of volatile components in guava and their relationship with the sensory properties of the fruit. They concluded that there is no one single group of volatile compounds that characterize the aroma in guavas, although they considered aldehydes to be important components in the development of the aroma. Pino et al. (*14*) reported the volatile components of guava fruit from Cuba, concluding that the major components quantified were either β-caryophyllene, limonene, 3-phenylpropyl acetate (*E*)-hexenyl acetate, or (*E*)-cinnamyl acetate. Additionally, Pino et al. (*15*) characterized the volatiles of Costa Rican guava fruit. They reported 173 components and included some GC-O results. The reported major constituents were β-carophyllene, α-terpineol, α-pinene, α-selinene, β-selinene, δ-cadinene, 4,11-selinadiene, and α-copaene.

Yen and Lin (*16*) found a total of 29 components in the aromatic profile of this fruit, with esters, terpenic hydrocarbons, and alcohols the most abundant components in the aromatic profile.

In one of the more recent works related to the volatile components in guava Paniandy et al. (*17*) described for the first time γ-butyrolactone and nerolidol as contributors to the aromatic profile in this fruit. Hexanal followed by γ-butyrolactone, (*E*)-2-hexenal, (*E,E*)-2,4-hexadienal, (*Z*)-3-hexenal, (*Z*)-2-hexenal, (*Z*)-3-hexenyl acetate, and phenol were the major components quantified in the headspace of this fruit. In the hydrodistilled oil, β-caryophyllene, nerolidol, 3-phenylpropyl acetate, and caryophyllene oxide were the components present in the highest concentrations.

Idstein and Schreier (*18*) reported a total of 154 compounds, with 116 of those being reported for the first time using a combination of GC, GC-FTIR, and GC-MS. Six carbon aldehydes and alcohols were predominant. These authors used a high-vacuum distillation of fresh quava puree followed by a liquid−liquid extraction.

The real contribution of each component to the aromatic quality of the fruit can only be known by studying the aroma activity of each component. Gas chromatography olfactometry (GC-O) has proven to be a powerful method for determining key aroma compounds in food (*19*−*23*). So far, little research involving the aromatically active components in guava fruit and guava essence by GC-O has been reported. The aim of the present work is to compare the aromatic profile of fresh puree and aqueous essence and to determine the most important contributors to the aroma of fruit and aqueous essence.

MATERIALS AND METHODS

Guava Samples. Unpasteurized guava puree and aqueous essence were obtained from a local manufacturer of aqueous essences located in Florida. The samples were taken to the laboratory within 2 h after industrial processing and frozen at −20 °C for ∼2 months. Both essence and puree were derived from the same fruit source. Aqueous essence was obtained by a proprietary process, which utilizes low temperature and a short steam distillation.

Extraction of Volatile Compounds. *Guava Fruit*. Volatile compounds were isolated using a liquid extraction technique. Cyclohexanone (10 mg/kg) was added to 120 g of puree, and volatile components were then extracted with 80 mL of methylene chloride (Sigma Chemical Co., St. Louis, MO) by stirring for 1 h with a magnetic stirrer at ∼2 °C. The resulting mixture was centrifuged for 10 min (5000 rpm, 3000*g*), the organic layer was dried with sodium sulfate and concentrated to ∼1 mL using a distillation−rectification system, followed by further concentration to 0.1 mL with a nitrogen flow.

Guava Essence. Volatile components present in 3 mL of essence with 10 mg/kg of cyclohexanone as internal standard were extracted twice using 2 mL of methylene chloride for each extraction, mixed between two glass syringes connected by a stainless steel luer lock adaptor, and combined. The final emulsion was centrifuged, and the organic layer was recovered and dried as above. This fraction was concentrated to 0.1 mL using a nitrogen flow. The extraction efficiencies for the guava essence and guava puree were ∼98% as determined by successive extractions.

GC-MS Analysis. The qualitative and quantitative analyses of the volatile compounds were carried out using a model 6890 gas chromatograph (GC) equipped with a 30 m × 0.25 mm i.d. HP-5 (5% cross-linked phenyl-methyl siloxane) column with 0.25 μm i.d. film thickness and an Agilent model 5973N MSD mass spectrometer (MS) with a 7683 autosampler (Agilent, Palo Alto, CA). The initial oven temperature was held at 40 °C for 6 min. It was then increased at 2.5 °C/min to 150 °C and finally at 90 °C/min to 250 °C; the injection port and ionizing source were kept at 250 and 280 °C, respectively. The split ratio was 10:1 with 2 μL of sample injected. There was a solvent delay of 2 min after which mass spectra were scanned from *m*/*z* 35 to 300, generating 5.27 scans/s. Compound identifications were made by comparison of the mass spectra and retention times with those of corresponding reference standards (Aldrich Chemical Co., St. Louis, MO; andBedoukian Research, Inc., Danbury, CT) for all compounds except (*Z*)-3-hexenoic acid and α-copaene that were identified by the NIST98 library (NIST, Gaithersburg, MD) and reported retention indices.

Quantification. For the purpose of quantifying identified components, linear regression models were obtained using standard dilution techniques with cyclohexanone as the internal standard. Samples were run in triplicate. Target ions were used in the identification and quantification of each component by the mass spectrometry data system. Standard reference compounds were used in all cases if commercially available. For the quantification of the two compounds that were not available, linear regression of similar components was used. The substitutions were (*Z*)-3-hexenoic acid by (*E*)-3-hexenoic acid and α-copaene by β-caryophyllene.

Multidimensional Gas Chromatography. GC/GC−Olfactometry. Two HP-5 (5% cross-linked phenyl-methyl siloxane) columns (30 m × 0.25 mm with 0.25 μm film thickness) were used as both the preparative column and the main column. The columns were installed in Hewlett-Packard 5890 and 5890 series II plus chromatographs and coupled via a CTS1 cryotrapping device (Gerstel, Inc., Baltimore, MD), which was maintained at −150 °C during repeated preparative runs to collect and concentrate the aromatic fraction. Preparative GC effluent was split 1/99 between the FID and the analytical column by a DCS device (Gerstel); the analytical GC effluent was split 1/1 between the mass spectrometer and the sniffing port. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The transfer line to the GC-O sniffing port was held at 300 °C. The volume of extract analyzed and oven program temperatures were the same as those described above for the GC-MS. Humidified air was added in the sniffing port at 100 mL/min. Compound identifications were made

by comparison of the mass spectra, retention times, and Kovats indices (as determined relative to *n*-alkanes from pentane to pentadecane) of the volatile components in both extracts with those of corresponding reference standards. The mass spectrometer retention times were compared with the retention times measured in the olfactometry runs, and both were compared to reference standards if available. To identify the components that were perceived by the panelists but did not present any detectable peak in the mass spectra, several preparative runs were made to concentrate the sample in the cryotrap. Data were collected using the ChemStation G1701AA data system (Hewlett-Packard, Palo Alto, CA).

Olfactometry Global Analysis (Frequency Response). GC-O frequency analysis was performed by using a modified version of the method reported by Pollien et al. (*24*). Three panelists analyzing each sample in triplicate for a total of nine analyses were used for the detection of aromatically active compounds, and verbal descriptors of the odor active components were recorded for both extracts. This modification increases the uncertainty of Pollien's method but uses experienced/trained assessors. Aromatically active components were those that were detected and described using identical terms by at least one panelist three times.

RESULTS AND DISCUSSION

Volatile Components in Commercial Guava Essence and Fresh Guava Fruit Puree. In the analysis of the aromatic profile of the commercial aqueous essence by GC-MS, a total of 44 components were identified and quantified including 17 alcohols, 17 esters, 3 ketones, 2 aldehydes, 2 acids, 1 furan, 1 acetal, and 1 terpene hydrocarbon. 2-Methyl-1-propanol, butanol, 3-hydroxy-2-butanone, acetal, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl butyrate, hexanol, and octanol were the components quantified in greatest concentration. Only 22 components were quantified in the puree including 6 alcohols, 5 esters, 1 ketone, 1 aldehyde, 2 acids, 1 furan, 1 lactone, and 5 terpenic hydrocarbons. Major components quantified in the puree correspond to 3-hydroxy-2-butanone, 3-methyl-1-butanol, ethyl butyrate, (*Z*)-3-hexen-1-ol, ethyl hexanoate, (*Z*)-3-hexenyl acetate, D-limonene, (*Z*)-ocimene, 2-phenylethyl alcohol, 3-phenylpropanol, cinnamyl alcohol, α-copaene, and *β*-caryophyllene (**Table 1**).

These results for guava puree agree with those found previously (*7*, *9*, *10*, *14*), where (*Z*)-3-hexenyl acetate, (*E*)-3-hexenyl acetate, limonene, (*Z*)-3-hexenol, 2-pentanone, cinnamyl alcohol, 3-phenylpropyl acetate, 3-phenylpropanol, and (*E*)-3-cinnamyl acetate were reported as the major constituents of guava fruit. Nevertheless, our results differ partly from those reported by Askar et al. (*6*) and Paniandy et al. (*17*), who reported hexanal as the major component quantified in the guava followed by *γ*-butyrolactone, (*E*)-2-hexenal, *β*-caryophyllene, nerolidol, 3-phenylpropyl acetate, and caryophyllene oxide. Hexanal could not be quantified in the present work because it was detected at trace levels and coeluted with ethyl butyrate under the chromatographic conditions used.

Hashinaga et al. (*9*) and Chyau et al. (*10*) published that immature guava fruit contained high levels of isobutanol, butanol, and sesquiterpenes. In ripe fruit, 1,8-cineole, (*E*)-2-hexenal, (*E*)-3-hexenal, ethyl hexanoate, and (*Z*)-3-hexenyl acetate were the major components. In the present study the fruits utilized for essence extraction were fully ripe, and the results are similar in that isobutanol, butanol, and sesquiterpenes were some of the components found in higher concentrations in both the essence and puree.

The comparative study between the aromatic profile in the commercial essence and the fresh fruit puree revealed that the aromatic fraction of the essence was richer in components with low molecular weight, especially alcohols, esters, and aldehydes. On the other hand, in fresh fruit puree, terpenic hydrocarbons and 3-hydroxy-2-butanone seemed to be the most important contributors to the aromatic profile. 2-Methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, butanol, hexanol, and octanol are the alcohols present in highest concentrations in the essence, whereas in the fresh fruit puree, cinnamyl alcohol and (*Z*)-3-hexen-1-ol were the major alcohols found. It should be noted that methylene chloride is not as polar as some other common solvents and, like any solvent extraction, may not provide a uniform extraction.

Table 1. Quantification of the Volatile Components in Commercial Guava Essence and Fresh Guava Puree by GC-MS

component	RT	Kovats index[b] (HP-5)	concn[a] (mg/kg) essence	fresh puree
ethyl acetate	2.14	613	nc	nd
2-methyl-1-propanol	2.32	619	199.07 ± 33.67	nd
1-butanol	2.75	653	39.41 ± 2.51	nd
2-pentanone	2.95	653	4.70 ± 0 0.75	nd
1-penten-3-ol	3.05	683	2.81 ± 0.08	nd
acetic acid	3.17	709	nd	tr
ethyl propanoate	3.33	709	6.11 ± 1.42	nd
n-propyl acetate	3.39	716	1.51 ± 0.27	nd
3-hydroxy-2-butanone	3.49	711	137.82 ± 6.19	22.63 ± 2.482
methyl butyrate	3.56	723	2.50 ± 0.17	nd
acetal	3.70	997	34.82 ± 1.29	nd
3-methyl-1-butanol	4.17	737	147.20 ± 7.44	1.03 ± 0.29
2-methyl-1-butanol	4.24	744	62.41 ± 6.52	nd
isobutyl acetate	4.95	753	0.89 ± 0.07	nd
pentanol	5.20	766	1.36 ± 0.20	nd
(*Z*)-2-penten-1-ol	5.37	767	1.74 ± 0.16	nd
ethyl butyrate	5.97	771	30.42 ± 1.53	1.13 ± 0.26
furfural	7.92	852	0.60 ± 0.05	0.57 ± 0.17
ethyl (*E*)-2-butenoate	8.28	855	0.32 ± 0.08	nd
(*E*)-2-hexenal	8.69	857	5.97 ± 0.77	nd
(*Z*)-3-hexen-1-ol	9.16	852	0.56 ± 0.06	6.79 ± 1.56
hexanol	10.34	865	19.52 ± 1.37	nd
γ-butyrolactone	12.50	891	nd	tr
methyl hexanoate	12.72	934	0.54 ± 0.02	nd
benzaldehyde	15.32	962	8.49 ± 1.77	0.65 ± 0.25
β-myrcene	16.27	989	nd	0.28 ± 0.01
6-methyl-5-hepten-2-one	16.80	987	1.53 ± 0.41	nd
ethyl hexanoate	17.41	997	9.5 ± 0.39	1.25 ± 0.40
(*Z*)-3-hexenyl acetate	17.94	985	6.89 ± 0.25	1.56 ± 0.24
hexyl acetate	18.30	1008	1.41 ± 0.27	tr
D-limonene	18.56	1031	nd	10.85 ± 1.10
1,8-cineole	18.97	1033	0.08 ± 0.01	nd
(*Z*)-*β*-ocimene	19.44	1037	nd	4.07 ± 0.50
hexanoic acid	20.58	1085	5.01 ± 0.47	nd
octanol	22.95	1075	12.06 ± 1.30	nd
methyl benzoate	23.75	1091	0.75 ± 0.10	nd
(*Z*)-3-hexenoic acid	24.41	1101	nd	tr
linalool	24.44	1101	1.30 ± 0.11	nd
2-phenylethyl alcohol	26.09	1116	2.51 ± 0.48	2.56 ± 0.10
ethyl benzoate	28.32	1170	0.33 ± 0.01	0.17 ± 0.01
terpinen-4-ol	28.86	1182	3.33 ± 0.22	tr
ethyl octanoate	29.47	1195	0.42 ± 0.07	nd
α-terpineol	29.90	1198	0.95 ± 0.01	nd
octyl acetate	30.40	1200	1.03 ± 0.06	nd
octanoic acid	31.72	1179	9.48 ± 1.49	nd
ethyl phenylacetate	32.50	1244	0.23 ± 0.04	nd
3-phenylpropanol	32.96	1253	6.10 ± 0.67	4.60 ± 0.36
cinnamyl alcohol	37.74	1341	2.21 ± 0.32	13.48 ± 0.77
2-phenylethyl propanoate	38.20	1350	0.84 ± 0.08	nd
α-copaene	38.49	1390	nd	6.23 ± 0.96
β-caryophyllene	40.81	1437	0.30 ± 0.05	22.57 ± 2.37

[a] nd, not detected; tr, traces; nc, not calculated. ± standard deviation with $n \geq 3$. [b] Directly from, or interpolated from, values obtained from Kondjoyan and Berdagué (*25*).

Table 2. Descriptors of the Volatile Components Detected by GC-O in Guava Essence and Guava Fruit Puree

component	Kovats index	descriptors	frequency of detection	
			essence	puree
2,3-butanedione/ethyl acetate	685	acid, buttery	7	9
2-methyl-1-propanol	694	floral, fruity, organic solvent	4	0
acetic acid	700	vinegar	0	9
3-penten-2-ol	718	plastic, glue, green	4	8
acetal	734	fruity	1	0
3-hydroxy-2-butanone	733	buttery, acid, pungent	4	9
3-methyl-1-butanol	744	pesticide, almond, solvent, dirty feet	5	9
2-methyl-1-butanol	755	ripe onion, buttery, dirty feet	5	7
isobutyl acetate	767	glue, plastic, green	9	9
hexanal	788	green, herbal	6	6
ethyl butyrate	789	fruity, strawberry, apple	9	9
2,3-butanediol	806	esticide, sulfury, onion	0	8
butyl acetate[a]	811	cheese, condensed milk, chestnut	5	0
3-methylbutanoic acid[a]	839	cheese, dirty feet, fecal	0	7
2-hexenal[a]	847	faint green, grass	9	4
(*Z*)-3-hexen-1-ol	852	cheese, nut, cereal, medicinal, nutty oil	4	9
hexanol	865	moldy, humid, banana	5	4
heptanal	888	dry fish, cheese, acid	4	8
3-methyl-2,4-pentanedione/2-butenyl acetate	897	boiled potato, cooked oil, nutty	6	9
furfural[a]	911	roasted nut	3	0
α-pinene	919	spicy, fruity, pine, mint	0	5
1-octen-3-ol[a]	974	rubber, mushrooms, wet earth	7	9
6-methyl-5-hepten-2-one/β-myrcene	980	metallic, wet rubber, resin, spicy	4	9
ethyl hexanoate	997	fruity, anise, floral	9	9
(*Z*)-3-hexenyl hexanoate	1007	banana candy	8	9
hexyl acetate/1-methoxycyclohexene	1014	spicy, herbal, sweet wine, rubbery	0	8
1,8-cineole/D-limonene[a]	1030	spicy, mint, fruity, antiseptic, menthol	5	9
NI[b]	1033	plastic, wet rubber	5	0
hexanoic acid	1037	pesticide, horse urine	6	5
ocimene	1056	wet cloth, fruity	0	4
octanol	1084	burnt matches, hair salon, spicy	5	9
methyl benzoate	1099	caramel, sharp sweet, menthol, green	5	7
(*E*)-2-octen-1-ol	1104	plasticine	0	3
Furaneol	1106	caramel, sweet	0	3
linalool	1112	lemon, parsley	3	3
NI	1122	burnt wood, green	0	5
2-phenylethyl alcohol	1130	roses, antiseptic cleaner, spoiled wine, tropical fruity	8	9
NI	1139	fruity, green, wet wood, dry flower	8	9
borneol[a]	1155	plastic, celery leaves, dry flowers	5	6
benzyl acetate[a]	1162	floral, burnt, boiled zucchini	4	3
NI	1166	fabric, nut, oily, dry fruit, dust	6	4
ethyl benzoate	1173	honey, caramel, floral	7	8
ethyl octanoate	1199	floral, green leafy, menthol, anise	0	8
3-phenylpropanol/ethyl phenylacetate	1252	fruity, spicy, cinnamon, floral, anise	5	9
NI	1257	dry sweat, cat urine, pungent	9	9
2-phenylethyl acetate	1264	floral, roses	4	0
NI	1274	spicy, woody, latex	0	4
(*E*)-cinnamaldehyde	1283	paint, cinnamon, oat	4	4
NI	1291	fruity, spicy, plastic, sweet	0	8
(*E*,*E*)-2,4-decadienal[a]	1317	floral, fruity, liquorice	6	0
NI	1320	dead bug, fat	5	0
cinnamyl alcohol	1329	fruity, floral, rose, woody	3	8
ethyl phenylpropanoate	1351	floral, fruity, spicy	4	0
α-copaene	1374	cinnamon, spicy, floral	3	8
(*Z*)-3-hexenyl hexanoate	1381	fruity, prune	6	4

[a] Tentative identification. [b] NI, unidentified compound.

Ethyl butyrate and, in lesser amounts, (*Z*)-3-hexenyl acetate, propyl acetate, and octyl acetate were the major esters found in the essence, and similar results were obtained in the fresh fruit puree, for which the esters quantified in highest concentrations were (*Z*)-3-hexenyl acetate, ethyl hexanoate, and ethyl butyrate.

The next step in the study was to determine which of those components were responsible for the guava aroma. For this purpose, GC-O was used.

GC-O. As mentioned above, it is important to consider the real contribution of the volatile components to the aroma. Because each component has a different aroma threshold, not all of the compounds quantified contribute to the characteristic aroma of the pink guava fruit, and from a total of 51 components quantified only 43 and 48 aromatic active components (**Table 2**) contributed to characterize the aroma of the commercial aqueous essence and fresh puree of the pink guava fruit, respectively. This means that there are compounds reported in **Table 1** that are not reported in **Table 2**. This is due to several factors. The GC-O work and quantitation were conducted using separate instruments, but, more importantly, the aroma thresholds play a role. A compound may be quantitated but have an aroma threshold such that it is not detected by the panelists, whereas a compound at much lower concentration may not be quantitated but could be perceived by numerous panelists.

Olfactometric analysis allowed the detection of components that could not have been quantified by GC-MS, including

hexanal, 2,3-butanediol, butyl acetate, 3-methylbutanoic acid, heptanal, furfural, α-pinene, 1-octen-3-ol, (*E*)-2-octen-1-ol, Furaneol, borneol, benzyl acetate, 2-phenylethyl acetate, (*E*,*E*)-2,4-decadienal, and (*Z*)-3-hexenyl hexanoate, and even for the identification of components that are described for the first time as constituents of the aromatic profile in pink guava fruit: 3-penten-2-ol and 2-butenyl acetate.

In the commercial essence, five components appeared to contribute most to the characteristic guava aroma because they were detected by the three panelists in all of the replicates: isobutyl acetate, ethyl butyrate, (*E*)-2-hexenal, and ethyl hexanoate. Other components that were detected by all of the panelists, although not in all of the replicates, include ethyl acetate, 1-octen-3-ol, (*Z*)-3-hexenyl hexanoate, 2-phenylethyl alcohol, and ethyl benzoate.

However, in the fruit puree 18 components characterized the aroma because they were perceived by the three judges in all of the replicates: ethyl acetate, acetic acid, 3-hydroxy-2-butanone, 3-methyl-1-butanol, isobutyl acetate, ethyl butyrate, (*Z*)-3-hexen-1-ol, 2-butenyl acetate, 1-octen-3-ol, 6-methyl-5-hepten-2-one, ethyl hexanoate, (*Z*)-3-hexenyl hexanoate, limonene, octanol, 2-phenylethyl alcohol, and 3-phenylpropanol.

Components that were perceived by all of the panelists, although not in all of the replications, need also to be considered as important contributors, including 3-penten-2-ol, 2-methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, heptanal, hexyl acetate, methyl benzoate, ethyl benzoate, ethyl octanoate, an unknown, cinnamyl alcohol, and α-copaene.

These results confirmed that not all of the components present in the highest concentrations contribute to the aroma of guava fruit. In commercial aqueous essence, components such as 2-methyl-1-propanol, butanol, 3-methyl-1-butanol, 2-methyl-1-butanol, and hexanol that were quantified in highest concentrations were not perceived by all of the panelists and could not be considered as most important contributors to the aroma.

With regard to fresh guava puree, components that were detected in low concentrations and even components that could not be quantified by GC-MS appeared as the principal contributors to the aroma of this fruit, because they were detected by all of the panelists in all of the replicates. These components are ethyl acetate, acetic acid, isobutyl acetate, 2-butenyl acetate, 1-octen-3-ol, 6-methyl-5-octen-2-one, octanol, and 3-phenylpropanol.

The main differences between the aromas of the commercial guava essence and the fresh fruit puree could be attributed to the components that were perceived by all of the judges in one of the samples and were not detected or had few detections in the other. According to the olfactometric results, the major differences between these samples could be related to 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 that were perceived with higher frequency of detections in the fruit puree than in the essence. Related to the commercial essence (*E*)-2-hexenal seems to be more significant to the aroma of this product than to the fresh fruit puree.

The importance of the olfactometric analysis in the study of the aromatic profile was evident, because this technique allowed the detection of components that could not be detected by GC-MS and the identifications of components that have been described for the first time as aromatic active contributors to guava aroma. In addition to these results, the main differences between the aroma of commercial essence and the aroma of fresh fruit puree could be established and are related to the components that were perceived with more frequency of detections in one sample as compared to the other.

LITERATURE CITED

(1) Gow-Chin, Y.; Hsin-Tang, L.; Pat, Y. Changes in volatile flavor components of guava puree during processing and frozen storage. *J. Food Sci.* **1992**, *57* (3), 679−685.

(2) Stevens, K.; Brekke, J. E.; Stern, D. J. Volatile constituents in guava. *J. Agric. Food Chem.* **1970**, *18*, 598−599.

(3) Wilson, C. W.; Shaw, P. E. Terpene hydrocarbons from *Psidium guajava*. *Phytochemistry* **1978**, *17*, 1435−1436.

(4) MacLeod, A. J.; Troconis, N. G. Volatile flavor components of guava. *Phytochemistry* **1982**, *21*, 1339−1342.

(5) Zheng, Y. Q.; Sun, Y. L.; Wu, Z. P.; Liu, M. X. Natural flavour components of *Psidium guajava* L. fruit. *Acta Bot. Sinica* **1987**, *29* (6), 643−648.

(6) Askar, A.; El-Nemr, S. E.; Bassiouny, S. S. *Alimentaria* **1986**, *25* (6), 162−167.

(7) Vernin, G.; Verin, E.; Vernin, C.; Metzger, J. Extraction and GC-MS-SPECMA data bank analysis of the aroma of *Psidium guajava* L. fruit from Egypt. *Flavour Fragance J.* **1991**, *6* (2), 143−148.

(8) Ekundayo, O.; Ajani, F.; Seppaenen-Laakso, T.; Laakso, I. Volatile constituents of *Psidium guajava* L. (guava) fruit. *Flavour Fragance J.* **1991**, *6* (3), 233−236.

(9) Hashinaga, F.; Shima, Y.; Itoo, S. Production of volatile components of guava during maturation. *Bull. Fac. Agric., Kagoshima Univ.* **1987**, *37*, 59−64.

(10) Chyau, C. C.; Chen, S. Y.; Wu, C. M. Differences of volatile and nonvolatile constituents between mature and ripe guava (*Psidium guajava* Linn) fruits. *J. Agric. Food Chem.* **1992**, *40*, 846−849.

(11) Bassols, F.; Demole, E. P. The occurrence of pentane-2-thiol in guava fruit. *J. Essent. Oil Res.* **1994**, *6* (5), 481−483.

(12) Koenig, T.; Ruff, C.; Kleinschnitz, M.; Schreider, P. Enantiomeric distribution of 2-pentanethiol in guava fruit (*Psidium guajava* L.) by multidimensional gas chromatography with sulfur chemiluminescence detection. *J. High Resolut. Chromatogr.* **1998**, *21* (6), 371−372.

(13) Ortega, A. G.; Pino, J. A. The volatile constituents of the guava (*Psidium guajava* L.). *Alimentaria* **1996**, *276*, 65−72.

(14) Pino, J. A.; Ortega, A.; Rosado, A. Volatile constituents of guava (*Psidium guajava* L.) fruits from Cuba. *J. Essent. Oil Res.* **1999**, *11* (5), 623−628.

(15) Pino, J. A.; Marbot, R.; Vásquez, C. Characterization of volatiles in Costa Rican guava [*Psidium* friedrichsthalianum (Berg) Niedenzu] fruit. *J. Agric. Food Chem.* **2002**, *50*, 6023−6026.

(16) Gow Chin, Y.; Hsin Tang, L. Changes in volatile flavor components of guava juice with high-pressure treatment and heat processing and during storage. *J. Agric. Food Chem.* **1999**, *47*, 2082−2087.

(17) Paniandy, J. C.; Chane-Ming, J.; Pieribattesti, J. C. Chemical composition of the essential oil and headspace solid-phase microextraction of the guava fruit (*Psidium guajava* L.). *J. Essent. Oil Res.* **2000**, *12* (2), 153−158.

(18) Idstein, H.; Schreier, P. Volatile constituents from guava (*Psidium guajava* L.) fruit. *J. Agric. Food Chem.* **1985**, *33*, 138−143.

(19) Priser, C.; Etiévant, P. X.; Nicklaus, S.; Brun O. Representative Champagne wine extracts for gas chromatography olfactometry analysis. *J. Agric. Food Chem.* **1997**, *45*, 3511−3514.

(20) 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 Fragance J.* **1998**, *13* (1), 49−55.

(21) Feng, Y. W.; Lavin, E. H.; Acree. T. E. The major resources of aroma of fresh soymilk studied by gas chromatography olfactometry. *IFT Annual Meeting: Book of Abstracts*; International Food Technologists: Chicago, IL, 1997; pp 73−74.

(22) Bazemore R.; McDaniels, M. OSME analysis (CGO) of aroma active volatiles in aqueous orange essence. *IFT Annual Meeting: Book of Abstracts*; International Food Technologists: Chicago, IL, 1995; p 257.

(23) Bazemore, R.; Goodner, K. L.; Roussef, R. L. Volatiles from unpasteurized and excessively heated orange juice analyzed with solid phase microextraction and GC-olfactometry. *J. Food Sci.* **2000**, *64* (5), 800−803.

(24) Pollien, P.; Ott, A.; Montignon, F.; Baumgarther, M.; Muñoz-Box, R.; Chaintreau, A. Hyphenated headspace gas chromatography-sniffing technique: Screening of impact odorants and quantitative aromagram composition. *J. Agric. Food Chem.* **1997**, *45*, 2630−2637.

(25) Kondjoyan, N.; Berdagué, J. L. *A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds*; Laboratorie Flavour: Theix, France, 1996.

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