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Physicochemical Characterization of a New Pineapple Hybrid (FLHORAN41 Cv.)

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The physicochemical characteristics (pH, total and soluble solids, and titratable acidity), sugars, organic acids, carotenoids, anthocyanins, volatile compounds, and cell wall polysaccharides of a new pineapple hybrid (FLHORAN41 cultivar) were measured throughout maturation and compared with the Smooth Cayenne cv. At full maturity, the FLHORAN41 cv. has a higher titratable acidity and soluble solids content than the Smooth Cayenne cv. The golden yellow flesh and red−orange to scarlet shell of ripe FLHORAN41 cv. fruits are due to carotenoid and anthocyanin levels that are, respectively, 2.5 and 1.5 times higher than those of the flesh and shell of the ripe Smooth Cayenne cv., respectively. During maturation of the FLHORAN41 cv., there was an increase in all classes of aroma compounds (mainly terpene hydrocarbons and esters), although their relative proportions were similar in both cultivars at full maturity. Cell wall polysaccharides undergo little change during maturation.

KEYWORDS: ***Ananas comosus*; pineapple; hybrid; FLHORAN41; Smooth Cayenne; maturity stage; carotenoids; anthocyanins; volatile compounds; cell wall polysaccharides**

INTRODUCTION

The pineapple *Ananas comosus* (L.) Merr. is a member of the Bromeliaceae family (monocotyledons) and comprises about 2000 species (*1*). With an annual worldwide production of over 14 million tons, it is the eighth most abundantly produced fruit in the world. The most important producers are Thailand, Philippines, Brazil, and China (*2*). In international trade, the numerous pineapple cultivars are grouped into four main classes, namely, Smooth Cayenne, Red Spanish, Queen, and Abacaxi, although there is much variation within each class. The fruit is a syncarp formed by the fusion of spirally arranged fruitlets produced by flowers of the inflorescence (*1*).

Among the many sensory characteristics of a fruit [shape, density, tenderness vs firmness, sweetness, acidity, sweetness/acidity balance, aroma (retronasal sensation perceived when masticating and then swallowing), s.o.], the colors of the shell and the flesh are of prime importance. Indeed, the consumer may in the first instance be attracted by the shell color and then, after cutting but prior to consumption, by the color of the flesh. The Smooth Cayenne pineapple cultivar is distributed worldwide and normally has a pale, yellowish flesh and a pale, brown−orange shell at commercial maturity. In addition to these general sensory characteristics, consumers are nowadays more and more concerned with the nutritional qualities of what they eat. Among fruit nutrients (vitamins, minerals, phenolics, and s.o.), the carotenoids (responsible for the yellow to orange color of fruits) are considered desirable since their antioxidant properties could help prevent some human diseases (e.g., cardiovascular and cerebral diseases, cancers, vision disorders, s.o.) (*3*). If breeding aims were focused on disease and insect resistance for many years, cultivar selection is now also taking into account the appearance, organoleptic, and nutritional characteristics of the fruit.

A wide hybridization program was initiated by CIRAD-FLHOR in 1988 (*4*) to improve overall pineapple quality; it produced several thousands of hybrids, among which 200 small plants were chosen. Finally, a selection program conducted on the above plants between 1990 and 1998 resulted in the isolation of a new cultivar (FLHORAN41 cv.). The female parent (i.e., the seed parent) was the Smooth Cayenne cv. (clone "HA 10"), a typical Hawaian type of pineapple, and the male parent (i.e., the pollen parent) was the Manzana cv. (clone "CO 24"), a variety grown for local consumption at a high altitude, primarily in Columbia (*5*, *6*). Depending on storage conditions, the FLHORAN41 cultivar develops a red−orange to scarlet shell color at maturity (**Figure 1**), making this cultivar potentially quite attractive to the consumer. Furthermore, its flesh at maturity appears as golden yellow (**Figure 2**) while the Smooth Cayenne cv. has a pale, yellowish flesh.

The aim of this work was therefore to carefully characterize this new cultivar throughout maturation and compare it with

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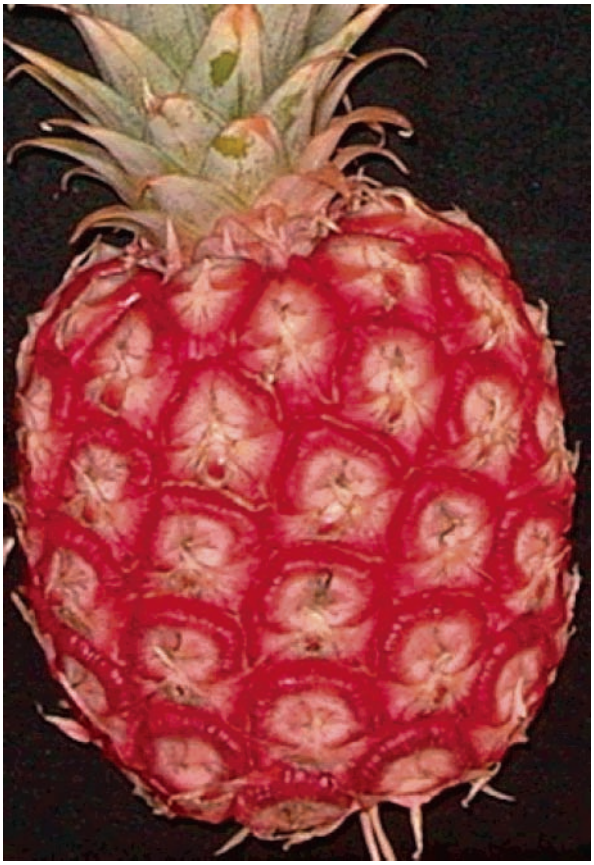

Figure 1. Ripe fruit—shell (FLHORAN41 cv.).

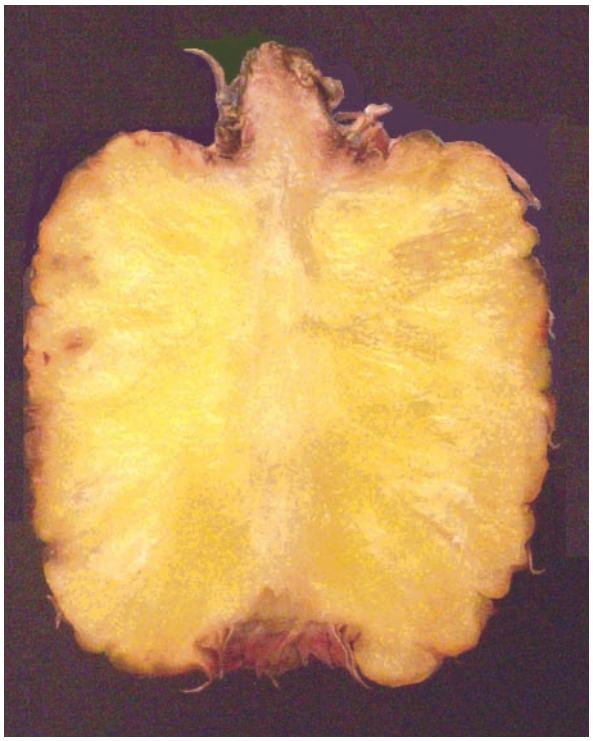

Figure 2. Ripe fruit—flesh (FLHORAN41 cv.).

the well-known Smooth Cayenne cv. Physicochemical characteristics of the flesh (pH, soluble solids, total solids, titratable acidity, sugars, and organic acids) were analyzed during maturation. Furthermore, because the flesh of this new cultivar is golden yellow at maturity, hydrocarbon carotenoids and xanthophylls were measured and partly identified in the flesh and shell of the fruit during maturation. Although anthocyanins have been widely studied in fruits (*7*−*9*), to our knowledge, the evolution of these compounds in the pineapple shell during development has only been qualitatively reported (optical density) in Smooth Cayenne (*10*). Because the FLHORAN41 shell turns red−orange to scarlet at full maturity, anthocyanins were also measured in the shell during maturation and partly characterized. Flesh volatile compounds and cell wall polysaccharides were also analyzed throughout maturation.

MATERIALS AND METHODS

Materials. Pineapples were grown at the CIRAD-FLHOR Rivière Lézarde Experimental Station (Martinique, French West Indies; climate typically tropical; latitude 15° N, longitude 62° W, altitude 80 m) on a halloysite soil supplemented before plantation with Mg and Ca and fertilized before flowering with a 1.8 K_2O/N ratio. Fruits were picked at five stages of maturity from 90 to 140 days after flowering. Fruits were then characterized on the basis of shell colors and °Brix/acid ratios, and five fruits were selected for each of the following stages of maturity: very green (90 days after flowering), green (115 days), turning (122 days), ripe (130 days), and very ripe (140 days).

Preparation of Samples. The fruits were first peeled, and the central core was removed. The flesh was crushed in a blender (Waring, United States) and frozen before analyses of total and soluble solids, pH, titratable acidity, sugars, organic acids, carotenoids, volatile compounds, and cell wall polysaccharides. The shell was freed of most of the adhering flesh with a scalpel and a tooth brush, cut into small pieces with scissors, rapidly rinsed with distilled water, dried with filter paper, and then pulverized in liquid nitrogen with a Dangoumill 300 ballmill (Prolabo, Paris, France) for 5 min (top impact frequency) and frozen before analysis of carotenoids and anthocyanins.

Solvents and Chemicals. All solvents were of the highest analytical grade. Reference compounds [carotenoids, anthocyanins, volatile compounds, and *n*-alkanes (C_5−C_{27})] were from Extrasynthèse (Genay, France) and Sigma-Aldrich Chimie (Saint Quentin Fallavier, France).

Analysis of Hydrocarbon Carotenoids and Xanthophylls. These compounds were extracted according to a previously published procedure (*11*). In brief, flesh puree or shell powder (5 g) was added to 50 mg of sodium carbonate and then extracted for 5 min under stirring (red light) with ethanol−hexane (1:1) (35 mL) (*12*). The slurry was then filtered on a sintered glass crucible (porosity 4), and the filtration cake was washed successively with ethanol−hexane (1:1) (35 mL), ethanol (2 × 12.5 mL), and hexane (2 × 12.5 mL). Solvent fractions were pooled and then washed with 10% NaCl (2 × 50 mL), to facilitate layer separation, and with distilled water (3 × 50 mL). The organic phase was brought to dryness under vacuum at 40 °C and finally dissolved in 500 μL of hexane−chloroform (90:10, v/v).

Carotenoids were separated by high-performance liquid chromatography (HPLC) using an Agilent system (1100 series) including a quaternary pump, a manual Rheodyne 7525i injector, an automated gradient controller, and a diode array detector. Spectra were recorded from 370 to 550 nm. The column was reverse phase Lichrospher RP-18 (Merck, Darmstadt, Germany) (5 μm packing) (250 mm × 4.6 mm id) protected with a guard column of the same material. The carotenoids were eluted under the following conditions: injected volume, 20 μL; oven temperature, 30 °C; solvent mixture [ethanol (10%), acetonitrile (85%), methylene chloride (2.5%), and hexane (2.5%), isocratic] at a flow rate of 0.7 mL min^{-1} for 10 min and then a 40 min gradient to solvent proportions of methanol (10%), acetonitrile (45%), methylene chloride (22.5%), and hexane (22.5%) (*13*). The column was reequilibrated under the initial isocratic conditions for 20 min at a flow rate of 1.5 mL min^{-1} and finally for 5 min at 0.7 mL min^{-1}. Identification of carotenoids was achieved by comparing their spectral data (λ_{max} and $\%A_{III}/A_{II}$) with those of reference compounds and literature (*13*) and by injection and coinjection of reference compounds.

Quantification was carried out by external standardization with calibrated solutions of all-*trans*-β-carotene, which were injected under the same HPLC conditions as extracts. Total carotenoid contents were calculated as the sum of carotenoid peak areas expressed as all-*trans*-β-carotene equivalents (*14*).

Table 1. Physicochemical Characteristics of FLHORAN41 Cv. Flesh during Maturation

	FLHORAN41 cv.					Smooth Cayenne cv.
	maturity stage					
	very green	green	turning	ripe	very ripe	ripe
moisture content[a]	91.3 ± 0.8[d]	88.4 ± 1.1	86.2 ± 1.0	83.8 ± 0.9	82.4 ± 0.9	85.1 ± 1.1
pH	3.46 ± 0.15	3.48 ± 0.19	3.52 ± 0.18	3.63 ± 0.22	3.68 ± 0.18	3.40 ± 0.20
titratable acidity[b]	10.6 ± 1.3	11.3 ± 1.4	11.6 ± 1.2	12.3 ± 1.2	11.9 ± 0.9	9.3 ± 1.4
soluble solids[a]	7.2 ± 0.8	11.0 ± 0.9	13.2 ± 0.9	16.6 ± 1.1	18.0 ± 1.0	13.6 ± 0.9
glucose[a]	1.2 ± 0.1	1.1 ± 0.1	1.7 ± 0.1	2.8 ± 0.1	3.4 ± 0.1	*e*
fructose[a]	1.1 ± 0.1	1.0 ± 0.1	1.8 ± 0.1	2.9 ± 0.1	3.4 ± 0.2	
sucrose[a]	2.5 ± 0.1	6.0 ± 0.2	6.7 ± 0.2	6.9 ± 0.2	6.7 ± 0.3	
citric acid[c]	0.57 ± 0.06	0.62 ± 0.06	0.60 ± 0.08	0.60 ± 0.08	0.72 ± 0.08	0.63 ± 0.06
malic acid[c]	0.21 ± 0.03	0.24 ± 0.02	0.20 ± 0.03	0.15 ± 0.02	0.21 ± 0.03	0.12 ± 0.02
oxalic acid[c]	0.19 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.01
phosphoric acid[c]	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02

[a] %/fw. [b] mequivalent 100 g^{-1} fw. [c] % of anion (fw). [d] Significant differences defined as $p < 0.05$. [e] Not determined.

Analysis of Anthocyanins. Shell powder (2 g) was extracted with methanol−1.5 M HCl (85:15, v/v) (3 × 20 mL) under magnetic stirring with intermittent filtration on sintered glass crucible (porosity 4). Extracts were combined and then dried under vacuum at 30 °C. To avoid hydrolysis of anthocyanins, distilled water (2 × 5 mL) was added during concentration and drying. The dried extract was finally dissolved in 1 mL of methanol and analyzed by HPLC.

Anthocyanins were separated by HPLC using the same system as described above. The spectra were recorded from 370 to 670 nm. The anthocyanins were eluted under the following conditions: flow rate, 1 mL min^{-1}; injected volume, 20 μL; oven temperature, 30 °C; solvent A, water/formic acid (98:2); solvent B, acetonitrile/water/formic acid (80:18:2). The proportions of solvent A in the gradient were as follows: isocratic 97% for 7 min; from 97 to 80% in 15 min; 80 to 68% in 8 min; 68 to 65% in 5 min; 65 to 60% in 5 min; 60 to 50% in 5 min; 50 to 20% in 5 min; and 20 to 97% in 5 min.

Total anthocyanin concentrations were obtained by measuring the absorbance of extracts at $\lambda = 518$ nm and expressed as cyanidin-3-*O*-glucoside equivalents ($\epsilon = 29600$) (*7*, *15*). Acidic hydrolysis of anthocyanins was performed with 2 M trifluoroacetic acid for 75 min at 120 °C (*16*). After they were dried under an air stream, the liberated anthocyanidins were analyzed by HPLC as described above. Sugars liberated from anthocyanins were converted to their alditol acetates (*17*) and analyzed by gas chromatography (GC) according to Hoebler et al. (*18*) with inositol as the internal standard. Identification of anthocyanins and anthocyanidins was achieved by comparing their spectral data (λ_{max}) with those of reference compounds and by injection and coinjection of reference compounds.

Extraction of Volatile Compounds. The flesh puree (20 g) was homogenized using a Potter Elvejhem homogenizer for 5 min with distilled water (20 mL) and pentane/dichloromethane (2:1) (40 mL) to which 2-heptanol (10 μg) was added as the internal standard. Phase separation was achieved by centrifugation at 9000*g* for 5 min. The upper organic phase was recovered, dried over anhydrous sodium sulfate, and finally concentrated at 37 °C with a 25 cm Vigreux distillation column to a volume of 0.5 mL.

GC-Mass Spectrometry (MS) Analysis. Solvent extracts were analyzed by GC-MS using a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer with electron ionization mode (EI) generated at 70 eV. The ion source and quadrupole temperatures were 230 and 150 °C, respectively, and the filament emission current was 1 mA. Volatile compounds were separated on a DB-Wax (column A, J&W Scientific, Folsom, CA) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film) and on a DB-1 (column B, J&W Scientific) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). The oven temperature was increased from 40 °C at a rate of 3 °C min^{-1} up to 250 °C where it was held for 20 min. The on-column injector was heated from 20 to 245 °C at 180 °C min^{-1}. The detector temperature was 245 °C. Helium was the carrier gas at 1.1 mL min^{-1}. Electron impact mass spectra were recorded in the 40−600 amu range at 1 s $interval^{-1}$. The injected volumes were 1 μL of concentrated extract. The compounds were identified on the basis of linear retention indices on both columns (DB-Wax and DB-1) (*19*) and EI mass spectra (Wiley 275.L library) from the literature or from authentic standard compounds. Concentrations were expressed as μg 2-heptanol equivalents 100 g^{-1} of fresh weight (fw), response factors being taken as 1.0 for all compounds with reference to the internal standard. Linear retention indices were calculated with reference to *n*-alkanes (C_5−C_{27}). Concentrations are given as the average of triplicates.

Preparation of Alcohol Insoluble Residues (AIRs). AIRs were prepared as follows: ethanol (400 mL) was added to aliquots of purees (100 g), and after thorough mixing, the slurry was boiled for 30 min and then filtered on a sintered glass crucible (porosity 4). The residue was then successively washed with ethanol/water (80:20) (200 mL), ethanol (100 mL), acetone (100 mL), and ether (50 mL). Finally, the residue was dried (24 h) in a vacuum oven (50 °C) and weighed.

Analytical Methods. Soluble solids (°Brix) were determined with a hand refractometer at room temperature. The pH was measured by a pH meter (pH-Vision 6071, Jenco Elec. Ltd., Taiwan), and the total acidity was determined by titration with 0.1 M NaOH. Total solids were determined by drying a sample (2 g) for 3 h at 60 °C and then for 24 h in a vacuum oven at 50 °C. Sugars and organic acids were analyzed by HPLC as described in ref *20*. Neutral monosaccharides were released from AIRs (5 mg) by hydrolysis with 2 M trifluoroacetic acid for 75 min at 120 °C (*16*). They were also submitted to Saeman hydrolysis as described by Hoebler et al. (*18*), i.e., 72% (w/w) sulfuric acid, 25 °C, 45 min, and then 1 M sulfuric acid, 100 °C, 2 h. Sugars were then converted to their alditol acetates (*17*) and analyzed by GC according to Hoebler et al. (*18*) with inositol as the internal standard. Uronic acids were measured without deesterification after preliminary dissolution in concentrated sulfuric acid by the *m*-phenylphenol procedure (*21*, *22*).

RESULTS AND DISCUSSION

Physicochemical Characteristics. Table 1 shows the main physicochemical characteristics of flesh of the FLHORAN41 cv. at five stages of maturity as compared to the ripe Smooth Cayenne cv. Total soluble solids of FLHORAN41 cv. flesh gradually increased from 90 to 140 days after flowering in a very similar way to the maturing Smooth Cayenne (*23*). In our experiment, the ripe Smooth Cayenne was less sweet than the corresponding hybrid fruit. In parallel to the change in total soluble solids, the sum of total soluble sugars (i.e., glucose, fructose, and sucrose) rose continuously during maturation. More precisely, sucrose greatly increased from the very green to the green stage of maturity while glucose and fructose rose continuously from the green to the very ripe stage. Such changes have already been shown for the Smooth Cayenne cv. by Dull (*24*), who also demonstrated the invertase effect in the progressive enzymatic hydrolysis of sucrose.

Table 2. Carotenoid and Anthocyanin Contents of Flesh and Shell of FLHORAN41 Cv. during Maturation

	FLHORAN41 cv.					Smooth Cayenne cv.
	maturity stage					
	very green	green	turning	ripe	very ripe	ripe
all-*trans*-β-carotene[a,c]	6 ± 1[e] (36)[f]	17 ± 3 (38)	104 ± 10 (34)	181 ± 20 (31)	204 ± 22 (32)	72 ± 11 (29)
total carotenoids[a,c]	16 ± 3	45 ± 5	259 ± 32	580 ± 42	628 ± 54	246 ± 24
all-*trans*-β-carotene[b,c]	315 ± 28 (18)	430 ± 32 (25)	516 ± 38 (24)	666 ± 47 (23)	639 ± 49 (22)	572 ± 42 (22)
total carotenoids[b,c]	1781 ± 84	1736 ± 87	2212 ± 94	2915 ± 104	2852 ± 109	2545 ± 97
anthocyanins[b,d]	97 ± 4	106 ± 7	114 ± 11	129 ± 9	125 ± 7	82 ± 4

[a] Flesh. [b] Shell. [c] Expressed as μg all-*trans*-β-carotene equivalent 100 g^{-1} fw. [d] Expressed as μg cyanidin-3-*O*-glucoside equivalent g^{-1} fw. [e] Significant differences defined as $p < 0.05$. [f] Values in parentheses are the relative proportions.

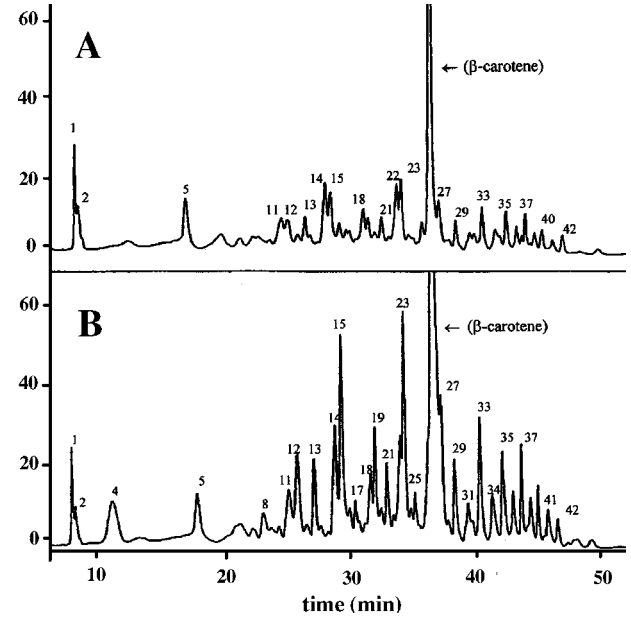

Figure 3. HPLC chromatograms of flesh carotenoids: (**A**) Smooth Cayenne cv.; (**B**) FLHORAN41 cv.

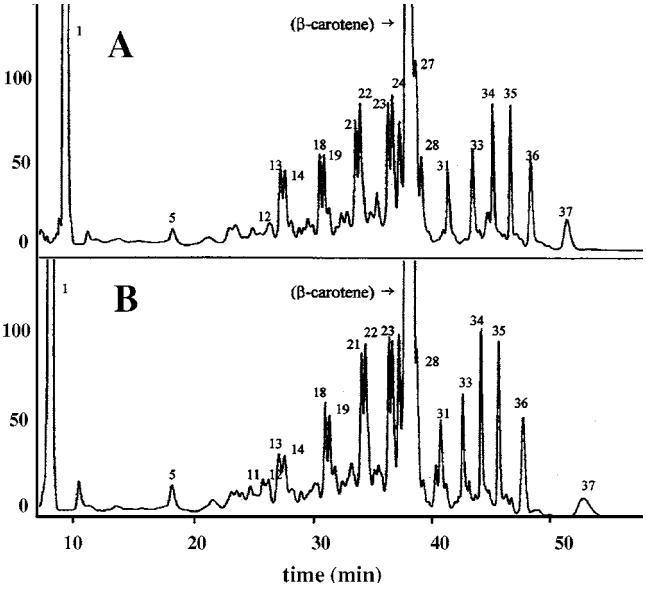

Figure 4. HPLC chromatograms of shell carotenoids: (**A**) Smooth Cayenne cv.; (**B**) FLHORAN41 cv.

Contrary to what was observed for the maturing Smooth Cayenne (*23*, *25*) where the titratable acidity doubled between 90 days after flowering and maturity, no such increase occurred in the flesh of the FLHORAN41 cv. during maturation. This is reflected by the moderate increase of citric acid in the FLHORAN41 cv. from 90 to 140 days (26%) as compared with a 3-fold increase in the citric acid content of Smooth Cayenne during the corresponding maturation period (*23*). In agreement with previous authors (*23*), malic acid was more or less stable during this period while pH increased very slightly.

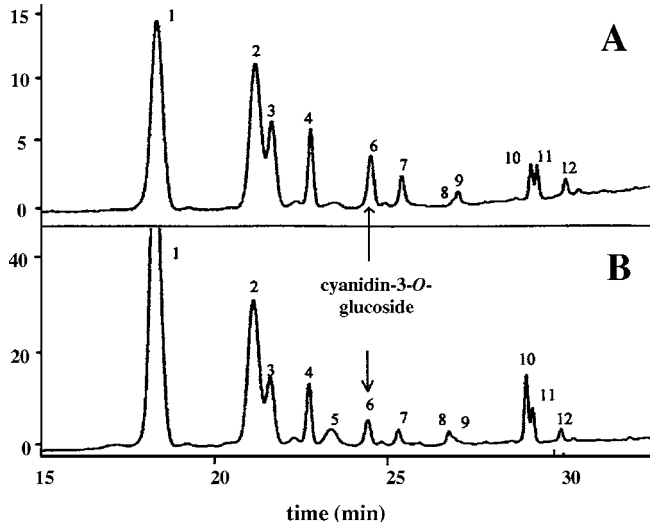

Figure 5. HPLC chromatogram of shell anthocyanins: (**A**) Smooth Cayenne cv.; (**B**) FLHORAN41 cv.

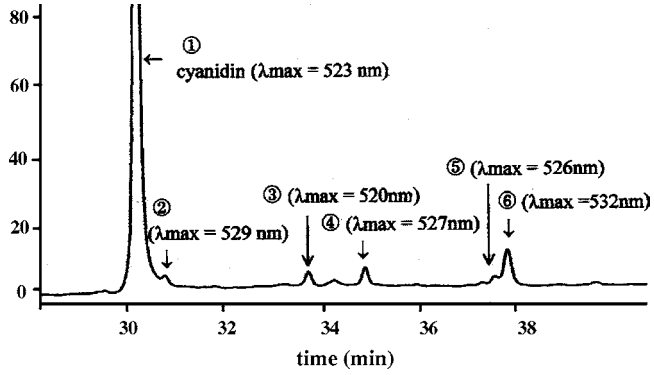

Figure 6. HPLC chromatogram of shell anthocyanidins (ripe FLHORAN41 cv.).

Hydrocarbon Carotenoids and Xanthophylls. These were extracted from the flesh of the two cultivars and separated by HPLC (**Figure 3**; ripe fruits): 42 peaks were detected, most of them exhibiting typical carotenoid spectra. Qualitatively speaking, chromatograms from the two cultivars were similar at all stages of maturity, carotenoids being more concentrated in the ripe FLHORAN41 cv. extract. Peak 4 was not found in Smooth Cayenne, while all-*trans*-zeaxanthin, all-*trans*-β-cryptoxanthin, and lycopen were found to be absent in the flesh of both cultivars. Only all-*trans*-β-carotene (31−38% of the total

Table 3. Flesh Volatile Compounds[a] during Maturation

				FLHORAN41 cv.					Smooth Cayenne cv.
				maturity stage[b]					
peak no.	compound	LRI[c]	LRI[d]	very green	green	ripe	very ripe	ripe	reliability of identification[e]
1	methyl butanoate	975	715			40	25	45	1
2	methyl 2-methylbutanoate	1015	738		tr[f]	65	40	20	2
3	α-pinene	1023	918	10	10	15	15	tr	1
4	ethyl butanoate	1037	794	5	tr	10	10	5	1
5	2-methyl-3-buten-2-ol	1043	-	tr	tr	5	5	5	2
6	ethyl 2-methylbutanoate	1052	835	tr	tr	5	tr	tr	2
7	butyl acetate	1071	807	tr		tr			1
8	hexanal	1079	775	5	5	tr	tr	tr	1
9	methyl pentanoate	1085	810			tr			2
10	β-pinene	1103	960	20	10	10	20	15	1
11	sabinene	1116	960	5	5	5	10	10	1
12	β-myrcene	1158	980	10	10	10	15	15	1
13	methyl hexanoate	1181	915	tr	5	10		20	1
14	limonene	1193	1007	1300	810	975	1410	1410	1
15	β-phellandrene	1199	1007	20	25	15	10	15	1
16	unknown 1[g]	1207		tr	5	5	5	tr	
17	ethyl hexanoate	1228	984			10		5	1
18	unknown 2	1235		10	5	5	10	5	
19	(*E*)-β-ocimene	1243	1030	5	5	40	15	5	1
20	*p*-cymene	1257	1006	tr	tr	tr			
21	methyl 2-hydroxy-2-methyl butanoate	1277			5	60	80	10	2
22	ethyl 2-hydroxypropanoate	1333				tr	tr		2
23	methyl 3-hydroxy-3-methyl butanoate	1361				5	5		2
24	methyl octanoate	1375	1018	tr	10	5		tr	2
25	nonanal	1381	1086	tr	10	5		tr	1
26	ethyl octanoate	1427	1170			5		5	1
27	methyl 3-hydroxybutanoate	1468	947			tr	5	tr	2
28	α-copaene	1478	1390			15	5	10	2
29	decanal	1479	1170	5	5	5		5	1
30	unknown 3	1486		5	5	5		5	
31	dimethyl malonate	1498	1027			15	10	40	2
32	methyl 3-(methylthio)propanoate	1508			tr	180	25	180	2
33	unknown 4	1510				10	15	5	
34	methyl 2-methyl-3-oxobutanoate	1517			tr	50	65	15	2
35	unknown 5	1528			tr	35	45	25	
36	unknown 6	1547				20	25	5	
37	ethyl 3-(methylthio)propanoate	1551		tr	tr	30	5	10	2
38	unknown 7	1555				5	5	5	
39	2,5-dimethyl-4-methoxy-3(2*H*)-furanone	1577			5	30	20	10	2
40	(*E*)-β-caryophyllene	1578	1415			10	tr		1
41	γ-butyrolactone	1599	1278		5	50	55	20	1
42	methyl 3-hydroxyhexanoate	1634	1019		tr	30	20	40	2
43	unknown 8	1670				15	15	5	
44	methyl 3-acetoxyhexanoate	1676	1170		5	75	50	115	2
45	γ-hexalactone	1678	1010		5	70	50	110	1
46	α-zingiberene	1711	1478		tr	5	5	10	2
47	unknown 9	1719		5	tr	30	40	40	
48	unknown 10	1732				5	10		
49	δ-cadinene	1741	1517	5		10	5	10	1
50	methyl 4-hydroxybutanoate	1755				5	tr		2
51	methyl 5-acetoxyhexanoate	1759			10	10	46	95	2
52	δ-hexalactone	1763	1051		tr	15	25	20	1
53	unknown 11	1782				10	10	10	
54	unknown 12	1827				10	10	15	
55	unknown 13	1830				tr	tr	tr	
56	hexanoic acid	1846	894	tr	5	10	10	15	1
57	γ-octalactone	1890	1518			tr	tr	10	2
58	methyl 5-acetoxyoctanoate	1906		10	10	30	30	35	2
59	δ-octalactone	1939	1250			10	15	15	2
60	unknown 14	1950				10	20	5	
61	unknown 15	1964		5	tr	5	5	5	
62	unknown 16	1985				5	tr		
63	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	2019	1020		5	195		70	1
64	ethyl tetradecanoate	2043	1784	10	100	10	10	15	2
65	octanoic acid	2059	1160	tr	5	15	10	10	1
	total concentration			**1435**	**1085**	**2390**	**2386**	**2575**	

[a] Micrograms of 2-heptanol equivalent 100 g^{-1} fw. [b] Turning stage of maturity not analyzed. [c] Linear retention index from DB-Wax. [d] Linear retention index from DB-1. [e] Key for reliability of identification: 1 = identified by linear retention index and mass spectrum of reference compounds; 2 = tentatively identified by linear retention index and mass spectrum similar to published data. [f] tr = traces (<5 μg 100 g^{-1}). [g] Mass spectra of unknown compounds are listed in **Table 4**.

Table 4. Mass Spectra of Unknown Compounds

unknown compd no.	*m/z* (%)
1	88 (100), 43 (86), 57 (75), 56 (39), 69 (23), 101 (19), 117 (18)
2	43 (100), 88 (56), 83 (22), 59 (15), 130 (15), 55 (9), 99 (9)
3	71 (100), 43 (57), 116 (33), 59 (23), 84 (20), 101 (17), 55 (10)
4	43 (100), 87 (37), 100 (25), 59 (22), 117 (19), 74 (16), 41 (9)
5	43 (100), 117 (39), 85 (25), 69 (18), 55 (12), 97 (8), 57 (8)
6	43 (100), 104 (53), 89 (23), 131 (18), 71 (17), 45 (6)
7	85 (100), 57 (30), 59 (19), 31 (14), 97 (8), 73 (8)
8	74 (100), 43 (53), 55 (23), 102 (22), 32 (17), 109 (12), 84 (11)
9	43 (100), 131 (50), 99 (36), 74 (29), 55 (20), 112 (18), 129 (18)
10	72 (100), 43 (98), 81 (55), 55 (45), 57 (26), 109 (10)
11	85 (100), 43 (92), 57 (47), 83 (22), 59 (22), 74 (20), 115 (15)
12	74 (100), 43 (53), 55 (23), 102 (22), 32 (17), 109 (12), 84 (11)
13	59 (100), 43 (61), 82 (53), 67 (51), 55 (16), 109 (14), 89 (13)
14	43 (100), 145 (26), 103 (23), 111 (9), 71 (5)
15	59 (100), 82 (62), 67 (50), 43 (48), 71 (33), 55 (14), 109 (7)
16	43 (100), 117 (58), 159 (31), 85 (23), 112 (15), 74 (9), 144 (7)

carotenoids) (**Table 2**) was unequivocally identified. The total carotenoid content of ripe Smooth Cayenne flesh agreed with that reported in the Mauritius cv. (*26*). The content in ripe and very ripe FLHORAN41 cv. fruits was about 2−2.5 times higher than in Smooth Cayenne cv. Thus, it seems that the deep golden yellow of mature FLHORAN41 flesh (**Figure 2**), when compared to Smooth Cayenne, could be partly explained by its higher total carotenoid content.

From the very green to the green stage, the total carotenoid content in flesh of the FLHORAN41 cv. is more or less stable, but from the green to the ripe stage, there is a tremendous increase in both all-*trans*-β-carotene and total carotenoids (11 and 13 times, respectively). Gortner (*10*) already mentioned such a change in the Smooth Cayenne cv. from 30 days before maturity to the fully mature stage. This period of maturity acquisition is directly correlated to the change in soluble solids of the fruit, with a rapid increase from the green to ripe stage of maturity (**Table 1**).

Carotenoids were also extracted from the shell of the two cultivars and separated by HPLC (**Figure 4**; ripe fruits). Qualitatively speaking, both chromatograms were similar at all stages of maturity. Apart from all-*trans*-β-carotene, one other major peak (1), only very weakly represented in pineapple flesh (**Figure 5**), was tentatively identified as lutein according to its spectrum (λ_{max} 422, 444, and 472 nm, A_{III}/A_{II} = 63%) (*27*). Whatever the cultivar and the stage of maturity, total carotenoid contents are higher in the shell (**Table 2**) than in the flesh but unlike the flesh no difference is found between the cultivars. Once again, total carotenoids, including all-*trans*-β-carotene, increased by 1.5 times from the green to the ripe stage, although this increase was less pronounced than for the flesh (10 times). This behavior was not in agreement with Gortner (*10*), who reported that shell carotenoids decrease constantly from 100 days before maturity to full maturity. Finally, according to Gortner (*10*), the color of a ripe pineapple shell is the result of both the degradation of chlorophylls and an equilibrium between carotenoids and anthocyanins.

Anthocyanins. Because the characteristic shell color (from red−orange to scarlet) of the FLHORAN41 cv. (**Figure 2**) could be attributable to the qualitative and quantitative composition of carotenoids and anthocyanins, the shell anthocyanin content of the FLHORAN41 cv. was analyzed during maturation and compared to that of ripe Smooth Cayenne (**Table 2**). The total anthocyanin concentration in the FLHORAN41 cv. shell rises slightly from the very green to ripe stage (from 97 to 129 μg g^{-1} of cyanidin-3-*O*-glucoside equivalent), to a level 1.5 times higher than that in the ripe Smooth Cayenne shell (**Table 2**). In contrast, Smooth Cayenne shell anthocyanins have been reported to decrease during maturation (*10*). Thus, it seems that since the shell carotenoid composition and content of the two cultivars were very similar at the ripe stage, the red−orange to scarlet color of the ripe FLHORAN41 cv. shell is due to its higher anthocyanin content as compared to the Smooth Cayenne cv. HPLC chromatograms of shell anthocyanins from the two ripe cultivars are presented in **Figure 5**. Twelve peaks having typical anthocyanin absorbance spectra were observed, and their relative concentrations were similar in both cultivars apart from peak 1, which was more concentrated in the FLHORAN41 cv., and peak 5, which was not detected in the Smooth Cayenne cv. Of these twelve peaks, only one was formally identified as cyanidin-3-*O*-glucoside (peak 6; retention time and spectral data identical to the reference standard); cyanidin-3-*O*-rutinoside and pelargonidin-3-*O*-glucoside were not detected. Tentative identification of anthocyanidins was achieved by acid hydrolysis and HPLC analysis (**Figure 6**). Of the six detected aglycones having typical anthocyanidin spectra, only cyanidin was unequivocally identified (peak 1) representing 86% of the total anthocyanidin content; no pelargonidin was detected. Therefore, one can say that the ripe FLHORAN41 cv. shell anthocyanins are essentially cyanidin glycosides. The anthocyanin sugar moieties of the ripe FLHORAN41 cv. shell were analyzed by GC after acid hydrolysis: glucose, xylose, galactose, arabinose, and rhamnose were found in relative proportions of 1/0.21/0.10/0.09/0.04, respectively. As cyanidin-3-*O*-glucoside represents only 2−5% of the total anthocyanin content, we suggest that the major anthocyanins (i.e., peaks 1−3; **Figure 5**) are cyanidin di- and triglucosides and/or mixed glycosides such as xylosylglucosides or galactosylglucosides. This hypothesis agrees with the results of Saito and Harborne (*28*), who mentioned the presence of cyanidin-3,5-diglucoside and cyanidin-3,5,3′-triglucoside in the flowers and leaves of *A. comosus*.

Volatile Compounds. They were extracted from the flesh of FLHORAN41 and Smooth Cayenne cvs. by the pentane−dichloromethane (2/1) azeotropic mixture and then separated and identified by GC-MS (**Table 3**). The ranges in standard deviations (given in parentheses) according to concentration ranges were as follows: 5−20 (10−18%), 20−200 (8−14%), 200−500 (6−10%), 500−1000 (4−7%), and 1000−2000 μg 100 g^{-1} fw (2−5%). The yield of total volatiles from the flesh of FLHORAN41 almost doubled between the very green to the green and the ripe to the very ripe stages, reaching ∼2.4 mg 100 g^{-1}: a value similar to that of ripe Smooth Cayenne fruit. Almost all classes of compounds rose by ∼50−60% from the green to the mature stage, although a smaller increase was found for terpene hydrocarbons. Umano et al. (*29*) observed a similar

Table 5. Yield and Polysaccharide Composition of Flesh AIRs during Maturation

	FLHORAN41 cv.					Smooth Cayenne cv.
	maturity stage					
	very green	green	turning	ripe	very ripe	ripe
AIR (%/fw)	2.6 ± 0.1[a]	2.2 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	2.0 ± 0.1
composition[b]						
uronic acids	6.1	5.1	5.0	4.7	5.2	4.0
rhamnose	0.7	0.4	0.3	0.3	0.3	0.3
fucose	0.3	0.4	0.4	0.4	0.4	0.4
arabinose	6.8	7.9	7.7	7.5	7.4	7.4
xylose	9.7	11.5	11.5	11.3	12.2	11.6
mannose	3.4	3.7	3.9	3.9	3.6	3.7
galactose	7.9	9.4	8.0	7.6	7.9	5.9
glucose (noncellulosic)	2.0	2.4	2.0	1.9	1.5	2.1
glucose (cellulosic)	21.2	22.3	22.1	22.6	21.5	25.6
total	58.1	63.1	60.9	60.2	60.0	61.0

[a] Significant differences defined as $p < 0.05$. [b] %/dry weight.

increase from 0.6 to 0.9 mg 100 g^{-1} between green and ripened fruits of an unknown cultivar; the lower content at the ripe stage due to an extraction procedure (distillation under reduced pressure) is known to afford a poor recovery of terpenes (*30*).

Of the 65 peaks detected, 49 were identified, and as previously reviewed (*31*), these were mainly aliphatic, hydroxy, and acetoxy esters (~40−50% of the total concentration in ripe fruits) and terpenes. Sixteen unknowns were also detected, and their mass spectral data are listed in **Table 4**. Limonene (14) was by far the most abundant compound but, surprisingly, greatly decreased from the very green to the ripe stage. Ripe FLHORAN41 and Smooth Cayenne flesh have more or less the same qualitative composition, except that *n*-butyl acetate (7), ethyl 2-hydroxypropanoate (22), and (*E*)-β-caryophyllene (40) were only detected in the FLHORAN41 cv. Considering the quantitative distribution, ripe FLHORAN41 flesh possesses higher concentrations of (*E*)-β-ocimene (19), γ-butyrolactone (41), 2,5-dimethyl-4-methoxy-3(2*H*)furanone (mesifurane, 39), 2,5-dimethyl-4-hydroxy-3(2*H*)furanone (Furaneol, 63), and some esters (mainly 2, 21, 34, and 37) than ripe Smooth Cayenne flesh. Conversely, some esters (13, 31, 44, and 51) were found at higher concentrations in the ripe Smooth Cayenne cv., with the concentration of methyl 5-acetoxyhexanoate (51) being nine times higher than in ripe FLHORAN41 flesh.

According to the flavor thresholds of methyl 2-methylbutanoate (2) and ethyl 2-methylbutanoate (6) (25 and 0.6 ng 100 g^{-1}, respectively, *32*), these two compounds found at 65 and 5 μg 100 g^{-1}, respectively, could be important aromatic compounds of ripe FLHORAN41 flesh. Methyl 3-(methylthio)propanoate (32) and ethyl 3-(methylthio)propanoate (37), known to have a characteristic pineapple flavor (*31*), were present in ripe fruits at concentrations similar to those found by Teai and Claude-Lafontaine (*33*) in a Polynesian pineapple. In the FLHORAN41 cv., they strongly decreased in concentration from the ripe to the very ripe stage of maturity. These two thioesters, having flavor thresholds of 18 and 0.7 μg 100 g^{-1}, respectively (*32*), could also be important flavor compounds of both ripe cultivars.

Five lactones (41, 45, 52, 57, and 59) were also identified, of which the concentrations greatly increased with maturation; ripe Smooth Cayenne flesh was richer in γ-hexalactone and γ-octalactone but poorer in γ-butyrolactone than the corresponding FLHORAN41 fruits.

The Furaneol (63) concentration in ripe FLHORAN41 flesh (195 μg 100 g^{-1}) was found to be similar to data in the literature (360−740 μg 100 g^{-1}, *34*). This compound, having a characteristic strawberry flavor (*32*), was found at a higher concentration in the ripe FLHORAN41 cv. than that in the ripe Smooth Cayenne cv. (70 μg 100 g^{-1}) but completely disappeared between the ripe and very ripe stages. According to its flavor threshold (2.1 μg 100 g^{-1}, *35*), this compound may be an important flavor compound of both ripe cultivars.

Cell Wall Polysaccharides. AIRs were prepared from the flesh of the FLHORAN41 cv. at five different stages of maturity and compared to the AIR of ripe Smooth Cayenne (**Table 5**). Contrary to Singleton and Gortner who noted a slight decrease of Smooth Cayenne AIRs from 50 days before to full maturity (*23*), the FLHORAN41 AIR contents were roughly the same throughout maturation and slightly higher than that of Smooth Cayenne. Acid and neutral polysaccharides ranged from 58.1 to 63.1% of AIR dry matter. Cellulose represented from 35 to 38% of the total polysaccharides, the Smooth Cayenne cv. being a little richer. On reaching maturity, the polysaccharide composition of the FLHORAN41 AIR did not change much, exhibiting a typical monosaccharide cell wall distribution with dominant proportions of xylose followed by decreasing amounts of galactose, arabinose, and uronic acids and minor proportions of noncellulosic glucose, rhamnose, and fucose. A similar composition was found by Smith and Harris (Smooth Cayenne cv.; flesh of fruit 2) (*36*), and these authors mentioned that uronic acids were composed of equal proportions of galacturonic and glucuronic acids. Finally, the monosaccharide composition of ripe FLHORAN41 AIR is roughly the same as that of Smooth Cayenne except for a slightly higher proportion of galactose (7.6/5.9%/dw).

This new pineapple hybrid (FLHORAN41 cv.) as compared to the Smooth Cayenne cv. has, at full maturity, higher titratable acidity and soluble solids content. Its golden yellow flesh is 2.5 times richer in carotenoids, which, due to their provitamin antioxidant nature, is a favorable characteristic of this hybrid. Its shell develops an attractive red−orange to scarlet color due to a higher anthocyanin content. Flesh aroma compounds were in similar relative proportions in both cultivars.

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