

Characterization of Glycosidically Bound Aroma Compounds in the African Mango (*Mangifera indica* L.)

Jide Adedeji,[†] Thomas G. Hartman,[‡] J. Lech,[‡] and Chi-Tang Ho^{*,†}

Department of Food Science and The Center for Advanced Food Technology, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903

Glycosidically bound volatile constituents of the African mango (*Mangifera indica*) 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 volatile components from the glycosidically bound fraction were released by enzymatic hydrolysis with almond β -glucosidase. Volatile components from the glycosidically bound fraction were analyzed by gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS). A total of 33 compounds were reported in the glycosidically bound fraction including 8 monoterpene alcohols, 5 aldehydes, 4 acids, 7 esters, and 5 C₁₃ norisoprenoids.

INTRODUCTION

Mango closely follows the banana (*Musa* sp.) as the most important commercial tropical fruit cultivated worldwide for fresh market consumption (MacLeod and de Tonconis, 1982; MacLeod and Pieris, 1984).

The economic importance and widespread consumption of the mango fruit (*Mangifera indica*) have attracted a notable amount of investigations into its flavor composition (Angelini et al., 1973; Hunter et al., 1974; Bandyopadhyay and Gholap, 1973; Gholap and Bandyopadhyay, 1980, 1984; MacLeod and de Toconis, 1982; Engel and Tressl, 1983a; MacLeod and Pieris, 1984; MacLeod and Synder, 1985). Some authors have noted the interesting varietal variations that exist in the aroma chemistry of this fruit (MacLeod and Pieris, 1984; Engel and Tressl, 1983a; Idstein and Schreier, 1985; Sakho et al., 1985). However, their efforts had been directed at the characterization of only the free volatile compounds in mango. There have been no papers on the glycosidically bound volatiles in the African mango.

Recently, the importance of glycosidically bound volatile constituents and their contributions to fruit aroma is receiving increasing attention by many workers (Wilson et al., 1984; Gunata et al., 1985; Schwab and Schreier, 1988a,b; Schwab et al., 1989; Adedeji et al., 1991). It has been noted by many authors that these glycosidically bound volatiles in fruits and plant tissues (subsequent to their release by enzymatic and/or chemical hydrolysis) increase the yield of essential oils and serve as a controlled release mechanism in the liberation of free volatiles during fruit ripening and climacteric; also, glycosides serve as precursors to free fruit volatiles and aroma.

This paper reports the existence and characterization of the glycosidically bound volatiles in the African mango.

EXPERIMENTAL PROCEDURES

Reagents. The solvents (*n*-pentane, diethyl ether, methanol, and acetonitrile) were of HPLC grade from Fisher Scientific Co. (Springfield, NJ). Standard of *n*-paraffins (C₆-C₂₆) was 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 mango fruits were collected from the 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. facility, samples were kept under frozen storage and later thawed prior to analysis.

Separation of the Free and Bound Aroma Compounds. Mango fruits were washed and the skin and flesh removed from the hard stone. The recovered flesh was blended in a high-shear blender with distilled water at ambient temperature. About 350 mL of puree obtained was centrifuged at 9000 rpm for 15 min; 150 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 50 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 and discarded.

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 discarded as was the previously obtained free fraction eluate. 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 the bound fraction before concentration.

GC and GC-MS Analyses of Volatile Compounds. A Varian 3400 gas chromatograph equipped with a fused silica column (60 m \times 0.32 mm i.d.; df = 0.25 μ m; DB-1; J&W Scientific) and a flame ionization detector was used to analyze the volatile components in the 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, raised to 280 °C at 2 °C/min and held for 10 min. Semiquantitative determinations were carried out with a Varian 4270 integrator. The estimated concentration was obtained according to the internal standard method where the ratio of the weight of internal

[†] Department of Food Science.

[‡] The Center for Advanced Food Technology.

Table I. Volatile Compounds in Glycosidically Bound Fraction of Mango

compound	RI (DB-1)	estimated concn in fruit pulp, ppm
acetaldehyde	609	1.952
2-butanol	692	0.389
butyric acid	731	0.459
propyl 3-hydroxybutanoate	972	0.030
butyl 3-hydroxybutanoate	980	0.005
isobutyl 3-hydroxybutanoate	992	0.069
benzaldehyde	995	0.186
benzyl alcohol	1047	0.007
2-phenylethyl alcohol	1102	0.380
epoxylinalool	1163	0.005
<i>p</i> -cymen-8-ol	1169	0.006
benzoic acid	1191	0.003
myrtenol	1194	0.013
safranal	1244	0.441
ipsdienol	1266	0.273
<i>p</i> -cymen-7-ol	1278	0.004
9-hydroxymegastigma-4,6-dien-3-one	1281	0.023
perillyl alcohol	1295	0.005
thymol	1310	0.004
ethyl benzaldehyde	1340	0.033
<i>endo</i> -isocamphenone	1320	0.018
eugenol	1345	0.028
α,α -dimethylbenzeneethanol	1422	0.044
3-isopropenyl-2,5-dimethyl-3,4-hexadien-2-ol	1446	0.063
ethyl <i>o</i> -hydroxyphenyl acetate	1496	0.006
<i>trans</i> -verbenol	1545	0.020
3-hydroxy- β -damascone	1566	0.046
9-hydroxymegastigma-4,7-dien-3-one	1580	0.042
9-hydroxymegastigma-4,6,7-trien-3-one	1594	0.062
vomifoliol	1686	0.041
ferulic acid	1881	0.019
palmitic acid	1956	0.013
3-hydroxypropyl oleate	2154	0.026

standard to the area percentage of internal standard is calculated against the area percent of unknown peak for each of the identified compounds. This value is divided by the weight of sample used to give a factor which is then used to multiply area percent for each individual peak. Linear retention indices were calculated against *n*-paraffin standards (C₆-C₂₆) as references (Majlat et al., 1974).

GC-MS analysis was accomplished by using a Varian 3400 GC 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 at 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 isobutane as reagent gas was used to confirm the molecular weight of many components which do not yield molecular ions in the electron ionization (EI) mode.

RESULTS AND DISCUSSION

Table I shows the glycosidically bound volatile compounds identified in the African mango *M. indica*. The identification was accomplished by searching the library database of the National Bureau of Standards (NBS) or using published literature and data (Swigar and Silverstein, 1981; MSDC, 1974; Heller and Milne, 1980; Ten Noever de Brauw, et al., 1983; MacLafferty and Staufner, 1989; Honkanen et al., 1965; McFadden et al., 1965; Marlatt et al., 1991; Sefton et al., 1989; Winterhalter, 1990). 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 33 volatile compounds were identified in the glycosidically bound fraction of the African mango; the

aroma profile is characterized by 8 monoterpene alcohols, 7 aldehydes, 4 acids, 7 esters, and 5 C₁₃ norisoprenoids.

Studies in the characterization of free aroma volatiles of mango have shown that certain monoterpene hydrocarbons are important to mango flavor (MacLeod and de Toconis, 1982; Engel and Tressl, 1983; Sakho et al., 1985). Esters and other carbonyls have been reported to impart a fruity note, and lactones and some fatty acids are important to mango flavor (MacLeod and de Toconis, 1982; MacLeod and Pieris, 1984; MacLeod and Snyder, 1985; Engel and Tressl, 1983; Sakho et al., 1985). In this study of the glycosidically bound volatiles of mango, none of these contributors to the mango free volatiles have been found, yet after the enzymatic hydrolysis of our glycosidically bound sample, the characteristic aroma of mango was evident. This finding further complicates an already complex aroma variability found in different varieties, cultivars, geographic location, and state of fruit maturity in the mango. Some authors have suggested that because of the complexity of components, there can be no typical flavor component or flavor formulation for mangoes (MacLeod and Snyder, 1985; Engel and Tressl, 1983; Hunter et al., 1974).

We have reported the presence of esters such as propyl 3-hydroxybutanoate, butyl 3-hydroxybutanoate, isobutyl 3-hydroxybutanoate, and 3-hydroxypropyl oleate in the bound fraction of mango. Engel and Tressl (1983) reported also large differences in the ester fractions of Alphonso and Baladi varieties. They found that the Baladi variety contains the complete series of ethyl esters of the even-numbered fatty acids from C₂ to C₁₆ in relatively high amounts, while they were absent or present only in trace amounts in the Alphonso mangoes. Ethyl 3-hydroxybutanoate and ethyl 3-hydroxyhexanoate are known already as constituents of other tropical fruits like pineapple (Creveling et al., 1986), passion fruit (Winter and Kloti, 1972), and wood apple (MacLeod and Pieris, 1981). All three hydroxy-substituted esters of butanoic acid had been reported in hog plum (Adedeji et al., 1991).

Some researchers have suggested that the ratio of palmitic to palmitoleic acid determines the flavor quality of the ripe mango, a ratio of less than 1 resulting in strong aroma and flavor (Bandyopadhyay and Gholap, 1973). It is interesting to note the presence of palmitic acid and 3-hydroxypropyl oleate in this study.

Epoxylinalool, myrtenol, safranal, ipsdienol, perillyl alcohol, thymol, *endo*-isocamphenone, and *trans*-verbenol are all hydroxyl, carbonyl, and acetate monoterpenes in which the hydroxyl groups attached to sugars condense with the alcohol or phenol groups of the aglycons. The identification of these classes of compounds is consistent with the chemistry of fruit glycosides. This explains the absence of monoterpene hydrocarbons and especially car-3-ene, which has been reported by several authors (MacLeod and de Toconis, 1982; Engel and Tressl, 1983) as playing an important role in the aroma chemistry of mango. Lactones are notably absent in the bound fraction of the African mango, despite our search for such spectra (Honkanen et al., 1965; McFadden et al., 1965).

9-Hydroxymegastigma-4,6-dien-3-one, 3-hydroxy- β -damascone, 9-hydroxymegastigma-4,7-dien-3-one, 9-hydroxymegastigma-4,6,7-trien-3-one, and vomifoliol are important C₁₃ norisoprenoids identified in the glycosidically bound fraction of the African mango; their mass spectra match completely with the published spectra of Winterhalter (1990). Many researchers have reported the existence of these extremely low odor threshold compounds as significant aroma contributors in such fruits as grapes

(Williams et al., 1982; Sefton et al., 1989), apples (Schwab and Schreier, 1988b), papayas (Schwab et al., 1989), tomato (Marlatt et al., 1991), and quince (Winterhalter and Schreier, 1988). These C₁₃ norisoprenoids are believed to be degradation products of carotenoids.

ACKNOWLEDGMENT

This is New Jersey Agricultural Experiment Station Publication No. 10205-3-91 supported by state funds. We thank The Center for Advanced Food Technology for mass spectrometric analysis support.

LITERATURE CITED

- Adedeji, J.; Hartman, T. G.; Rosen, R. T.; Ho, C.-T. Free and glycosidically bound aroma compounds in hog plum (*Spondias mombins* L.). *J. Agric. Food Chem.* **1991**, *39*, 1494-1497.
- Angelini, P.; Bandyopadhyay, C.; Rao, B. Y. K.; Gholap, A. S.; Bazinet, M. L. 1973. Studies on aroma of ripe mango pulp: Identification of aroma bearing compounds. Presented at the 33rd Annual Meeting of the Institute of Food Technologists, Miami Beach, FL; 1973; Abstract 366.
- Bandyopadhyay, C.; Gholap, A. S. Relationship of aroma and flavor characteristics of Mango (*Mangifera indica* L.) to fatty acid composition. *J. Sci. Food Agric.* **1973**, *24*, 1497.
- Creveling, R. K.; Silverstein, R. M.; Jennings, W. G. Volatile components of pineapple. *J. Food Sci.* **1968**, *33*, 284.
- Engel, K. H.; Tressl, R. Studies on the volatile components of two mango varieties. *J. Agric. Food Chem.* **1983a**, *31*, 796-801.
- Engel, K. H.; Tressl, R. Formation of aroma components from non volatile precursors in passion fruit. *J. Agric. Food Chem.* **1983b**, *31*, 998-1002.
- Gholap, A. S.; Bandyopadhyay, C. Fatty acid biogenesis in ripening mango (*Mangifera indica* L. Var. Alphonso). *J. Agric. Food Chem.* **1980**, *28*, 839.
- Gholap, A. S.; Bandyopadhyay, C. Characterization of green aroma of raw mango (*Mangifera indica* L.). *J. Sci. Food Agric.* **1984**, *28*, 885.
- Gunata, Y. Z.; Bayonove, R.; Baumes, R. L.; Cordinnier, R. E. The aroma of grapes. 1. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985**, *331*, 83-90.
- Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectral Data Base*; U.S. Department of Commerce: Washington, DC, 1980.
- Honkanen, E.; Moisio, T.; Karnoven, P. The mass spectra of some aliphatic lactones. *Acta Chem. Scand.* **1965**, 370-374.
- Hunter, G. L. K.; Bucek, W. A.; Radford, T. Volatile components of canned alphonso mango. (*Mangifera indica*). *J. Food Sci.* **1974**, *39*, 900-903.
- Idstein, H.; Schreier, P. Volatile constituents of alphonso mango (*Mangifera indica*). *Phytochemistry* **1985**, *24*, 2312-2316.
- Maciel, M. I.; Hansen, T. J.; Aldinger, S. B.; Labows, J. N. Flavor chemistry of cashew apple juice. *J. Agric. Food Chem.* **1986**, *34*, 923-927.
- MacLeod, A. J.; de Toconis, N. G. Volatile flavor components of Cashew "Apple" (*Anacardium occidentale*). *Phytochemistry* **1982**, *21*, 2527-2530.
- MacLeod, A. J.; Pieris, N. M. Volatile flavor components of wood apple (*Feronia limonia*) and a processed product. *J. Agric. Food Chem.* **1981**, *29*, 49.
- MacLeod, A. J.; Pieris, N. M. Comparison of some mango cultivars. *Phytochemistry* **1984**, *23*, 361-366.
- MacLeod, A. J.; Snyder, C. H. Volatile components of two cultivars of mango from Florida. *J. Agric. Food Chem.* **1985**, *33*, 380-384.
- Marlatt, C.; Chien, M.; Ho, C. T. C₁₃ Norisoprenoids bound as glycosides in tomato. *J. Essent. Oil Res.* **1991**, *3*, 27-31.
- Majlat, P.; Erdos, Z.; Takacs, J. Calculation and application of retention indices in programmed temperature gas chromatograph. *J. Chromatogr.* **1974**, *91*, 89-110.
- McFadden, W. H.; Day, E. A.; Diamond, M. J. Correlations and anomalies in mass spectra. Lactones. *Anal. Chem.* **1965**, *37*, 89-92.
- McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS registry of mass spectra data*; Wiley-Interscience: New York, 1989.
- MSDC. *Eight Peak Index of Mass Spectra*, 2nd ed.; Mass Spectrometry Data Center: Reading, U.K., 1974.
- Sakho, M.; Crouzet, J.; Seck, S. Volatile components of African mango. *J. Food Sci.* **1985**, *50*, 548-550.
- Schwab, W.; Schreier, P. Aryl-β-D-glucosides from carica papaya fruit. *Phytochemistry* **1988a**, *27*, 1813-1816.
- Schwab, W.; Schreier, P. Simultaneous enzyme catalysis extraction (Sece): A versatile technique for the study of flavor precursors. *J. Agric. Food Chem.* **1988b**, *36*, 1238-1242.
- Schwab, W.; Mahr, C.; Schreier, P. Studies on the enzymic hydrolysis of bound aroma components from carica papaya fruit. *J. Agric. Food Chem.* **1989**, *37*, 1009-1012.
- Sefton, M. A.; Skouroumonis, R. A.; Massey-Westrop, R. A.; Williams, P. A. Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust. J. Chem.* **1989**, *43*, 2071-2084.
- Swigar, A. A.; Silverstein, R. M. *Monoterpenes*; Aldrich Chemical Co.: Milwaukee, WI, 1981.
- Ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of mass spectra of volatile components in foods*; Central Institute for Nutrition and Food Research: Zeist, The Netherlands, 1983.
- Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westrop, R. A. Use of C₁₈ reversed phase liquid chromatography for the isolation of monoterpene glycosides and norisoprenoid precursors from grape juice and wines. *J. Chromatogr.* **1982a**, *235*, 471-480.
- Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westrop, R. A. Novel monoterpenes disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry* **1982b**, *21*, 2013.
- Wilson, B.; Strauss, C. R.; Williams, P. J. Changes in free and glycosidically bound monoterpenes in developing muscat grapes. *J. Agric. Food Chem.* **1984**, *32*, 919.
- Winter, M.; Kloti, R. Volatile constituents of passion fruit. *Helv. Chim. Acta* **1972**, *55*, 1916.
- Winterhalter, P. Bound terpenoids in the juice of the purple passion fruit (*Passiflora edulis* Sims). *J. Agric. Food Chem.* **1990**, *38*, 452-455.
- Winterhalter, P.; Schreier, P. J. Free and bound C₁₃ norisoprenoids in quince (*Cydonia Oblonga*, Mill). *J. Agric. Food Chem.* **1988**, *36*, 1251-1256.

Received for review November 4, 1991. Accepted February 5, 1992.