JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Characterization of Odor-Active Compounds in Guava Wine

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ABSTRACT: The volatile compounds of guava wine were isolated by continuous solvent extraction and analyzed by GC−FID and GC−MS. A total of 124 volatile constituents were detected, and 102 of them were positively identified. The composition of guava wine included 52 esters, 24 alcohols, 11 ketones, 7 acids, 6 aldehydes, 6 terpenes, 4 phenols and derivatives, 4 lactones, 4 sulfur-compounds, and 5 miscellaneous compounds. The aroma-active areas in the gas chromatogram were screened by application of the aroma extract dilution analysis and by odor activity values. Twelve odorants were considered as odor-active volatiles: (*E*)-β-damascenone, ethyl octanoate, ethyl 3-phenylpropanoate, ethyl hexanoate, 3-methylbutyl acetate, 2-methyltetrahydrothiophen-3-one, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, ethyl (*E*)-cinnamate, ethyl butanoate, (*E*)-cinnamyl acetate, 3-phenylpropyl acetate, and ethyl 2-methylpropanoate.

KEYWORDS: Guava wine, key odorant, gas chromatography−mass spectrometry, gas chromatography−olfactometry, aroma extract dilution analysis (AEDA), odor activity value

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the most popular tropical fruits cultivated and consumed worldwide. The fruits are consumed fresh and are largely used in food industry for the production of canned fruit, jam, and concentrated juice. Fruits could also be used in wine production. Taking into account the production of guava in Cuba, this beverage could come to be a product of important economic interest for producers and not merely as a complementary activity to wine elaboration. Although volatile compounds of guava have been studied extensively,[1−3] there is no information published to date on the volatiles of guava wine. Aroma compounds are especially important in fruit wine as they contribute to the quality of the final product.

Wine volatile fraction is extremely complex, due to the great number of compounds present, which may have different volatilities and, moreover, may be found in a wide range of concentrations.[4,5] Various techniques have been applied to evaluate volatile compounds in wines, and a review of them is available.[6,7] Solvent extraction is one widely used technique for the extraction of volatile compounds in wine.[8,9]

It has been shown for a considerable number of foods that all of the volatiles present in a food are not able to interact with human olfactory receptors. Instead, only a smaller number of the so-called key odorants is obviously detected by the human odorant receptors and, consequently, participate in the creation of the respective aroma impression in the brain.[10] An approach to separate odor-active volatiles from the bulk of odorless food volatiles is GC-olfactometry on serial dilutions of aroma distillates, such as aroma extract dilution analysis (AEDA).[10] Dilution to odor threshold techniques, such as AEDA, are useful methods for the screening of important odorants in foods, but these methods neither permit a study on the influence of the food matrix on odorant binding nor permit a study on the interactions of odorants when matching the overall odor impression of the food. These limitations are resolved when the concentrations of the individual odorants are correlated with the respective odor thresholds using the odor activity value (OAV) concept.[10]

In a few studies, application of dilution to odor threshold, such as AEDA[11] or calculation of odor activity values,[12,13] on guava fruits is available. Yet, no attempts were made to investigate the volatiles of guava wine with regard to their aroma activity.

The aim of the present work was, therefore, to analyze the volatile compounds in guava wine using continuous solvent extraction and to characterize the odor-active volatiles by employing AEDA and determination of the odor activity values.

MATERIALS AND METHODS

Chemicals. Reference substances of the odorants identified were obtained from the commercial sources given in parentheses: ethyl acetate, 1,1-diethoxyethane, 3-methylbutanal, 3-methyl-2-butanone, ethyl propanoate, ethyl 2-methylpropanoate, propyl acetate, 2-pentanone, methyl butanoate, isobutyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, butyl acetate, hexanal, 2-methylpropan-1-ol, 3-pentanol, 3-methylbutyl acetate, ethyl pentanoate, 1-butanol, ethyl crotonate, pentyl acetate, 2-methylbutan-1-ol, 3-methylbutan-1-ol, ethyl hexanoate, 3-methyl-3-buten-1-ol, 1-pentanol, 2-methyltetrahydrofuran-3-one, ethyl pyruvate, hexyl acetate, 3-hydroxy-2-butanone, ethyl (*E*)-3-hexenoate, (*Z*)-3-hexenyl acetate, 4-methylpentan-1-ol, 3-methylpentan-1-ol, 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, nonanal, 2-butoxyethanol, ethyl octanoate, acetic acid, 2-furfural, butyl lactate, 1-heptanol, 6-methyl-5-hepten-2-ol, benzaldehyde, ethyl 3-hydroxybutanoate, 4-methyl-5-vinylthiazol, ethyl nonanoate, 1-octanol, 2,3-butanediol, γ-butyrolactone, ethyl 2-furoate, acetophenone, ethyl decanoate, ethyl benzoate, 1-nonanol, methyl salicylate, 1-decanol, ethyl phenylacetate, 2-phenylethyl acetate, hexanoic acid, benzyl alcohol, 2-phenylethanol, 3-phenylpropyl acetate, γ-nonalactone, α-terpineol, γ-decalactone, (*E*)-cinnamyl acetate, eugenol, 1-hexadecanol, indole, vanillin, 6-methylcoumarin, methyl vanillate, benzyl benzoate, and hexadecanoic acid (Sigma-Aldrich, St. Louis, MO); diethyl succinate, 3-phenylpropan-1-ol, octanoic acid, ethyl 3-phenylpropanoate, nonanoic acid, decanoic acid, and ethyl

Received: October 28, 2010
Accepted: March 19, 2011
Published: March 19, 2011

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(*E*)-cinnamate (Merck, Darmstadt, Germany); 2-methylbutanal, ethyl sorbate, and ethyl nicotinate (Fluka, Buchs, Switzerland); and limonene, *p*-cymene, (*E*)-3-hexenyl acetate, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, terpinen-4-ol, γ-hexalactone, (*E*)-β-damascenone, 4-vinyl-2-methoxyphenol, 4-vinylphenol, 2-methyltetrahydrothiophen-3-one, and linalool were a gift from Dallant (Barcelona, Spain).

Pentane, anhydrous sodium sulfate, and absolute ethanol were obtained from Merck (Darmstadt, Germany). A C_8-C_{32} *n*-alkane mixture, used for determination of linear retention indices, was obtained from Sigma−Aldrich (St. Louis, MO). For sensory evaluation, each chemical standard was first dissolved in 1 mL of absolute ethanol and then diluted to the desired concentrations using as solvent an 11% ethanol solution. The water used was twice distilled and boiled for at least 1 h prior to use.

Wine Making. Guava fruits var. Suprema Roja were harvested at the ripe maturity stage from the 2010 crop season and immediately transported to the laboratory. The general characteristics of the fruit pulp were: soluble solids, 11.0 ± 0.1; total acidity, 0.69 ± 0.02, and pH, 4.2 ± 0.1. Fresh fruits (25 kg) were carefully peeled, cut in pieces, and passed through a colloid mill. The milled fruit was added at 10% (w/w) to a wort containing brown sugar (190 g/L), diammonium hydrogen phosphate (1 g/L), and anhydrous citric acid (2 g/L). Next, the wort was transferred into two stainless steel tanks (100 L) for the fermentation using dried bakery yeast (1 g/L, Fermipan Lefersa, La Habana). Fermentation was performed in duplicate at controlled temperatures (26 ± 2 °C), and it was considered complete when the Brix level was stable. After fermentation, the guava wine was racked by adding 0.4 g/L sodium bisulfite and clarified by adding 0.1 g/L agar. After 5 days, the guava wine was decanted, and it was stored at 25 °C for 1 month. The process described before is a typical method for fruit wine making in Cuba. The general characteristics of guava wine were: alcohol, 10.8 ± 0.3%; residual extract, 12.7 ± 0.5 g/L; volatile acidity, 0.13 ± 0.02 g/L as acetic acid; and pH, 3.2 ± 0.1.

Standard Chemical Analysis. Soluble solids, total acidity (such as anhydrous citric acid), and pH value were performed in the fruit pulp according to standard method.[14] Alcohol, extract, pH, and volatile acid (such as acetic acid) were performed in guava wine according also to standard methods.[14]

Isolation of Volatile Compounds. The extraction procedure was based on the method described previously.[15] Briefly, 200 mL of guava wine diluted up to 1 L with distilled water was spiked with 50 μL of methyl nonanoate in an 11% hydroalcoholic solution at 40 μg/mL as the internal standard. The guava wine was continuously extracted with 120 mL of *n*-pentane during 6 h at room temperature. The extract was dried over anhydrous sodium sulfate and concentrated first in a micro-Kuderna-Danish concentrator to 1 mL and finally under a stream of pure N_2. The extract was subsequently stored at −20 °C in a glass vial equipped with a Teflon-lined cap before the analysis. Each sample was extracted in triplicate.

The recovery and repeatability of the extraction procedure was tested for some compounds [3-methylbutan-1-ol, limonene, ethyl butanoate, ethyl hexanoate, (*Z*)-3-hexen-1-ol, ethyl octanoate, decanal, and ethyl dodecanoate]. The compounds were added at 50 μg/L to an 11% alcohol solution, and triplicate analyses were performed. The average recoveries were 55−70%, and the relative standard deviations were <10%.

GC−FID and GC−MS Analyses. The GC−FID analysis was accomplished with a Konik 4000 A instrument (Konik, Barcelona) equipped with DB-Wax (30 m × 0.25 mm, 0.25 μm film thickness; J & W Scientific, Folsom, CA) and DB-5 ms (30 m × 0.25 mm, 0.25 μm film thickness; J & W Scientific, Folsom, CA) capillary columns, working with the following temperature program and conditions: 50 °C for 2 min, ramp of 4 °C/min up to 250 °C; injector and detector temperatures 250 °C; carrier gas helium (1 mL/min); detector FID; injections 1 μL in split mode with 1:10 ratio. The relative quantities of the volatiles were expressed as peak area percents in the GC−FID chromatogram. For some volatile compounds [(*E*)-β-damascenone, ethyl octanoate, ethyl 3-phenylpropanoate, ethyl hexanoate, 3-methylbutyl acetate, 2-methyltetrahydrothiophen-3-one, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, ethyl (*E*)-cinnamate, ethyl butanoate, (*E*)-cinnamyl acetate, 3-phenylpropyl acetate, linalool, ethyl decanoate, ethyl benzoate, γ-nonalactone, 2-phenylethyl acetate, and ethyl 2-methylpropanoate], chemical aroma standard mixtures were prepared in 11% v/v hydroalcoholic model solution to bracket the concentrations of each individual compound in guava wine. Standard curves according to the internal standard method were created for these compounds to obtain more exactly data. All analyses were replicated three times.

GC-MS was performed with a HP-6890 instrument gas-chromatograph (Hewlett-Packard Co., Palo Alto, CA) interfaced with a HP-5973 mass-selective detector fitted with a DB-Wax capillary column (30 m × 0.25 mm, 0.25 μm film thickness; J & W Scientific, Folsom, CA). Analytical conditions were the same as GC−FID analyses. Injector and transfer line temperatures 230 °C; carrier gas helium at 1 mL/min; injections 1 μL in split mode with 1:10 ratio. The detection by the mass spectrometer was performed in the electron impact (EI) mode (70 eV ionization energy). The acquisition was performed in scanning mode (mass range *m*/*z* 35−400 u). Identification of the constituents was based on comparison of the linear retention indices with those of authentic samples, comparing their linear retention indices relative and on computer matching against commercial libraries (NIST 02, Wiley 275, Palisade 600 and ADAMS 2001) and FLAVORLIB home-made library mass spectra built up from pure substances and components of known oils. Some of the identifications were confirmed by the injection of the chemical standards into the GC−MS system. Linear retention indices of the compounds were calculated using an *n*-alkane series.

Gas Chromatography−Olfactometry Analysis (GC−O). GC−O analyses were performed with a gas chromatograph Konik 4000A instrument (Konik, Barcelona) equipped with DB-Wax (30 m × 0.25 mm, 0.25 μm film thickness; J & W Scientific, Folsom, CA). Analytical conditions were the same as in GC−FID analyses. The end of the capillary column was connected to a deactivated Y-shaped glass splitter dividing the effluent into two equal parts, which were transferred via two deactivated fused silica capillaries (50 cm × 0.25 mm) to a sniffing port and an FID, respectively. The sniffing port, mounted on a detector base of the GC, consisted of a cylindrically shaped aluminum device (40 mm × 25 mm i.d.) with a beveled top and a central drill hole housing the capillary. Nitrogen (30 mL/min) was used as the makeup gas. The injection volume was 1 μL. During a GC−O run, the nose of the panelist was placed closely above the top of the sniffing port and the odor of the effluent was evaluated. If an odor was recognized, the retention time was marked in the chromatogram, and the odor quality was assigned. The GC−O analyses were performed, at least, by two panelists.

Aroma Extract Dilution Analysis (AEDA). The guava wine extract was stepwise diluted to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, .., 1:1024 of the original solutions.[16] Each dilution was submitted to GC−O, using capillary DB-Wax. Analytical conditions were the same as in GC−FID analyses. The odor-active compounds were located in the chromatograms, and each odorant detected was assigned an FD factor representing the highest dilution in which the odorant was detectable. The FD factors obtained by two panelists were averaged.

Odor Detection Threshold Determinations. A previously described multiple paired comparison test was used.[17] Samples were prepared in capped, wide-mouthed, 50 mL glass bottles. A group of 30−50 unscreened and untrained assessors was used for determining the odor thresholds. In each case, panels were replicated a sufficient number of times, so that a minimum of 100 responses were obtained for

each concentration used in determining a particular threshold. The test involved presenting the assessors with several samples, along with an 11% ethanol solution for reference. Each sample was compared in smell individually with the reference to determine a possible difference. Six samples were presented to each judge during each session. The first bottle was the reference, and the next five coded bottles contained four different dilutions and an 11% ethanol solution identical to the reference. The four dilutions were placed in order of increasing concentrations to prevent fatigue. The position of the 11% ethanol solution coded sample among the different samples was arbitrarily changed from day to day. The statistical analyses for determining the odor detection threshold values involved calculating the concentration corresponding to 50% positive responses from the total judgments. The calculation was made from the linear regression of percentage detection against log concentration. The 95% confidence limit calculated for the threshold values was used as a measure of error.

RESULTS AND DISCUSSION

All of the volatiles from guava wine, isolated by continuous extraction with *n*-pentane, were evaluated by three guava wine experts by smelling a drop of the extract onto a cardboard smelling strip as done by perfumers. After evaporation of the solvent, all three experts agreed that the distillate evoked the characteristic odor of guava wine, thereby indicating that the method used for aroma isolation was appropriate.

A total of 124 volatiles were detected in guava wine, and 102 of them were positively identified (Table 1). Positive identifications were achieved by comparison of linear retention indices and mass spectra with those of standard reference compounds analyzed under identical experimental conditions. Tentative identifications were based on matching linear retention indices and mass spectra of unknowns against those reported in commercial libraries. The composition of guava wine included esters (52), alcohols (24), ketones (11), acids (7), aldehydes (6), terpenes (6), phenols and derivatives (4), lactones (4), sulfur-compounds (4), and miscellaneous compounds (5).

Alcohols were by far the dominant class in terms of total amount in guava wine, and they accounted for 78% of the total volatile composition. In this class, 3-methylbutan-1-ol, 2-methylbutan-1-ol, and 2-phenylethanol showed the highest contents. These compounds are an important group of volatile compounds produced by yeast cells during alcoholic fermentation.[18] The identified alcohols have been reported in guava fruit,[1−3] excepting 3-pentanol, 3-methyl-3-buten-1-ol, 4-methylpentan-1-ol, 3-methylpentan-1-ol, 3-ethoxypropan-1-ol, 2-buthoxyethanol, and 2,3-butanediol.

After alcohols, esters (19% of the total volatile composition) were clearly the dominant constituents in guava wine. These compounds can both originate from the raw material[1−3] (primary compounds) and be synthesized during alcoholic fermentation by yeast[18] (secondary compounds). Their concentrations are dependent upon several factors, mainly juice composition, fermentation temperature, yeast strains, and aeration degree.[4,18] Among these compounds, 3-methylbutyl acetate and ethyl acetate were the major esters, which is typical of a product obtained from fermentation by yeast. The identified esters have been reported in guava fruit,[1−3] excepting ethyl pyruvate, ethyl 2-hydroxypropanoate (ethyl lactate), ethyl sorbate, ethyl 2-hydroxy-3-methylbutanoate, ethyl 3-hydroxybutanoate, 2-hydroxy-4-methylpentanoate, 2-methylbutyl lactate, 3-methylbutyl lactate, diethyl succinate, ethyl 9-decenoate, ethyl 4-acetyloxybutanoate, 1,3-propylene diacetate, ethyl nicotinate, ethyl

Table 1. Volatiles Identified in Guava Wine

compound	RI_P[a]	RI_A[a]	identity[b]	peak area %
ethyl acetate	867	605	A	2.44
1,1-diethoxyethane	870	726	A	0.18
2-methylbutanal	897	653	A	<0.01
3-methylbutanal	900	655	A	0.01
3-methyl-2-butanone	919	657	A	0.01
ethyl propanoate	921	717	A	0.16
ethyl 2-methylpropanoate	930	751	A	0.09
propyl acetate	935	707	A	0.24
2-pentanone	938	688	A	0.03
methyl butanoate	961	729	A	0.01
2,4,5-trimethyl-1,3-dioxolane[c]	985		B	0.02
3-methylpropyl acetate	987	768	A	0.31
ethyl butanoate	1031	805	A	0.49
ethyl 2-methylbutanoate	1033	851	A	<0.01
ethyl 3-methylbutanoate	1035	859	A	0.01
butyl acetate	1064	811	A	0.02
hexanal	1067	802	A	0.01
3-ethoxy-2-butanone[c]	1069		C	0.02
2-methylpropan-1-ol	1083	626	A	0.11
3-pentanol	1086		A	0.01
3-methylbutyl acetate	1115	881	A	2.82
ethyl pentanoate	1117	901	A	0.01
1-butanol	1136	653	A	0.04
ethyl (*E*)-crotonate	1153	855	A	0.01
pentyl acetate	1179	915	A	0.01
limonene	1189	1029	A	0.09
2-methylbutan-1-ol	1200	742	A	21.16
3-methylbutan-1-ol	1215	741	A	31.74
ethyl hexanoate	1217	998	A	1.45
β-phellandrene[c]	1223	1030	B	<0.01
3-methyl-3-buten-1-ol	1236	731	A	0.01
1-pentanol	1240	771	A	0.05
2-methyltetrahydrofuran-3-one	1243		A	<0.01
p-cimene	1245	1025	A	<0.01
ethyl pyruvate	1247		A	0.02
hexyl acetate	1265	1009	A	0.29
3-hydroxy-2-butanone	1278	718	A	0.01
ethyl (*Z*)-3-hexenoate	1279		A	0.02
ethyl (*E*)-3-hexenoate	1287		A	0.02
(*Z*)-3-hexenyl acetate	1300	1005	A	0.04
(*E*)-3-hexenyl acetate	1308		A	0.14
4-methylpentan-1-ol	1310	833	A	0.05
3-methylpentan-1-ol	1324		A	0.14
ethyl 2-hydroxypropanoate[c]	1350	815	B	0.16
1-hexanol	1353	871	A	2.44
(*E*)-3-hexen-1-ol	1355	855	A	0.35
3-ethoxypropan-1-ol[c]	1357		C	0.12
(*Z*)-3-hexen-1-ol	1371	859	A	0.91
nonanal	1388	1101	A	0.02
2-butoxyethanol	1395	909	A	0.05
ethyl 2-hydroxy-3-methylbutanoate	1422	1062	A	0.36
ethyl octanoate	1425	1197	A	1.25
acetic acid	1442	645	A	0.07
2-furfural	1453	836	A	0.01

dx.doi.org/10.1021/jf2011112 |*J. Agric. Food Chem.* 2011, 59, 4885–4890

Table 1. Continued

compound	RI_P[a]	RI_A[a]	identity[b]	peak area %
butyl lactate	1455		A	0.09
1-heptanol	1458	967	A	0.15
6-methyl-5-hepten-2-ol	1463	992	A	0.01
ethyl sorbate	1501	1089	A	0.11
benzaldehyde	1505	960	A	0.10
2-methyltetrahydrothiophen-3-one[c]	1507	998	B	0.31
ethyl 3-hydroxybutanoate	1510	931	A	0.02
4-methyl-5-vinylthiazol[c]	1513		B	0.02
ethyl 2-hydroxy-4-methylpentanoate[c]	1518	1078	B	1.16
ethyl nonanoate	1530	1320	A	0.07
linalool	1544	1097	A	0.02
1-octanol	1550	1068	A	0.09
2-methylbutyl lactate[c]	1555		B	0.08
3-methylbutyl lactate[c]	1558		B	0.23
2,3-butanediol	1568	789	A	0.15
2,5-dimethyl-4-methoxy-3(2*H*)-furanone	1579	1061	A	0.51
terpinen-4-ol	1582	1177	A	0.01
edulan I[c]	1590	1309	B	0.02
γ-butyrolactone	1594	918	A	0.12
ethyl 2-furoate	1597	1047	A	0.05
3-(methylthio)-propyl acetate[c]	1620	1123	B	<0.01
acetophenone	1623	1065	A	0.01
ethyl decanoate	1637	1396	A	0.18
ethyl benzoate	1647	1173	A	0.58
1-nonanol	1660	1169	A	0.02
diethyl succinate	1666	1179	A	0.86
2,6,6-trimethyl-2-cyclohexen-1,4-dione[c]	1660	1143	B	0.02
γ-hexalactone	1675	1059	A	0.01
ethyl 9-decenoate	1677	1389	B	0.05
α-terpineol	1680	1189	A	0.04
γ-terpineol[c]	1683	1349	C	<0.01
3-(methylthio)-propan-1-ol[c]	1690	982	B	0.02
ethyl 4-acetyloxybutanoate[c]	1694		C	0.09
1,3-propylene diacetate	1700	1089	B	0.05
methyl salicylate	1730	1192	A	<0.01
1-decanol	1734	1270	A	0.03
ethyl phenylacetate	1745	1247	A	0.02
ethyl nicotinate	1789	1224	A	<0.01
2-phenylethyl acetate	1792	1258	A	1.06
(*E*)-β-damascenone	1805	1385	A	0.02
hexanoic acid	1840	1000	A	0.01
benzyl alcohol	1864	1032	A	0.07
ethyl 3-phenylpropanoate	1879	1355	A	0.18
ethyl 3-hydroxyoctanoate	1884	1330	A	0.02
2-phenylethanol	1899	1107	A	15.93
3-phenylpropyl acetate	1942	1373	A	1.82
γ-nonalactone	2006	1361	A	0.03
3-phenylpropan-1-ol	2032	1232	A	4.39
octanoic acid	2051	1170	A	0.19
ethyl 3-hydroxydecanoate	2068	1530	A	0.05
ethyl (*E*)-cinnamate	2095	1467	A	0.03
γ-decalactone	2125	1467	A	0.02
(*E*)-cinnamyl acetate	2150	1446	A	0.04
eugenol	2152	1359	A	0.01

Table 1. Continued

compound	RI_P[a]	RI_A[a]	identity[b]	peak area %
diethyl 2-hydroxypentanodionate[c]	2160		C	0.05
nonanoic acid	2162	1271	A	0.03
4-vinyl-2-methoxyphenol	2181	1323	A	0.01
ethyl 2-hydroxy-3-phenylpropanoate[c]	2249	1455	B	1.72
decanoic acid	2270	1386	A	0.43
2-phenylethyl methoxyacetate[c]	2320		C	0.02
4-vinylphenol	2372	1229	A	0.04
1-hexadecanol	2400	1876	A	0.02
indole	2425	1291	A	0.03
3-oxo-α-ionone[c]	2509		C	0.02
6-methylcoumarin	2518	1555	A	<0.01
vanillin	2569	1394	A	0.03
methyl vanillate[c]	2598	1687	B	<0.01
benzyl benzoate	2604	1760	A	0.03
hexadecanoic acid	2895	1960	A	0.04

[a] RI_P and RI_a: Experimental retention index on capillary columns DB-Wax and HP-5 ms. [b] The reliability of the identification proposal is indicated by the following: A, mass spectrum and RI agreed with standards; B, mass spectrum and RI agreed with database or literature; C, mass spectrum agreed with mass spectral database. [c] Tentative identification.

3-hydroxyoctanoate, ethyl 3-hydroxydecanoate, diethyl 2-hydroxypentanedienoate, ethyl 2-hydroxy-3-phenylpropanoate, 2-phenylethyl methoxyacetate, and methyl vanillate, which probably should be originated during the fermentation (secondary compounds).

Lactones are formed from the corresponding hydroxy acids.[19] These compounds, particularly γ-lactones, are important compounds in terms of their contribution to the flavor of many fruits.[1,2] They constituted 0.2% of the total volatile composition. The four lactones detected were reported in guava fruit.[1−3]

Fatty acids accounted for 0.8% of the total volatile composition. They may arise from autoxidation of saturated lipids constituents of fruits, whose production was increased with the thermal treatment.[16] A total of seven acids were identified, among which decanoic acid, octanoic acid, and nonanoic acid were the most abundant. All of the acids have been found in guava fruit.[1−3] Acids probably contribute little to the flavor, because they generally have high odor detection thresholds.[20]

Terpenes are typical volatiles in many fruits including guava.[1,2,21] This class represented only 0.2% of the total volatile compounds. Among the six terpenes detected, all were previously found in guava fruit.[1−3,21] No sesquiterpene compounds were detected in guava wine.

Aldehydes and ketones represented in guava wine 0.1% and 0.6% of the total volatile composition, respectively. In total, 6 aldehydes and 11 ketones were detected, benzaldehyde and 2,5-dimethyl-4-methoxy-3(2*H*)-furanone being the major ones. The 4-methoxy-2,5-dimethyl-3(2*H*)-furanone, commonly named mesifuran, was previously reported in guava fruit,[1−3] and its odor has been described as caramel-like.[13]

Four phenols and their derivatives were detected (0.4% of the total volatile composition). Of them, vanillin and methyl vanillate were not previously reported in guava fruit.[1−3] Volatile phenols detected in guava wine samples can originate from cinnamic, *p*-coumaric, and ferulic acids by enzymatic or thermal decarboxylation.[5]

Table 2. Most Odor-Active (FD ≥ 32) Volatiles Identified in Guava Wine[a]

compound	odor threshold (μg/L)	content (μg/L)	odor quality[a]	FD factor	OAV[b]
ethyl 2-methylpropanoate	15[c]	17.7	fruity	32	1
ethyl butanoate	20[d]	92.0	fruity	64	5
3-methylbutyl acetate	30[d]	531.3	banana-like	512	18
ethyl hexanoate	14[c]	273.7	fruity	1024	19
ethyl octanoate	5[c]	235.4	fruity	1024	47
2-methyltetrahydrothiophen-3-one	4.9[c]	58.2	chlorine, wet	32	12
linalool	25.2[c]	3.4	citrusy, flowery	32	<1
2,5-dimethyl-4-methoxy-3(2*H*)-furanone	16	95.9	caramel	64	6
ethyl decanoate	200[c]	34.5	brandy	32	<1
ethyl benzoate	575[c]	108.4	flowery	32	<1
2-phenylethyl acetate	250[d]	200.3	rose, honey	32	<1
(*E*)-β-damascenone	0.05[d]	3.4	cooked apple	1024	68
ethyl 3-phenylpropanoate	21.5[c]	430.4	fruity, honey	1024	20
3-phenylpropyl acetate	100	342.1	flowery, fruity	64	3
γ-nonalactone	30[e]	5.1	coconut-like	32	<1
ethyl (*E*)-cinnamate	1.1[c]	6.3	fruity, honey	64	6
(*E*)-cinnamyl acetate	2	8.2	flowery, balsamic	128	4

[a] Odor quality perceived at the sniffing port. [b] Odor-activity values were calculated by dividing the concentrations by the respective odor threshold. [c] Data taken from ref 6. [d] Data taken from ref 23. [e] Data taken from ref 24.

Sulfur-compounds constituted 0.4% of the total volatile composition. Four compounds were identified: 2-methyltetrahydrothiophen-3-one, 4-methyl-5-vinylthiazol, 3-(methylthio)-propyl acetate, and 3-(methylthio)-propanol, which are reported for the first time in a guava product.[1−3]

Five other volatiles of different chemical classes were identified: 1,1-diethoxyethane, 2,4,5-trimethyl-1,3-dioxolane, *p*-cymene, edulan I, and indole. All of these compounds constituted 0.2% of the total volatile composition. Of them, only *p*-cymene had been previously reported in guava fruit.[1−3]

The results of the AEDA study are given in Table 2, in which odor zones are arranged following their elution order from the polar column. As is summarized in the table, the AEDA yielded 17 odor regions with flavor dilution (FD) factors ≥32. All of the odorants have been satisfactorily identified on the basis of their retention index and mass spectra. There are four compounds with highest FD factors, among which it is possible to find some ubiquitous byproducts of yeast, such as ethyl hexanoate, ethyl octanoate, and ethyl 3-phenylpropanoate, and a carotenoid degradation compound such as (*E*)-β-damascenone.[22] Another group of odorants with high FD factors was 3-methylbutanoate acetate (secondary compound) together with (*E*)-cinnamyl acetate, which has been found in guava fruit[1−3,21] and is an important contributors of its flavor.[11−13] Other odorants with FD = 64 were ethyl butanoate, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, 3-phenylpropyl acetate, and ethyl (*E*)-cinnamate. Ethyl butanoate and 2,5-dimethyl-4-methoxy-3(2*H*)-furanone were also detected by AEDA as potentially important to the aroma of guava fruit,[11] while 3-phenylpropyl acetate was also found by OAV as potentially important to the aroma of guava fruit.[12] A last major group, with FD = 32, was constituted by ethyl 2-methylpropanoate, 2-methyltetrahydrothiophen-3-one, linalool, ethyl decanoate, ethyl benzoate, 2-phenylethyl acetate, and γ-nonalactone. Of them, linalool, ethyl benzoate, and γ-nonalactone were also detected as potentially important to the aroma of guava fruit.[11] This is the second time that 2-methyltetrahydrothiophen-3-one has been identified in an AEDA experiment, since its previous report in red wine from Rioja.[21]

Taking into account the above-mentioned limitations of AEDA, the odor activity value (OAV) concept[10] was applied to the odorants of guava wine. However, it is necessary that the threshold of the components is determined in a matrix as close as possible to the food itself. For this reason, the odor thresholds for nearly all of the volatiles under investigation were determined in ethanol 11% or taken from papers with similar conditions[6,23,24] (Table 2). The results suggested that 12 odorants should contribute to the characteristic aroma of guava wine because their contents clearly exceeded their odor thresholds in ethanol 11% (Table 2). Following this procedure, the compound with the highest OAV was identified as (*E*)-β-damascenone, exhibiting an intense aroma like that of cooked apples. Ethyl octanoate, with a characteristic fruity odor, was the second most significant component according to its OAV. Other odorants with higher OAV were ethyl 3-phenylpropanoate, ethyl hexanoate, 3-methylbutyl acetate, and 2-methyltetrahydrothiophen-3-one. Another five odorants, linalool, ethyl decanoate, ethyl benzoate, 2-phenylethyl acetate, and γ-nonalactone, presented OAV < 1, which probably means that AEDA overemphasizes the role of some slightly polar compounds, as has been recently discussed.[25] The potentially important odorants obtained with the odor activity approach are a refinement of that provided by the AEDA and correct some of the defects of the AEDA technique.

In conclusion, 12 odorants were considered as odor-active volatiles in guava wine: ethanol, (*E*)-β-damascenone, ethyl octanoate, ethyl 3-phenylpropanoate, ethyl hexanoate, 3-methylbutyl acetate, 2-methyltetrahydrothiophen-3-one, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, ethyl (*E*)-cinnamate, ethyl butanoate, (*E*)-cinnamyl acetate, 3-phenylpropyl acetate, and ethyl 2-methylpropanoate, by application of the aroma extract dilution analysis and by odor activity values. Sensory studies need to be done to determine the actual contribution of these volatile

compounds to guava wine, including model and omission experiments.

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