

Free and Glycosidically Bound Aroma Compounds in Hog Plum (*Spondias mombins* L.)

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Free and glycosidically bound volatiles from tropical fruit *Spondias mombins* were characterized. Isolation and separation of components were accomplished by adsorption on a nonionic resin Amberlite XAD-2 by column chromatography and elution by various selective solvents. Aglycons from the glycosidically bound fraction were released by enzymatic hydrolysis with almond β -glucosidase. Volatile components from both free and glycosidically bound fractions were analyzed by gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS). Retention indices and concentrations were calculated by using a basic program. Tridecane was used as internal standard. Totals of 46 and 28 volatiles were reported in the free and glycosidically bound fractions, respectively. Sensory evaluation (olfactory) of the individual chemical components of free and glycosidically bound fractions was accomplished by sniffing the eluate at the end of a GC sniff port. Correlations of the GC-MS analysis with the sensory data revealed the character flavor compound as isobutyl 3-hydroxybutanoate.

INTRODUCTION

There is a never-ending need to characterize and synthesize new aroma compounds. Most studies have been undertaken with the aim of identifying the substances responsible for the characteristic aroma and flavor. Work in this direction has revealed that aromatic components of fruits are present either in a free form or in the form of glycosides bound to sugars in grape varieties (Williams et al., 1982a; Wilson et al., 1984; Gunata et al., 1985), apple fruit, (Schwab and Schreier, 1988b), passion fruit (Engel and Tressl, 1983b), quince (Winterhalter and Schreier, 1988), and carica papaya (Schwab and Schreier, 1988a; Schwab et al., 1989).

The hog plum tree (*Spondias mombins* L.) is a medium-sized deciduous tree cultivated for its pleasantly acid fruits widespread in tropical Africa and some parts of tropical America (Keay et al., 1964). The fruits are edible and plumlike (ellipsoid 2.5-4.0 cm long) with a thin yellow skin and pulp containing a large stone. It is consumed fresh as a snack. Despite its characteristic sweet unique aroma, there has been no report on the extraction, isolation, and characterization of its aroma constituents. This study reports the volatile components in the free fraction of *S. mombins* as well as the glycosidically bound fraction volatiles subsequent to its release with β -glucosidase.

EXPERIMENTAL PROCEDURES

Reagents. The solvents (*n*-pentane, diethyl ether, methanol, and acetonitrile) were of HPLC grade from Fisher Scientific Co. (Springfield, NJ). Standards of *n*-paraffins (C_6 - C_{26}) were obtained from Alltech Associates (Deerfield, IL). Amberlite XAD-2 (20-60 mesh) and tridecane were obtained from Aldrich Chemical Co. (Milwaukee, WI). Almond β -glucosidase was obtained from Sigma Chemical Co. (St. Louis, MO).

Fresh mature ripe fruits of *Spondias* were collected from a western Nigerian rain forest and immediately frozen and kept in that condition for shipment accompanied on a nonstop flight to John F. Kennedy International Airport (New York). Upon

arrival at the U.S. laboratory facility, samples were kept under frozen storage and later thawed prior to analysis.

Separation of the Free and Bound Aroma Compounds. Fruits of *Spondias* (265 g) were washed and the skin and flesh removed from the hard stone. The recovered flesh was blended in a high-shear blender with (250 mL) distilled water at ambient temperature. About 500 mL of puree obtained was centrifuged at 9000 rpm for 15 min; 300 mL of turbid juice obtained was then passed through a bed of Celite 545 (J. T. Baker Chemical Co., Philipsburg, NJ) by vacuum filtration. The clear juice obtained was then passed through a solvent-washed (Gunata et al., 1985) Amberlite XAD-2 column [1 cm (i.d.) \times 59 cm] with a flow rate of 2.0 mL/min. The column was then rinsed with 250 mL of distilled water to eliminate sugars, acids, and other water-soluble compounds. A free volatile fraction was eluted with 500 mL of pentane/ether (1:1) at a flow rate of 2.0 mL/min. The glycosidically bound fraction was subsequently eluted with 500 mL of methanol. The methanol eluate was then concentrated to dryness by a stream of nitrogen. The dried material was then dissolved in 100 mL of 0.2 M citric-phosphate buffer solution (pH 5). The buffered mixture was washed twice with 50 mL of pentane/ether (P/E) to remove possibly existing free volatiles. The P/E wash extracts were combined with the previously obtained free fraction eluate. The combined P/E was dried over anhydrous sodium sulfate and then concentrated to a final volume of 0.5 mL with nitrogen. The glycosidically bound component dissolved in buffer solution was hydrolyzed with almond β -glucosidase (150 mg, 5.3 units/mg) at 37 °C for 72 h. The liberated aglycons were extracted with 150 mL of P/E (1:1) three times. The extract was dried over anhydrous sodium sulfate and concentrated to a final volume of 0.5 mL with a stream of nitrogen.

Tridecane (1 mL of a 1.0 mg/mL solution in ether) was added as internal standard to both free and glycosidically bound fractions before concentration.

GC and GC-MS Analyses of Volatile Compounds. A Varian 3400 gas chromatograph equipped with a fused silica column [60 m \times 0.25 mm (i.d.); d_f = 0.25 μ m; Durawax 4; J&W Scientific] and a flame ionization detector was used to analyze the volatile components in free and glycosidically bound fractions. The operating conditions were as follows: injector and detector temperatures were 250 and 280 °C, respectively; helium carrier flow rate, 1.0 mL/min; temperature program, 40 °C held for 3 min and then to 280 °C at 2 °C/min and held for 10 min. Quantitative determinations were carried out with a Varian 4270 integrator. Linear retention indices were calculated against *n*-paraffin standards (C_6 - C_{26} ; Alltech Associates) as references

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Table I. Free Volatile Compounds Identified in *Spondias*

compound	RI (DB-1)	approx fruit pulp, ppm
ethanol	649	385
2-methyl-1-propanol	653	374
butanal	662	27
butyric acid	681	29
methyl propyl ketone	737	2
2-methyl-2-pentanol	751	12
2-methylpropanal	789	26
pentanal	791	32
1,2,3-trimethylcyclopentane	802	18
cis-3-hexen-1-ol	844	3
3-methyl-2-butanone	849	14
hexanol	858	19
hexanoic acid	890	14
ethylbutanoate	898	4
ethyl 3-hydroxybutanoate	928	4
ethyl caproate	942	8
propyl 3-hydroxybutanoate	979	3
benzaldehyde	1003	30
methyl benzoate	1064	12
butyl 3-hydroxybutanoate	1080	9
linalool	1082	4
isobutyl 3-hydroxybutanoate	1091	30
benzyl acetate	1147	6
linalool oxide	1195	6
methyl salicylate	1217	15
butyl caproate	1229	5
hexyl butanoate	1252	1
ethyl caprylate	1278	2
α -terpineol	1289	6
cis,cis-2,2,7,7-tetramethyl-3,5-octadiene	1293	2
ethyl 3-hydroxyhexanoate	1312	9
benzyl butanoate	1318	3
butyl benzoate	1324	2
α -copaene	1353	3
hexyl hexanoate	1356	4
methyleugenol	1360	2
1,2,3-trimethoxy-4-allylbenzene	1365	3
β -selinene	1419	4
γ -selinene	1438	6
1,2,3-trimethoxy-5-allylbenzene	1462	4
patchulane	1494	1
citronellyl acetate	1544	6
geranyl acetate	1560	3
phenylmethyl benzoate	1739	123
palmitic acid	1950	5
1-tetradecanol	2099	6

(Majlat et al., 1974). GC-MS analysis was accomplished by using a Varian 3400 gas chromatograph directly coupled to a Finnigan MAT 8230 high-resolution mass spectrometer. The column and temperature program were the same as described above. Mass spectra were obtained by electron ionization 70 eV and an ion source temperature of 250 °C. Mass range was 35–350 scanned at 1.0 s/decade. The filament emission current was 1 mA, and spectra were recorded on a Finnigan MAT SS 300 data system.

Chemical ionization (CI) using methane and isobutane as reagent gases was used to confirm molecular weight of many components which do not yield molecular ions in the electron ionization (EI) mode.

GC Sensory Analysis. A Varian 3400 GC was equipped with a 30 m \times 0.53 mm (i.d.) wide-bore DB-5 capillary column with 1.5- μ m film thickness. The end of the column was fitted with a J&W Scientific Y splitter presstight connector which was connected to two lengths of fused silica tubing, the diameters and lengths [0.1 mm (i.d.) \times 1 m and 0.32 mm (i.d.) \times 1 m] of which were adjusted empirically to provide a split ratio of approximately 30:1 (sniff port, FID). The sniff port was composed of a heated transfer line through which the fused silica tubing was carried out of the GC oven to make the end of the column accessible for sensory analysis (sniffing). The temperature of the heated transfer line was maintained at 280 °C to prevent condensation of eluting GC peaks.

Table II. Volatile Compounds in Glycosidically Bound Fraction from *Spondias*

compound	RI (DB-1)	approx fruit pulp, ppm
ethanol	651	538
3-hydroxybutanal	582	5
2-methyl-2-pentanol	716	13
pentanal	780	19
hexanol	919	80
ethyl 3-hydroxybutanoate	921	499
2-pentanol	933	16
propyl 3-hydroxybutanoate	980	269
benzaldehyde	1004	250
butyl 3-hydroxybutanoate	1078	10
linalool	1080	9
isobutyl 3-hydroxybutanoate	1093	38
benzyl alcohol	1117	37
hexanoic acid	1128	67
ethyl 3-hydroxyhexanoate	1186	2
methyl 2-hydroxybenzoate	1236	19
benzoic acid	1259	13
octanoic acid	1262	14
pentyl butanoate	1286	2
ethyl undecanoate	1290	5
butyl benzoate	1326	10
styrene glycol	1372	6
cinnamic acid	1450	747
4-hydroxycinnamic acid	1469	5
3-methoxy-4-hydroxycinnamic acid	1481	22
1-methylethyl benzoate	1550	14
3-phenyl-2,4-pentanedione	1633	5
palmitic acid	1951	3

RESULTS AND DISCUSSION

Tables I and II, respectively, show the free and glycosidically bound volatile compounds identified in *Spondias*. The identification was accomplished by searching the library database of the National Bureau of Standards (NBS), using published literature and data (Swigar and Silverstein, 1981; MSDC, 1974; Heller and Milne, 1980; Ten Noever de Brauw et al., 1983; McLafferty and Stauffer, 1989). Methane CI-GC-MS and isobutane CI-GC-MS spectra were used to confirm molecular weights of components that do not yield molecular ions in the EI mode.

A total of 46 volatile compounds were identified in the free fraction of *Spondias* comprising 19 esters, 8 alcohols, 4 aldehydes, 2 ketones, 3 acids, and other miscellaneous compounds. All of these compounds are reported for the first time as *S. mombins* volatile compounds. However, ethyl butanoate, hexanol, cis-3-hexen-1-ol, ethyl caprylate, linalool, linalool oxide, α -copaene, ethyl 3-hydroxybutanoate, α -terpineol, butyric acid, and hexanoic acid had been reported in the African mango *Mangifera indica* (Sakho et al., 1985). It is important to note that the values presented in the tables are only approximate ranges due to the vacuum filtration step which may have caused some loss in highly volatile components as well as differences in polarities of these components relative to the internal standard.

M. indica, a taxonomically close relative to *Spondias*, has a flavor profile characterized by the presence of free acids: acetic (0.7%), butyric (6%), hexanoic (5%), and the ester, ethyl 3-hydroxybutanoate (5%). These compounds are part of the contribution of lipid metabolism to the aroma of mango fruit (Sakho et al., 1985). It is suggested that these compounds are derived in *S. mombins* through the same mechanism of lipid metabolism. A homologous series of 3-hydroxybutanoates were noted in *Spondias* free fraction volatiles, such as ethyl 3-hydroxybutanoate, propyl 3-hydroxybutanoate, butyl 3-hydroxybutanoate, isobutyl 3-hydroxybutanoate, and hexyl

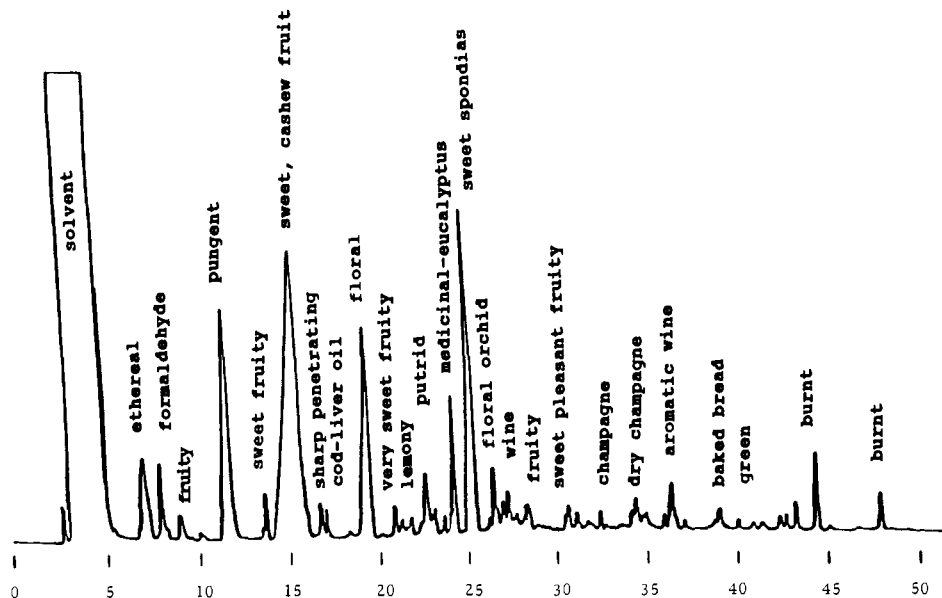


Figure 1. Chromatogram of the free fraction volatiles with odor descriptions.

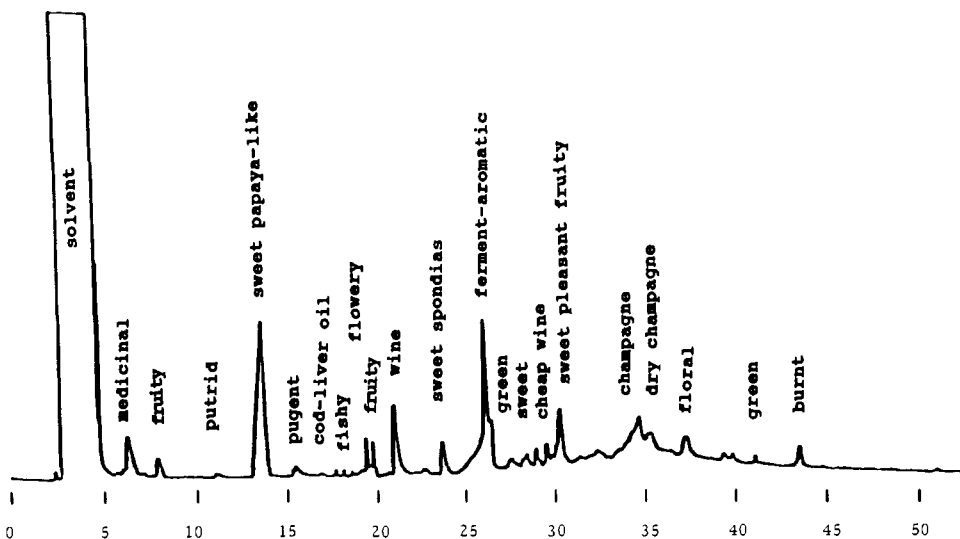


Figure 2. Chromatogram of the glycosidically bound fraction with odor descriptions.

3-hydroxybutanoate. These have all previously been reported in two varieties (Alphonso and Baladi) of mango (Engel and Tressl, 1983a). However, Idstein and Schreier (1985) failed to identify the hydroxy-substituted esters of butanoic acid in the Indian Alphonso variety of mango. This is not surprising as several authors had noted the varietal variability of volatiles in mango (Engel and Tressl, 1983a; MacLeod and Pieris, 1984; Idstein and Schreier, 1985; Hunter et al., 1974; Sakho et al., 1985).

A general overview of the flavor profile of *S. mombins* reveals that it has aroma compounds quite similar to that of the cashew apple (*Anacardium occidentale*) fruit whose major components are esters, some alcohols, aldehydes, ketones, and acids as reported by Maciel et al. (1986) and MacLeod and Troconis (1982). Maciel et al. (1986) also reported the presence of sulfur dioxide, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, and it is quite unusual that no sulfur-containing compounds were identified from *S. mombins*, judging from its aroma which showed some sulfuryl notes. It is possible that sulfur-containing species may be present at levels significant with respect to sensory threshold but are at concentrations below the analytical detection limits of this investigation.

Also, the possibility may exist for a loss of sulfur-containing compounds during vacuum filtration.

Table II shows volatile compounds identified in the bound fraction of *S. mombins* after enzymatic treatment (β -glucosidase). The identification was accomplished as stated for the free fraction. A total of 28 compounds are listed comprising 6 hydroxy-substituted esters, 7 alcohols, 3 aldehydes, 7 acids, and other miscellaneous compounds. All are reported for the first time as aglycons of the *Spondias* fruit. It should be noted that the glycosidically bound fraction had no flavor prior to enzymatic hydrolysis, and only after enzymatic treatment was the characteristic sweet and fruity aroma perceived. This finding is in agreement with results of many authors working with various other fruits (Williams et al., 1982a,b; Engel and Tressl, 1983b; Wilson et al., 1984; Gunata et al., 1985; Schwab and Schreier, 1988b). Benzaldehyde, butanol, hexanol, benzyl alcohol, and benzoic acid are some aroma active compounds identified in the bound fraction; these compounds have been reported as glycosides in apples and papaya fruits by Schwab and Schreier (1988) and Schwab et al. (1989), respectively, using simultaneous enzyme catalysis extraction and treatment with enzyme

emulsion to release these aglycons. Most compounds identified have free hydroxys which are conjugated to glucose. An example is butyl 3-hydroxybutanoate, which has the free hydroxy in the 3-position. However, other compounds such as benzaldehyde exist as conjugates such as cyanohydrins. It is interesting to note the presence of cinnamic acid, 4-hydroxycinnamic acid, and 3-methoxy-4-hydroxycinnamic acid in the glycosidically bound fraction of *Spondias* as these acids are well-known for their contribution to fruity and balsamic aroma in fruits; their total absence in the free fraction is quite surprising.

Sensory sniffing of the GC effluent of the free and glycosidically bound fraction volatiles gave many interesting aroma descriptions (Figures 1 and 2) which can generally be described as fruity and pleasantly sweet. Other varied notes included lemony, floral, pungent, fishy-cod liver oil, sweet papaya-like, various intensities of winelike odor, and the late appearance of a baked-bread odor. Of particular interest and importance was the peak which gave a typically sweet *S. mombins* odor which was identified as the aroma character peak of *S. mombins* and identified as isobutyl 3-hydroxybutanoate on correlation with mass spectral data. In addition, hexanol, ethyl 3-hydroxybutanoate, propyl 3-hydroxybutanoate, benzaldehyde, butyl 3-hydroxybutanoate, linalool, and isobutyl 3-hydroxybutanoate at different concentrations are present in both the free and glycosidically bound fractions of *S. mombins*, and it is reasonable to suggest that they all contribute to the fruity pleasantly sweet aroma profile of *Spondias*.

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