Free and Glycosidically Bound Volatile Compounds from Two Banana Cultivars: Valery and Pequeña Enana

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Headspace composition and glycosidically bound volatile compounds from two banana cultivars, Valery and Pequeña Enana, have been studied. Glycosides were isolated from aqueous extracts of banana pulp by means of an Amberlite XAD-2 column. Volatile compounds were released by enzymatic hydrolysis using almond β-glucosidase and further analyzed by HRGC/MS. Twenty-five main aglycons were analyzed for the first time in banana fruit pulp. These aglycons can be grouped in two biogenetically different groups: fatty acids and shikimic acid-derived compounds. Alcohols, such as decan-1-ol and 2-phenylethanol, and acids, such as 3-oxo-pentanoic acid, 3-methylbutanoic acid, and benzoic acid, were quantitatively the most important aglycons in glycosides isolated from both banana cultivars.

Keywords: *Aroma; flavor; banana; headspace; enzymatic hydrolysis; glycosides; volatiles*

INTRODUCTION

Flavor and aroma are properties of food that strongly influence its acceptance by consumers. This is especially true for tropical and subtropical fruits. Banana is a tropical fruit with a pleasant flavor, widely consumed throughout the world. Analytical research on the aroma compounds of this fruit carried out for 30 years was reviewed by Engel et al. (1990). The characteristic aroma of bananas arises from a complex mixture of compounds, esters of short-chain fatty acids such as acetates, butanoates, and 3-methylbutyl esters (Macku and Jennings, 1987; Pérez et al., 1993). Other key flavor compounds for bananas present at extremely low levels, at least in the free form, have just been identified (Shiota, 1993).

Recent studies on fruits and vegetables have shown that a significant portion of volatile flavor compounds may occur in many plants as nonvolatile precursors, such as glycosides (Gunata et al., 1985; Schwab and Schreier, 1988; Williams et al., 1989; Latza et al., 1996). The study of these glycosidically bound volatiles in fruits is important from two different points of view: one is a contribution to the understanding of flavor biogenesis during fruit ripening and another, from a more practical approach, is useful information for predicting the flavor of fruit products such as juices and wines (Stahl-Biskup et al., 1993). Free volatile compounds of sensory significance may be released from these odorless precursors by acidic or enzyme-mediated hydrolysis (Williams, 1993). The enzymatic hydrolysis pathway is present in plants due to the presence of a glycosidase complex, which includes a β-glucosidase activity (Heidlas et al., 1984; Aryan et al., 1987). In fact, β-D-glucosides appear to constitute a major part of the plant's glycosidic pool (Gunata et al., 1990; Shoseyov et al., 1990).

Although a number of studies have been carried out on the qualitative and quantitative aroma composition of bananas, information about precursors and the biogenesis of the aroma constituents is scarce. The role of the amino acids leucine, isoleucine, and phenylalanine as precursors of branched-chain and phenolic alcohols has been reported (Tressl and Drawert, 1973; Drawert and Berger, 1981), and the substrate selectivity of enzymes such as alcohol dehydrogenase and alcohol acyltransferase in relation to the aroma composition of bananas has been studied by several authors (Olías et al., 1995; Wyllie et al., 1996), but no studies have been carried out on glycosidically bound volatiles in bananas.

Musa acuminata (AAA) cv. Pequeña Enana is a banana cultivar belonging to the subgroup Cavendish extensively cultivated in the Canary Islands (Spain), with more than 10 000 cultivated ha and an average production of 400 000 tons per year. In this study the aroma composition of this banana cultivar has been compared to that of Valery bananas, one of the most important banana cultivars from Costa Rica, which has been recently introduced in the Spanish market. Free and glycosidically bound volatile compounds of both banana cultivars were studied, and main qualitative and quantitative differences were established. To the best of our knowledge, this is the first paper reporting the occurrence of glycosidically bound volatiles in banana.

EXPERIMENTAL PROCEDURES

Materials. Ripe banana fruits from Costa Rica, *M. acuminata* cv. Valery, and fruits from Canary Islands, *M. acuminata* subgroup Cavendish cv. Pequeña Enana, were purchased in a local market. Fruits were carefully matched according to degree of ripeness. Maturity of the fruits was determined by measuring fruit color and firmness.

Banana skin color was evaluated using a Minolta CR-200 portable tristimulus colorimeter (Minolta, Ramsey, NY), and expressed as *L*, *a**, *b** values. Three measures from two opposite zones were done for each fruit.

Firmness was measured as penetration force with a Zwick 3303 penetrometer, using a 5 mm plunger tip, and expressed as newtons (N). Five measures per fruit were performed.

Analysis of Free Volatile Compounds. *Headspace Concentration of Volatiles.* Banana fruits (1 kg) were placed in a desiccator at 25 °C and swept with purified air for 1 h, flow rate 50 mL/min. The effluent gas was passed through the internal standard container, and then volatiles were trapped in a Tenax TA trap (100 mg, 60/80 mesh, Chrompack). Methyl

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octanoate was selected as internal standard. Adsorbent traps were conditioned prior to use by heating them at 300 °C for several hours and again at 220 °C with passage of the carrier gas. Three samples per cultivar were analyzed. Blank tests were carried out.

Desorption Method. The desorption of volatiles trapped in the Tenax TA was carried out in the opposite direction to adsorption by using a Chrompack thermal desorption cold trap injector (TCT). The temperatures and times of injection were controlled by an injector control unit. Desorption was carried out by heating the trap at 220 °C for 5 min. Volatiles were then transported by the carrier gas with a desorption flow rate of 7 mL/min to a fused silica cold trap previously cooled to −110 °C with liquid nitrogen for 5 min, where they condensed. Finally, the samples were injected into the capillary GC system by flash heating the cold trap at 170 °C.

Volatile HRGC/MS Analysis. The TCT was installed on the GC/MS system, equipped with a J&W DB-Wax fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). The column temperature was held at 40 °C for 6 min and then increased to 200 °C at 2 °C/min. The carrier gas (helium) flow rate was 1 mL/min. Quantitation was performed by normalization of the values obtained from the integrator to that of methyl octanoate. Peak areas were converted to nanograms per gram of banana fruit per 3 L of headspace [ng (g of FW)$^{-1}$ 3 L^{-1}]. The end of the fused silica column was inserted directly into the ion source block. A Fisons MD800 mass selective detector and a MassLab V1.1 data system was used for mass spectrometric analyses. The spectra were recorded at an ionization voltage of 70 eV and a ion source temperature of 200 °C. Sample components were verified by comparison of the mass spectral data with those of authentic reference compounds or tentatively identified by mass spectrum matching using the NIST mass spectral library collection.

Analysis of Glycosidically Bound Volatile Compounds. *Isolation of Glycosidic Extracts*. Five hundred grams of banana pulp, from five different fruits, was sliced and blended in 500 mL of distilled water containing 0.2 M glucono-δ-lactone as a highly specific glycosidase inhibitor (Matsuura and Obata, 1993). The mixture was centrifuged at 13000*g* for 30 min at 15 °C, and vacuum filtered by using Celite 545 (10 g/25 mL of banana extract) and Whatman No. 1 filter paper. The juice obtained was passed through a solvent-washed (Gunata et al., 1985) Amberlite XAD-2 (200 × 10 mm) column, with a flow rate of ∼2 mL/min. After washings with 100 mL of distilled water and 250 mL of hexane, isolation of glycosides was performed by elution with 250 mL of methanol. This eluate was concentrated to dryness (Vigreux column, 45 °C) under reduced pressure, redissolved in 30 mL of 0.2 M citrate−phosphate buffer (pH 5.0), and washed with dichloromethane (3 × 5 mL) to remove any remaining volatile compound.

Enzymatic Hydrolysis. Glycosidically bound compounds were enzymatically hydrolyzed to release aglycons. The hydrolytic activities of two different enzymes were compared, almond β-glucosidase from almond (Sigma) and pectinase from mold (Sigma). In an analogous experiment three incubation times, 24, 48, and 72 h, were compared. In a typical experiment 30 mg of β-glucosidase was used, and 24 h of incubation time at 38 °C was the standard condition. A blank test without addition of enzyme was also carried out. After hydrolysis, octanol was added as an internal standard, and the liberated aglycons were extracted with dichloromethane (3 × 5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure (Vigreux column, 45 °C) to a final volume of 1 mL. The concentrated products were esterified with diazomethane (CH_2N_2) (Cohen, 1984) prior to their GC analysis. Under these conditions phenolic and acidic functional groups were converted into their methoxy derivatives and methyl esters, respectively. A control sample, nonesterified with diazomethane, was also analyzed. Three samples per cultivar were obtained according to this experimental procedure.

Capillary Gas Chromatography/Mass Spectrometry (HRGC/MS). Extracts were subjected to HRGC analyses on a Fison 8000 GC equipped with a J&W DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The column temperature was held at 40 °C for 5 min and then increased to 200 °C at 2 °C/min. The carrier gas (helium) flow rate was 1 mL/min. Quantitative determinations were obtained using octanol as an internal standard, and peak areas were expressed as nanograms per grams of banana fruit weight (ng/g of FW). Three samples per cultivar were analyzed.

A Fisons MD 800 mass selective detector and a MassLab V.1.1 data system was used for mass spectrometric analyses, as previously described under Volatile HRGC/MS Analysis.

RESULTS AND DISCUSSION

Free Volatile Compounds. From the point of view of objetive flavor quality meassurement, the sum of released volatiles can be used as a quality parameter. Table 1 shows the free volatile compounds observed in the headspace of both banana cultivars, in their elution order (t_R). The table includes the relative quantity of each compound, expressed as ng (g of FW)$^{-1}$ 3 L^{-1}, of headspace, which was determined by using methyl octanoate as internal standard, without considering recovery of volatiles and GC-FID response factors. Flavor evaluation of bananas with a similar degree of ripeness, assessed by color and firmness meassurements, showed that fruits of cv. Pequeña Enana had slightly lower aroma intensity than Valery bananas did, as can be deduced from the total amount of volatiles analyzed in the headspace of each variety. Esters are quantitatively the dominant group of volatiles in ripe banana fruits (Engel et al., 1990). Acetates account for ∼40% of volatiles identified in the headspace of the two samples, ethyl acetate being the major component in Valery bananas (20.94%), while methyl acetate is the most abundant volatile compound in Pequeña Enana (32.06%). 3-Methylbutyl acetate, considered the character impact compound in banana flavor (Macku and Jennings, 1987), is one of the few compounds present in a higher amount in banana fruits from Canary Islands, while other short esters such as butyl acetate, with a fruity-estery odor description, are present only at very low levels in Pequeña Enana cultivar. Alcohols and carbonyl compounds impart green-woody notes that also contributed to banana flavor (Engel et al., 1990). The amounts of pentan-2-one detected in both samples were quite similar, being the second most abundant volatile compound in both banana cultivars. Clear differences were found in the alcoholic fraction with free alcohols accounting for 32% of the volatiles in Valery bananas and only 15% in fruits var. Pequeña Enana. Although comparison of both GC profiles showed no qualitative differences, the quantitative relationship among different compounds in any flavor fraction may have a drastic effect on final aroma (Pérez et al., 1993). In this sense, the headspace composition of cv. Valery showed a richer aroma profile, with a higher number of compounds contributing to the overall aroma intensity (concentration > 2%), which gives floral, fruity, sweet notes that are absent in the simpler aroma of Pequeña Enana bananas.

Analysis of Glycosidically Bound Volatiles. As described under Experimental Procedures, two hydrolytic enzymes, pectinase and β-glucosidase, and three incubation times were assayed. In a previous study on grape glycosides (Gunata et al., 1990), a comparison among two β-glucosidases and pectinase (Pectinol VR, Rhom) enzymes revealed a higher efficiency of pectinase in the hydrolysis of some glycosidic compounds such as monoterpene glycosides. Almond β-glucosidase has been sucessfully used to release volatile compounds from glycosidic extracts of different fruits: e.g., apple

Table 1. Volatile Compounds Identified in the Headspace of Ripe[a] Fruits from Two Banana Cultivars: Valery from Costa Rica and Pequeña Enana from Canary Islands (Spain)

		Valery		Pequeña Enana	
compound	t_R, min	ng (g of FW)$^{-1}$ 3 L^{-1}	%	ng (g of FW)$^{-1}$ 3 L^{-1}	%
(**1**) methyl acetate	4.7	1.270	7.75	2.615	32.06
(**2**) ethyl acetate	8.0	3.435	20.94	0.475	5.82
(**3**) butan-2-one	8.4	0.230	1.40	0.167	2.04
(**4**) benzene	9.5	0.166	1.01	0.134	1.64
(**5**) pentan-2-one	11.5	2.201	13.43	1.270	15.58
(**6**) butyl acetate	12.9	1.425	8.66	0.150	1.84
(**7**) toluene	13.9	0.256	1.52	0.096	1.17
(**8**) 3-methylbutyl acetate	16.6	0.416	2.53	0.764	9.37
(**9**) 2-methylpropanol	18.1	0.751	4.58	0.215	2.63
(**10**) pentyl acetate	19.2	tr		0.032	0.39
(**11**) pentan-2-ol	20.6	1.321	8.05	0.753	9.24
(**12**) 2-methylbutyl butanoate	21.5	0.014	0.08	0.013	0.19
(**13**) butan-1-ol	22.0	0.988	6.03	0.182	2.23
(**14**) hex-2-enal	24.9	0.013	0.08	0.043	0.52
(**15**) 2-pentyl 2-methylpropanoate	25.3	0.026	0.15	0.036	0.44
(**16**) 2-butyl butanoate	25.6	0.047	0.28	0.006	0.09
(**17**) 3-methylbutanol	26.0	2.082	12.69	0.193	2.36
(**18**) 3-methylbutyl butanoate	29.1	tr		0.008	0.09
(**19**) 2-buthoxyethanol	39.6	0.119	0.72	0.096	1.17
(**20**) acetic acid	42.2	0.428	2.61	0.376	4.61
(**21**) methyl decanoate	45.1	0.476	2.90		
(**22**) propanoic acid	53.7	0.190	1.16	0.118	1.44
(**23**) 3-methylbutanoic acid	56.2	tr		0.075	0.92
(**24**) butanoic acid	60.3	0.357	2.17	0.139	1.70
(**25**) pentanoic acid	66.6	0.226	1.37	0.193	2.36
sum		16.437		8.149	

[a] Fruit ripeness was assesed by color and firmness meassurement. Color: Valery, L, 64.22 ± 5.81; a^*, −0.54 ± 2.04; b^*, 39.54 ± 5.51). Pequeña Enana L, 70.9 ± 1.79; a^*, −4.24 ± 0.99; b^*, 48.69 ± 3.94). Firmness: Valery, (17.09 ± 4.13 N; Pequeña Enana (20.0 ± 6.37).

(Schwab and Schreier, 1988), sour cherry (Schwab et al., 1990), pineapple (Wu et al., 1991), and tomato (Marlatt et al., 1992). In the present study on banana glycosides we observed no significant differences in the type of volatile compounds released by both enzymes, but a larger quantity of aromatic aglycons was hydrolyzed by almond β-glucosidase. In the same way, the nature of the aglycon residue greatly affects the rate of hydrolysis. Glucosides with a tertiary alcohol as an aglycon residue are more slowly hydrolyzed than those with a primary alcohol (Gunata et al., 1990). Consequently, banana glycosidic extracts were incubated for 24, 48, and 72 h with β-glucosidase enzyme. An incubation period of 24 h, with 30 mg of almond β-glucosidase, was found to be sufficient to hydrolyze glycosides present in 500 g of banana fruit. The methanol fraction containing glycosidically bound volatile compounds had no odor. Only after enzymatic hydrolysis did this glycosidic fraction have the characteristic fruity, "banana-like" aroma. These results suggested that free volatile components, which are well-known as aroma constituents, were also present as glycosidically bound compounds in the fruit pulp.

Figure 1 shows a typical chromatogram of the glycosidically bound volatile compounds found in Pequeña enana banana. Relative amounts (ng/g of FW) of main bound volatile compounds identified in the pulp of both banana cultivars are listed in Table 2. The comparison of glycosidic extracts from both banana cultivars showed that total amount of glycosidically bound volatiles was also significantly higher in cv. Valery. A complementary analysis by HRGC/MS up to 250 °C to analyze less volatile compounds was carried out with each sample, and several hydrocarbons and fatty acids were identified. These are not included in Table 2 nor are they discussed further. In a blank test performed without enzyme addition, none of these bound volatiles were detected.

The aglycons listed in Table 2 belong to two different biogenetically derived groups: fatty acids and shikimate-derived compounds. From the first group several aliphatic alcohols and acids were identified in both samples. Glycosidically bound forms of simple aliphatic alcohols were first reported in apple (Schwab and Schreier, 1988). In the present study, hexanol and decan-1-ol were found to be present as glycosides in banana pulp. Decan-1-ol was quantitatively the most important bound volatile found in Valery bananas and was also a major compound in cv. Pequeña Enana. Another quantitatively important compound found in this group was γ-decalactone. This compound is a character impact compound in peach flavor (Crouzet et al., 1990) and had not been previously described as an aroma component of banana. Obviously, it may be derived from the corresponding glycosidically bound hydroxy acid. γ-Hydroxy acids can lose water spontaneously to yield a lactone under acidic condictions, and the possibility of lactonization happening during GC injection is not ruled out. C-6 and C-5 aliphatic acids were also present in similar amounts in both glycosidic extracts. Two metabolites of lipoxygenase oxidative pathway of C-18 unsaturated fatty acids were also identified among banana bound volatiles: 9-oxononanoic acid and jasmonic acid (Sanz et al., 1997). The first compound is a well-known component of cucumber aroma. Jasmonic acid is a product of 13-hydroperoxylinolenic acid metabolism, which was first identified as a key component of *Jasminum grandiflorum* essential oil and studied more recently as an endogenous plant growth regulator with a wide range of physiological functions that have not yet been fully elucidated. Several amino acid conjugates of jasmonic acid and glucosides of hydroxylated jasmonates have been described in plants (Hamberg and Gardner, 1992), but no previous evidence has been presented of glycosidically bound jasmonic acid in fruits.

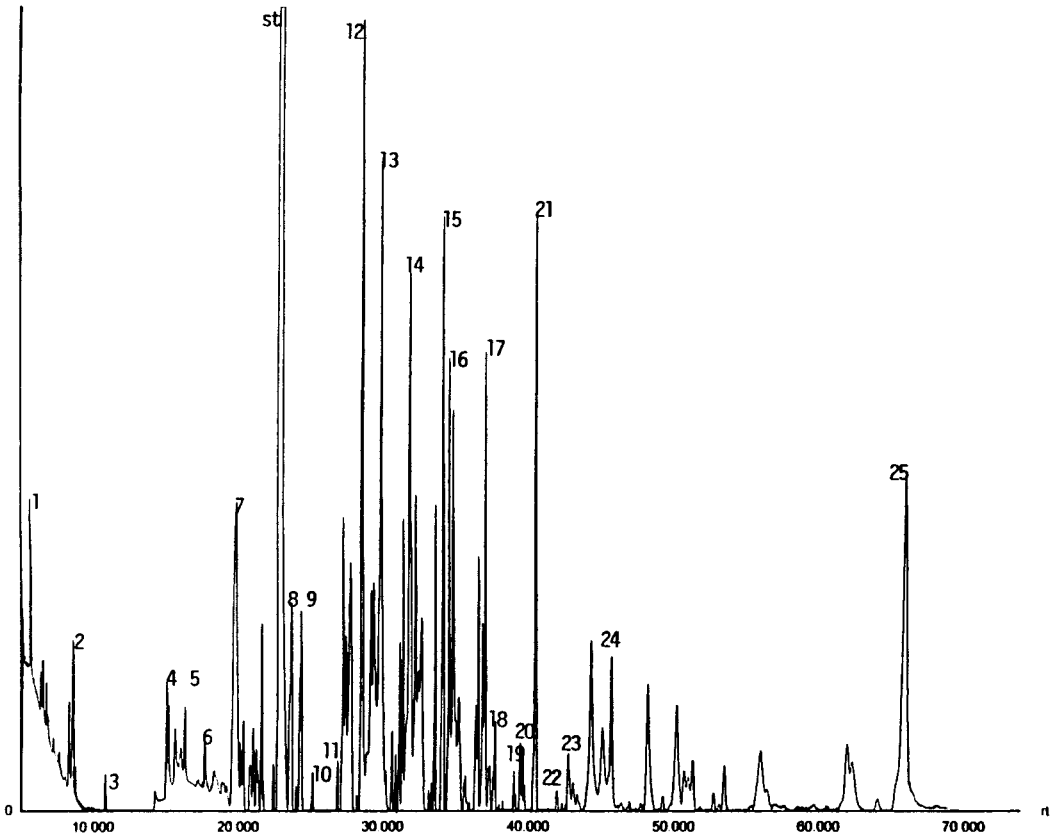

Figure 1. HRGC/MS separation on a fused silica DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) of aglycons obtained from Pequeña Enana banana after enzymatic hydrolysis of a glycosidic extract isolated by means of an Amberlite XAD-2 column. Peak numbering corresponds to compounds listed in Table 2.

Table 2. Glycosidically Bound Volatile Compounds Identified by HRGC/MS after Hydrolysis of Glycosidic Extracts Obtained from Ripe Fruit Pulp of Two Banana Cultivars: Valery and Pequeña Enana

		Valery		Pequeña Enana	
compound	t_R, min	ng (g of FW)$^{-1}$ 3 L^{-1}	%	ng (g of FW)$^{-1}$ 3 L^{-1}	%
(**1**) 3-methylbutanoic acid	5.6	8.42	3.17	12.20	8.65
(**2**) 4-methylhydroxypentan-2-one	8.5	18.72	7.04	2.74	1.94
(**3**) hexanol	10.8	7.16	2.69	1.18	0.83
(**4**) hexanoic acid	15.1	7.76	2.92	10.52	7.45
(**5**) hex-3-enoic acid	15.6	8.44	3.17	6.54	4.64
(**6**) hex-2-enoic acid	17.7	5.22	1.96		
(**7**) 2-(2-ethoxyethoxy)ethanol	20.8	4.48	1.68	4.16	2.95
(**8**) benzoic acid	23.6	24.88	9.36	5.42	3.84
(**9**) 2-phenylethanol	24.3	21.63	8.03	7.08	5.02
(**10**) 2,5,6-trimethyldecane	25.2	5.64	2.12	4.72	3.34
(**11**) phenylacetic acid	26.6	2.56	0.96	2.10	1.49
(**12**) decan-1-ol	29.8	32.00	12.05	12.36	8.77
(**13**) 3-oxo-pentanoic acid	31.8	26.56	10.00	12.64	8.97
(**14**) eugenol	32.2	10.24	3.84	8.00	5.67
(**15**) γ-decalactone	34.1	21.14	7.96	9.70	6.88
(**16**) 9-oxononanoic acid	34.6	13.52	5.09	1.68	1.19
(**17**) dodecanoic acid	37.1	10.11	3.80	1.92	1.36
(**18**) elimicine	37.7	1.30	0.48	5.42	3.84
(**19**) 3,4-dimethoxyacetophenone	38.2	1.02	0.38	4.18	2.96
(**20**) methyleugenol	39.4	2.86	1.07	0.94	0.66
(**21**) 2-furyloctanoic acid	40.5	21.24	7.99	13.38	9.49
(**22**) jasmonic acid	41.3	0.98	0.37	3.46	2.45
(**23**) 3,4,5-trimethoxyacetophenone	42.7	2.38	0.89	2.36	1.67
(**24**) tetradecanoic acid	45.7	7.36	2.77	8.22	5.83
(**25**) hexadecanoic acid	65.8	tr		tr	
sum		265.67		140.92	

[a] Compound numbers correspond to peak numbers in Figure 1.

An important fraction of the analyzed aglycons can be grouped as shikimic acid-derived compounds. The amino acid phenylalanine, one of the most characteristic free amino acids detected in ripe bananas, is a key metabolite in this shikimate pathway. By deamination and decarboxylation of 2-phenylalanine, 2-phenylethanol is formed. Through a different metabolic pathway involving phenylalanine ammonia-lyase, benzoic acids, phenolic acids, and their corresponding derivatives are produced from phenylalanine [reviewed by Sanz et al. (1997)]. 2-Phenylethanol, described as the major component in the bound fraction of tomato (Marlatt et al.,

1992), was identified in both banana cultivars, being one of the most abundant compound in the glycosidic extract of Valery fruits (21.6 ng/g of FW). Benzoic acid was also a major component of the glycosidically bound volatile fraction of Valery bananas, while phenylacetic acid was found in minor amounts in both extracts. The phenol derivatives, eugenol, methyleugenol, and elimicine, contribute to the full-bodied mellow aroma of ripe bananas. Van der Dries (1989) reported the occurrence of relatively large amounts of glycosidically bound eugenol in some species of the Lamiaceae family, and Wu et al. (1991) also reported the presence of glycosidically bound eugenol in pineapple. An important amount of glycosidically bound eugenol was found in both banana extracts. Elimicine and methyleugenol were also detected at lower levels. Other shikimate-derived compounds, 3,4-dimethoxyacetophenone and 3,4,5-trimethoxyacetophenone, were identified in the banana glycosidic extracts. Acetophenone was recently identified as a trace volatile compound in banana flavor (Shiota, 1993), and very similar acetophenone derivatives have been found in the form of glycosides in tomato (Marlatt et al., 1992).

Most of the bound volatiles found in this study are well-known flavor compounds previously identified in other fruits. The influence of these glycosides on banana flavor biogenesis or their hypothetical roles as transport vehicles of physiological active compounds through biological membranes remain to be elucidated. Further work is needed to isolate and identify specific structures of major glycosides in banana fruit.

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