Influence of cultivar, growth altitude and maturity stage on banana volatile compound composition

Pierre Brat[a]*, Ahmed Yahia[a], Marc Chillet[b], Christophe Bugaud[c], Frederic Bakry[a], Max Reynes[a], Jean-Marc Brillouet[a]

[a] Centre de coopération internationale en recherche agronomique pour le développement (Cirad), Département Flhor, TA 50 / PS4, Bld. De la Lironde, 34398 Montpellier Cedex 5, France

brat@cirad.fr

[b] Cirad-Flhor, Station de Neufchâteau, 97130 Capesterre-Belle-Eau, Guadeloupe, France

[c] Cirad-Flhor, BP 153, 97202 Fort-de-France, Martinique, France

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Abstract — **Introduction**. The quantitative volatile compound composition of bananas (*Musa acuminata*, AAA group, Cavendish subgroup) was investigated in two different cultivars (Robusta and Grande Naine) grown at two different altitudes [(90 and 500) m], and ripened at two different maturity stages (color grades 5 and 7). **Materials and methods**. Volatile compounds were separated and identified by coupled capillary gas liquid chromatography / mass spectrometry. After evaluating changes in volatile composition during ripening, the effect of both growth altitude and cultivar was examined. **Results and discussion**. For a partially mature fruit, the growth altitude showed different effects on the two cultivars. The Robusta cv. exhibited a higher aroma volatile compound concentration at high altitude, while that of the Grande Naine cv. was higher at low altitude. At the fully mature stage, this difference still existed but was not so distinct. **Conclusion**. Whatever the ripening stage, Robusta cv. exhibited the highest aroma volatile compound concentration. Discriminate sensory analysis closely related to differences in the volatile composition of samples.

France / Guadeloupe / *Musa* (bananas) / aromatic compounds / altitude / maturity

* *Correspondence and reprints*

Influence du cultivar, de l'altitude de la bananeraie et du stade de maturité sur la composition en composés volatils de la banane.

Résumé — **Introduction**. La composition quantitative en composés volatils de bananes (*Musa acuminata*, groupe AAA, sous-groupe Cavendish) a été étudiée pour deux cultivars différents (Robusta et Grande Naine) développés à deux altitudes différentes [(90 et 500) m] et à deux stades de maturité différents (notation 5 et 7 dans l'échelle de coloration). **Matériel et méthodes**. Des composés volatils ont été séparés et conjointement identifiés par chromatographie capillaire en gaz liquide et spectrométrie de masse. Après évaluation des changements en composés volatils intervenus pendant la maturation, les effets de l'altitude des plantations et du cultivar utilisé ont été examinés. **Résultats et discussion**. Pour un fruit partiellement mûr, l'altitude a eu différents effets sur les deux cultivars. La concentration en composés aromatiques volatils du cultivar Robusta a été la plus élevée en haute altitude, alors que celle de Grande Naine l'a été à basse altitude. Au stade de pleine maturité, cette différence était encore décelable, sans être aussi distincte. **Conclusion**. Quel que soit le stade de maturation, le cultivar Robusta a montré la concentration en composés aromatiques volatils la plus élevée. L'analyse sensorielle discriminante a été étroitement liée aux différences de composition en composés volatils des échantillons.

France / Guadeloupe / *Musa* (bananes) / composé aromatique / altitude / maturité

1. Introduction

Bananas are the third most consumed fruit in the world, after citrus fruits and grapes, with more than 74 million tons produced in 2000 [1]. European consumption is mainly limited to the subgroup Cavendish (*Musa acuminata*, AAA group) with the major cultivars Dwarf Cavendish, Grande Naine and Robusta [2]. Analytical research on the aroma compounds of this fruit has been carried out for more than 30 years [3], and the quantitative composition of the volatile compounds of different cultivars is now widely known and reviewed [4]. Irrespective of the method used (*e.g.,* static or dynamic headspace, or solvent extraction), the major volatile compounds are generally esters (isoamyl, isobutyl, and other uncommon esters), most of which are responsible for the fruity aroma [3, 5, 6], alcohols (pentan-2-ol, 3-methyl butanol) and ketones (pentan-2-one) [3, 7, 8].

The production of volatiles during ripening of bananas was studied by Macku and Jennings [9] who concluded that the total amount of banana volatiles followed a sigmoidal increase during ripening. Volatile composition is nevertheless highly dependent on geographical factors, as indicated by Cano *et al.* [10] and Shiota [3], who studied the influence of the area of cultivation. These latter authors highlighted how Cavendish bananas cultivated in Taiwan had a poorer appearance but stronger aroma and sweeter taste than those of the Philippines. It has been suggested on an empirical basis that, for a given cultivar at comparable maturity stages, bananas cultivated at a high altitude may have sensory characteristics different from those cultivated on a plain. To our knowledge, no comparative study on the influence of the growing altitude on sensory characteristics of banana has ever been conducted.

Two banana cultivars (Robusta and Grande Naine) were harvested at the 3/4 full stage [11] from open fields located on the plain, and in the hills in the French West Indies. Because bananas are preferentially consumed in the European market at color grades 5 and 7, the fruits were then matured up to these stages, and their volatile compound compositions were measured. Triangular difference tests were performed to discriminate the fruits.

2. Materials and methods

2.1. Plant materials

The fruit studied were bananas (*Musa acuminata*) from the AAA group, Cavendish subgroup [12], cultivated on four open fields in Guadeloupe (French West Indies, France). Two of the fields chosen were located in close proximity to one another on the plain in Bois Debout (altitude 90 m; average rainfall 2500 mm per annum; temperature 25–26 °C), and planted with Robusta (syn. Poyo), and Grande Naine (syn. Granda Enana) cultivars. The two other fields were located close to each other in a hilly area in Grand Marigot (altitude 500 m; average rainfall 3500 mm per annum; average temperature 22–23 °C), and planted with the same cultivars.

Three banana bunches were randomly harvested at the 3/4 full stage [11] from each field; the hands were separated and placed in cardboard boxes which were then shipped (14 °C) and delivered to an industrial ripening depot (Cavaillon, France) where they were ethylene-ripened to color grades 3–4 [13]. Each batch was then further ripened on the bench at 20 °C to color grade 5 (yellow with green tips) and color grade 7 (flecked) [13]. The fruits were then stored at (14 ± 1) °C, and 85–90% relative humidity [10] until being analyzed. Finally, fingers were randomly selected from each batch and all analyses including sensory study were carried out immediately after slicing.

2.2. Solvents and chemicals

Reference compounds, if available, and *n*-alkane (C_5–C_{27}) standards were from Aldrich Chimie (Saint-Quentin Fallavier, France). All chemicals and solvents (*n*-pentane and ether) were of analytical grade.

2.3. Extraction of volatile compounds

Three randomly picked fingers from each batch were finely sliced separately after

peeling and then crushed in a Waring blender (Waring, US). A pureed sample (5 g) was mixed with 5 mL of distilled water; then *n*-decanal (8.6 µg) was added as internal standard, followed by 50 mL of pentane / ether (1:1). The slurry was finally homogenized with a Potter Elvejhem homogenizer for 5 min. 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 2 mL.

2.4. GC–MS analysis

A Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer was used, with electron ionization mode (EI) generated at 70 eV. The ion source and quadrupole temperatures were 230 °C 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, USA) 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, Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film). 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 °C to 245 °C at 180 °C·min^{-1}. Detector temperature was 245 °C. Helium was the carrier gas at a flow of 1.1 mL·min^{-1}. Electron impact mass spectra were recorded in the 40–600 amu range at 1 s·interval^{-1}. Injected volumes were 1 µL of concentrated extract. Compounds were identified on the basis of linear retention indices on both columns (DB–Wax, and DB–1) [14] and EI mass spectra (Wiley 275.L library) from the literature or from authentic standard compounds. Since our aim was to measure the concentration of volatiles in each fraction, amounts are expressed as µg *n*-decanal equivalent·100 g^{-1} fresh weight, 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.

2.5. Taste panel procedure

Samples (10 g) of fresh pulp were laced into three-digit randomly coded plates and equilibrated at room temperature until presentation to the panellists. Triangle difference tests were conducted in a sensory analysis testing laboratory by an expert eighteen-member panel to detect the sensory differences between samples. They were therefore asked to taste samples at the 5 and 7 ripening stages, successively, according to growth altitude [(Robusta on plain / Robusta in hills) and (Grand Naine on plain / Grande Naine in hills)] and cultivar effects [(Robusta on plain / Grande Naine on plain) and (Robusta in hills / Grande Naine in hills)]. Training and tasting procedures generally followed those described by French Norm AFNOR [15, 16]. Flavor discrimination trials were carried out under red light, and no attempt to describe or compare the quality of the bananas was undertaken, as this is highly subjective and dependent on a wide range of factors.

2.6. Statistical analysis

The Pascal Schilch risk table was used to identify significant differences between samples. For an eighteen-member panel, the significant difference was calculated to be 11 and 10 correct answers at $P < 0.01$ and $P < 0.05$, respectively (*table II*).

3. Results and discussion

3.1. Changes in volatile composition during ripening

Volatile compounds were extracted by the azeotropic mixture pentane/ether (1:1) from fruits of the two cultivars at two different ripening stages, each cultivated at two different altitudes (*table I*). Each sample was extracted in triplicate and analyzed by GC–MS. The range in standard deviations (sd) according to concentration range were as follows: (5–20) µg·100 g^{-1} fresh weight, sd: (20–26)%; (20–200) µg·100 g^{-1} fresh weight, sd: (14–20)%; (200–500) µg·100 g^{-1} fresh weight, sd: (8–13)%; (500–1000) µg·100 g^{-1} fresh weight,

Table I.

Volatile compounds (µg·100g^{-1} fresh weight) recovered from two banana cultivars (Robusta and Grande Naine) at two maturity stages (color grades 5 and 7), and at two different growth altitudes (plain and hilly area) (Guadeloupe, French West Indies, France).

Volatile compounds		Linear retention index from		Color grade 5				Color grade 7				Key for R.I.[1]
				Robusta		Giant		Robusta		Giant		
		DB-Wax	Literature	Plain	Hills	Plain	Hills	Plain	Hills	Plain	Hills	
Aldehydes	hexanal	1063	1065	48	27	28	19	traces[e]	0	19	0	1
	(*E*)-hex-2-enal	1189	1202	68	47	36	38	19	6	20	14	1
	nonanal	1367	1374	2	3	3	3	4	2	3	3	1
Ketones	pentan-2-one	987	969	traces	89	30	19	426	341	306	223	1
	heptan-2-one	1160	1160	0	15	3	0	49	26	43	21	2
	(*Z*)-hept-4-en-2-one	1222	1228	0	18	4	0	58	16	49	19	2
Alcohols	2-methyl propanol	1078	1060	5	28	3	3	121	200	56	148	1
	pentan-2-ol	1105	1221	4	73	32	23	464	305	257	177	1
	butanol	1126	1103	19	31	15	10	68	101	76	83	1
	3-methyl butanol	1183	1168	33	180	22	25	353	350	185	291	2
	(*Z*)-hept-4-en-2-ol	1311	1302	7	17	4	3	57	17	42	22	2
	hexanol	1323	1331	64	47	75	35	35	39	46	33	1
	(*Z*)-hex-3-enol	1331	1351	2	0	1	1	4	6	4	5	2
	(*Z*)-hex-4-enol	1389	1382	16	0	3	2	4	16	3	12	2
Esters	ethyl acetate	922	872	0	9	5	8	12	51	33	38	1
	isobutyl acetate	1016	1010	7	30	6	5	232	431	122	500	2
	butyl acetate	1064	1059	18	23	8	6	76	171	135	153	1
	2-pentyl acetate	1067	1161	4	68	38	20	538	418	476	395	2
	isoamyl acetate	1108	1110	34	178	31	20	469	652	385	724	1
	isobutyl butanoate	1144	1139	traces	21	3	2	104	255	46	153	2
	isoamyl isobutanoate	1194	1187	traces	14	6	5	183	148	125	55	2
	butyl butanoate	1197	1207	0	27	12	10	82	104	117	92	1
	isoamyl butanoate	1244	1242	traces	183	32	26	401	549	259	412	1
	isoamyl isovalerate	1274	1287	0	24	0	0	103	139	31	0	2
Carboxylic acids	acetic acid	1399	1410	64	24	36	36	80	83	48	62	1
	propanoic acid	1490	1504	5	5	6	4	16	74	0	0	1
	isobutanoic acid	1515	1535	0	0	0	0	20	54	0	45	2
	butanoic acid	1580	1588	9	26	31	46	60	61	39	58	1
	isovaleric acid	1625	1636	13	38	58	46	86	63	27	52	2
	hexanoic acid	1802	1795	22	36	34	24	61	40	68	0	1
	octanoic acid	2012	2008	3	0	1	6	2	4	3	0	2
Phenolether derivatives	eugenol	2099	2115	84	42	41	7	163	246	194	314	1
	elimicine	2166	2150	30	110	55	70	273	271	563	317	2
	5-methoxyeugenol	2390	–	309	924	406	387	898	873	1096	1196	2
Total concentrations (µg·100 g^{-1} fresh weight)		–	–	867	2356	1067	908	5514	6111	4876	5618	–

[1] 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.

Traces: < 0.5 µg·100 g^{-1}.

Table II.

Results of triangular difference tests performed to discriminate fruits of Robusta and Grande Naine cvs. according to two factors: altitude effect or cultivar effect (Guadeloupe, French West Indies, France).

Factor considered		Color grade significance	
		5	7
Growth altitude effect	Robusta on the plain / Robusta in the hills	**	**
	Grande Naine on the plain / Grande Naine in the hills	**	ns
Cultivar effect	Robusta on the plain / Grande Naine on the plain	ns	**
	Robusta in the hills / Grande Naine in the hills	**	*

** Significant at $P < 0.01$, * significant at $P < 0.05$, ns: non-significant.

sd: (6–10)%; (1000–2000) µg·100 g^{-1} fresh weight, sd: (4–8)%. The most polar compounds (acetic acid and ethyl acetate) may be underestimated due to their higher solubility in the water phase than in the azeotropic mixture [17].

According to Macku and Jennings [9], whatever the cultivars and the growth altitude, most volatile compounds (*i.e.,* ketones, alcohols, esters and carboxylic acids) increased in concentration during ripening. Esters (mainly 2-pentyl, isoamyl and isobutyl acetates, and isoamyl butanoate and isobutanoate) probably cause the specific fruity aroma found in banana [3], and increase enormously during ripening [*e.g.,* Robusta cultivar on the plain (63 to 2198) µg·100 g^{-1}]. Tressl and Jennings [18] had already noted this behavior for all except 2-pentyl acetate. These authors also observed that the cycle for the acetate esters was out-of-phase with that observed for the butyrates (these esters probably not having a common precursor).

Alcohols (mainly pentan-2-ol, 3-methyl butanol and 2-methyl propanol) would also contribute to the succulent character of banana aroma [3], and their concentrations are from two to seven times higher at color grade 7 than grade 5. Pentan-2-one is one of the major volatile compounds of bananas, possessing a banana-like flavor at concen-

trations below 50 ppm [19]. It was poorly recovered at color grade 5 and was found at concentrations ranging from (223 to 426) µg·100 g^{-1} in fully mature fruit.

As previously reported by Miranda *et al.* [20], the full-bodied and mellow aroma of ripe bananas has been associated with phenolether derivatives (typically eugenol, 5-methoxyeugenol and elimicine) which probably originate from phenylalanine and caffeic acid precursors and are found at concentrations four to five times higher at color grade 7 than at color grade 5. In contrast, the concentrations in the banana puree of the two major aldehydes, hexanal, and (*E*)-hex-2-enal, decline during ripening, these two compounds possessing pungent fatty, green, and grassy odors (flavor thresholds in water of 0.3 and 4.9 µg·100 g^{-1}, respectively) [21].

3.2. Growth altitude effects on volatile composition

At color grade 5, the fruit of the Robusta cv. cultivated in the hills exhibit higher concentrations of ketones [(122 against 0) µg·100 g^{-1}], alcohols [(376 against 150) µg·100 g^{-1}], esters [(577 against 63) µg·100 g^{-1}] and phenolether derivatives [1076 against 423) µg·100 g^{-1}] than fruit cultivated on the plain (*table I*).

The only exceptions are aldehyde compounds with grassy odors, and carboxylic acids. Of the eight alcohols measured, 3-methyl butanol is present in the Robusta cv. sample grown in a hilly area at a much higher concentration than its retronasal odor threshold in water (180 vs. 25 µg·100 g^{-1}) [22]. Whatever the growth altitude at color grade 5, the fruit of the Grande Naine cv. showed similar concentrations of the various groups of volatiles (Giant on the plain~ Giant in the hills). These data were in agreement with the triangular test results (*table II*) for fruits of the Robusta cv. cultivated on the plain and in the hills which were found to be significantly different at grade 5; conversely, equivalent fruits from the Grande Naine cv. (on the plain or in the hills) were also found to be different, although their volatile compounds' distribution was similar.

Whatever the cultivar, the concentration of ester compounds at grade 7 was found to be in good agreement with the data of Jordán *et al.* [7] quantifying isoamyl acetate and butanoate at (485 ± 138) µg·100 g^{-1} and (713 ± 123) µg·100 g^{-1}, respectively (*table I*).

The major differences observed in the case of the fruit of the Robusta cv. at grade 5 were not so obvious at color grade 7. Except for esters, which were still found in higher concentrations in the fruit of the Robusta and Grande Naine cvs. cultivated in the hills with regard to those cultivated on the plain [(2919 against 2198) µg·100 g^{-1} and (2522 against 1730) µg·100 g^{-1}, respectively], and ketones, which showed a reverse behavior, other groups of volatiles were found in similar concentrations. These differences at both ripening stages were corroborated by the sensory analysis. The panel significantly discriminated between the two batches at $P < 0.01$ (*table II*).

3.3. Cultivar effects on volatile composition

A comparison of the Robusta and Grande Naine cvs. at color grade 5 when cultivated at low altitude produces mixed results. The Grande Naine cv. is characterized by higher proportions of ketones (none was identi-fied in the Robusta cv.), esters (mainly 2-pentyl acetate and isoamyl butanoate), and carboxylic acids than the Robusta cv. but a lower proportion of aldehydes [(67 against 117) µg·100 g^{-1}] (*table I*). Phenolether derivatives, that are responsible for the mellow aroma, and alcohols, that relate to the fruity notes of bananas, are present in similar amounts in both cvs. For fruits cultivated in the hills, at grade 5, the Robusta cv. was richer in ketones, alcohols and esters than the Grande Naine cv., and these fruits were actually discriminated by the triangular difference test (*table II*).

At color grade 7, contrasting differences were observed with the Robusta cv. on the plain possessing higher proportions of ketones, esters, and especially alcohols, which were present at a concentration about two times higher than that of the Grande Naine cv. When cultivated in the hills, the fruit of the Robusta cv. showed higher levels of ketones, alcohols and carboxylic acids than those of the Grande Naine cv., lower levels of phenolether derivatives, and similar amounts of esters. Again, these fruits were discriminated at $P < 0.05$ (*table II*).

4. Conclusion

Differences in the volatile composition of samples (*i.e.*, Robusta and Grande Naine cvs. on the plain and in the hills) at both color grades 5 and 7 were generally reflected in sensorial differences. At color grade 5, the growth altitude has differing effects on the two cvs.: the concentration of volatile compounds of Robusta cv. is higher at high altitude while that of Grande Naine cv. shows no significant difference whatever the growth altitude. When cultivated in the hills, the Robusta cv. exhibits a higher aroma concentration than the Grande Naine cv. These differences were similar, if less distinct, for fully mature fruit. These results clearly indicate that, whatever the ripening stage, the Robusta cv. grown at a high altitude possesses the highest concentrations of volatile compounds [(2356 and 6111) µg·100 g^{-1} at color grades 5 and 7, respectively].

Acknowledgments

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Influencia del cultivar, de la altitud de la plantación y del estado de madurez en la composición de compuestos volátiles del banano.

Resumen — **Introducción**. Se estudió la composición cuantitativa de compuestos volátiles de bananos (*Musa acuminata*, grupo AAA, subgrupo Cavendish) en dos cultivares diferentes (Robusta y Gran Enana) cultivados en dos altitudes diferentes [(90 y 500) m] y en dos fases de madurez diferentes (5 y 7 puntos en la escala de Von Loesecke). **Material y métodos**. Se separaron los compuestos volátiles y se identificaron conjuntamente mediante cromatografía capilar de gas-líquido y espectrometría de masas. Tras la evaluación de los cambios de compuestos volátiles producidos durante la maduración, se examinaron los efectos de la altitud de las plantaciones y del cultivar utilizado. **Resultados y discusión**. En fruta parcialmente madura, la altitud tuvo distintos efectos en ambos cultivares. La concentración de compuestos aromáticos volátiles del cultivar Robusta fue la más elevada en alta altitud, mientras que la de Gran Enana lo fue en baja altitud. En el estado de plena madurez, esta diferencia era aún perceptible, sin ser tan marcada. **Conclusión**. Cualquiera que sea el estado de maduración, el cultivar Robusta mostró la concentración más alta de compuestos aromáticos volátiles. El análisis sensorial discriminante estuvo estrechamente ligado a las diferencias de composición de compuestos volátiles de las muestras.

Francia / Guadalupe / *Musa* (bananos) / compuestos aromáticos / altitud / madurez