Identification of the Volatile Components Released by Fresh Coffee Berries at Different Stages of Ripeness

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The composition of volatile chemicals released by fresh coffee berries at different stages of ripeness for *Coffea canephora* var. *Robusta* and *Coffea arabica* was investigated by dynamic headspace collection and GC and GC/MS analyses. Green berries produced few compounds whereas red berries were characterized by high levels of terpenes for *C. arabica* and by both terpenes and sesquiterpenes for *C. canephora* var. *Robusta*. As berries dried out, these compounds decreased and oxygenated compounds became more abundant. The results showed changes in volatile blends released by the coffee berries during ripening. Red berry effluvia are already known to attract colonizing females of the coffee berry borer; thus, specific compounds identified will be considered as candidate chemicals involved in the attraction.

Keywords: *Fresh coffee berry; Hypothenemus hampei; Coffea canephora var. Robusta; Coffea arabica; dynamic headspace collection; GC/MS*

1. INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), is the most important pest of coffee berries, reducing drastically coffee production in almost all coffee-growing countries (Reid and Mansingh, 1985). Thus, an understanding of the host plant colonization process is of high interest. Plant-produced chemosignals are already known to be involved in the host colonization process within the Coleoptera (Wood, 1982; Byers, 1989), and in the case of CBB females behavioral tests have already shown that the host plant colonization is related with the stage of berry ripeness whatever the coffee berry variety (Ticheler, 1961; Giordanengo et al., 1993). When in choice situation, CBB females always prefer red berries, to green berries and olfaction tests have demonstrated that the effluvia released by ripe berries are involved in the berry choice and in the orientation behavior toward the suitable coffee berries, compatible with brood development (Giordanengo et al., 1993; Mathieu, 1995). Recently, Mathieu et al. (1996) reported a first identification of volatile compounds released by eight varieties of fresh red coffee berries and identified 28 compounds, but no report has been published on the comparison of volatile chemicals released at different stages of ripeness in coffee berries. Considering the fact that the coffee beverage is consumed worldwide, it is surprising that chemicals in fresh coffee berries have been so little studied. Most of the papers report on chemicals identified in roasted beans. Only two papers reported on volatile chemicals released by fresh coffee material, but they concerned beans and not the berries (Liardon et al., 1990; Spadone et al., 1990). Moreover, the coffee beans were ground and submitted to a distillation–extraction before chemical analyses. This paper reports the identification of volatile compounds produced by three different stages of ripeness in fresh coffee berries within two coffee species: *Coffea canephora* var. *Robusta* and *Coffea arabica* unknown variety.

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2. EXPERIMENTAL PROCEDURES

2.1. Plant Materials. *C. arabica* (variety unknown) was provided by Nestlé (Tours, France) and was then grown in a greenhouse (Versailles, France). The *C. canephora* Pierre var. *Robusta* Linden was sampled in Gadgi (New Caledonia). Three stages of ripeness were sampled. Characterization of these different stages was mainly based on berry color. Green berries (G) have reached maximal size and presented a vitrified endosperm. Red berries were uniformly red, at the stage before the pulp starts to dry. Within the red berry stage three stages of ripeness were considered (R1; R2; R3): R2 and R3 were visually equivalent to R1 the day R1 was sampled, but they were left on the trees and picked some days later. Dry berries (D) were sampled on the tree and not on the ground. The pulp of dry berries, though dry, remains sticky and firm. Green (G) and red Robusta berries (R1) were collected on the same day as they were present simultaneously in the field, whereas dry Robusta berries (D) were collected 2 months later. Under greenhouse conditions *C. arabica* trees bear the three different stages of green (G), red (R), and first dry (Da), berries, which were sampled on the same day. Only dry berries (Db) were picked a fortnight later.

2.2. Dynamic Headspace. Airborne volatiles were collected on resin, mainly because this collection method is considered to reflect as closely as possible the blends of volatiles released by plants or living materials (Golub and Weatherston, 1984; Blight, 1990). Moreover, dynamic headspace does not disturb the dynamic emission of volatile compounds in comparison with extraction or static headspace techniques (Etievant et al., 1986; Venema, 1986).

Samples were placed in a cylindrical glass container connected on one side with a trap filled with Supelpack 2 (16/50 mesh; resin Amberlite XAD-2 purified, Supelco) and on the other side with an air purifier filled with Amberlite XAD-16 (Supelco). The air flow was generated by a vacuum pump with air stream through the device from the air purifier cartridge to the trap cartridge (Figure 1). Five independent sets were available. Desorption of the resin contained in the trap cartridge was achieved by washing the resin in a minimal

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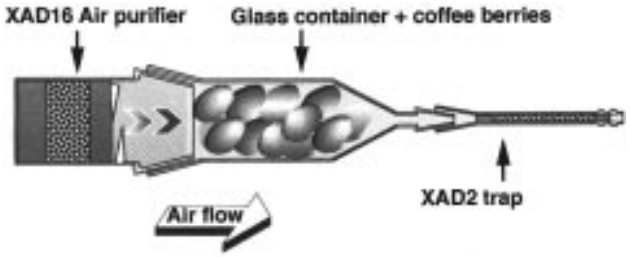

Figure 1. Device for dynamic headspace collection.

volume of CH_2Cl_2. After a few minutes of shaking, the solution was filtered over glass wool and then stored at −27 °C before analyses.

Effluvia samples were obtained under the following conditions: (for Robusta) temperature, 25 ± 2 °C; relative humidity, 80 ± 5%; number of berries, 1000; air flow, 40 mL min^{-1}; duration, 8 h; resin weight, 1 g; and volume of solvent, 1.8 mL; (for *C. arabica*) temperature, 25 ± 2 °C; relative humidity, 80 ± 5%; number of berries, 60; air flow, 40 mL min^{-1}; duration, 10 h; resin weight, 0.12 g; and volume of solvent, 0.5 mL.

Differences in experimental conditions were due to the fact that fewer *C. arabica* berries were available on the same tree compared with Robusta. As a consequence, experimental conditions were optimized for small samples.

2.3. Analytical Procedure. Compound identifications were achieved using the techniques mentioned in Table 1. Gas chromatographic analyses (GC) were carried out with a Hewlett-Packard 5890 gas chromatograph fitted with a split−splitless injector and a flame ionization detector. The fused silica capillary column used was a CPSil8 CB (25 m × 0.32 mm i.d., Chrompack) with temperature programmed from 30 to 55 °C at 15 °C min^{-1}, held for 7 min at 55 °C, and ramped from 55 to 210 °C at 8 °C min^{-1}. Injector and detector temperatures were, respectively, 225 and 240 °C. Helium was used as the carrier gas at 10 psi of pressure. Compounds were quantified with multilevel calibration curves (5, 50, 100, and 200 ng) obtained with the following compounds: 2-pentanone, 2-hexanone, 2-heptanone, limonene, linalool, and caryophyllene.

Gas chromatographic/mass spectrometric (GC/MS) analyses were carried out under the following two experimental conditions: (1) A Nermag R10-10C quadrupole mass spectrometer was coupled to a Girdel 32 gas chromatograph equipped with a split−splitless injector and a CPSil8 CB fused silica capillary column (25 m × 0.32 mm i.d., Chrompack) with temperature programmed at 40 °C for 2 min to 240 °C at 8 °C min^{-1}. Electron impact (EI) mass spectra were obtained at 70 eV, the instrument scanning from 40 to 350 amu. Chemical ionization (CI) mass spectra were generated at 92.5 eV using ammonia at a source pressure of 0.3 Torr, the instrument scanning from 60 to 400 amu. (2) A Varian Saturn II ion trap detector was coupled to a Varian 3400 gas chromatograph equipped with an SPI injector (40 to 240 °C at 240 °C min^{-1}) and a DB-5 fused silica capillary column (25 m × 0.32 mm i.d.) operated for 5 min at 40 °C and ramped from 40 to 240 °C at 5 °C min^{-1}. Mass spectra were obtained at 70 eV in EI mode.

3. RESULTS AND DISCUSSION

Table 1 reports results of chemical identification, the total amount of volatile compounds per berry (nanograms per berry), and the abundance of each identified compound per berry (nanograms per berry). Of the 45 compounds identified in this paper, 14 were detected and identified for the first time in the volatile blend released by fresh coffee berries. Only 12 of them have been previously reported in the volatile blend extracted from coffee beans (Spadone et al., 1990)

The chemicals identified can be split into five chemical groups: alcohols, ketones/aldehydes, acetates, terpenes, and sesquiterpenes. Chemical patterns of *C. arabica* and Robusta are shown Figure 2 as well as variations of percentages of each chemical group in relation with ripeness. Variations of quantities of each chemical group at different stages of ripeness are detailed in Figure 3.

In Robusta, the effluvia appeared to become more complex with the maturation process. Only 8 compounds were detected in green berry effluvia (G), whereas 19 compounds were identified in red berry effluvia (R1) and 29 in dry berry effluvia (D) (Table 1). The total amount of volatile compounds released by green berries was ∼16 ng/berry, whereas it reached 382 ng/berry for red berries and 1136 ng/berry for dry berries (Table 1). In Robusta, maturation of green berries into red berries appeared to be characterized by increases in the amounts and in the numbers of detected terpenes and sesquiterpenes (Figure 3). Red Robusta berries released mainly α-pinene (**22**), β-pinene (**25**), myrcene (**26**), limonene (**28**), caryophyllene (**40**), and humulene (**42**) (Table 1). In dry Robusta berries these compounds were either not detectable or present as traces. Dry Robusta berries produced mainly ketones and aldehydes, acetates, and alcohols, with the major components being 3-pentanone (**1**), 2-pentanone (**2**), hexanal (**12**), 2-pentyl acetate (**14**), and hexanol (**17**). Dry Robusta berries were also characterized by a high release rate of methyl salicylate (**35**).

In *C. arabica*, a single compound (**28**) was identified in the green berries (G). Ten were identified in red berries (R) and 14 in dry berries (Da and Db) (Table 1). The total amount of volatile compounds increased from 6 ng/berry for green berries to 233 ng/berry for red berries and reached 1094 (Da) to 1329 (Db) ng/berry for dry berries (Table 1). In the *C. arabica* studied here, no sesquiterpenes and no acetates were detected. Green and red berry effluvia were mainly made up of terpenes. *C. arabica* red berries released mainly limonene (**28**) and linalool (**34**), whereas dry *C. arabica* berries were characterized by release of alcohols, 2-pentanol (**3**), isopentanol (**6**), and 2-heptanol (**19**), and two particular compounds *cis*- (**32**) and *trans*-linalool oxide (**33**). Limonene (**28**), linalool (**34**), and the two linalool oxides (**32**, **33**) were identified for the first time in effluvia of both red and dry fresh berries of *C. arabica*. The increase of the total volatiles released by red *C. arabica* berries compared with green *C. arabica* berries was mainly due to an increase of the amount of terpene. The increase in total volatiles in dry berries was mainly due to the high release of oxygenated compounds. Methyl salicylate, the main component in dry Robusta effluvia, was missing in dry *C. arabica* effluvia, and this is probably a specific difference.

In both *Coffea* species as green berries matured into red berries, the emission of α-pinene (**22**), β-pinene (**25**), and limonene (**28**) increased. A decrease in terpene and (or) sesquiterpene abundance was also recorded with the maturation of red berries to dry berries for both *C. arabica* and Robusta. The increase of the amount of volatiles released by dry berries compared with red berries appeared to be a consequence of high quantities of oxygenated groups released (Figure 2), and this phenomenon seemed to characterize the maturation process of red berries to dry berries. Nevertheless, it should be noted that in *C. arabica*, it was due to alcohols, whereas in Robusta it was mainly due to ketones and aldehydes (Figure 3). Limonene (**28**) ap-

Table 1. Chemicals Identified from Effluvia Released by Fresh Coffee Berries

				C. canephora var. Robusta					*C. arabica*		
no.[a]		ID[b]	G[c]	R1	R2	R3	D	G	R	Da	Db
	no. of detected compounds		**8**	**19**	**23**	**27**	**29**	**1**	**10**	**14**	**14**
	total quantity (ng/sample)		15.57	382.9	463.5	236.3	1136	5.685	232.9	1094	1329
	compounds[d]										
1*	3-pentanone	1					42.9				
2	2-pentanone	1, 2, 3, 4	1.8		12.0	1.5	82.7				
3	2-pentanol	1, 2, 3, 4			14.4	16.5	15.5			26.6	54.9
4	3-hydroxy-2-butanone	1, 2, 3, 4				37.0	18.9		25.4	24.2	24.2
5	3-methyl-3-butenol	1					13.5		13.0	105.7	354.8
6	isopentanol	1, 4					10.6		13.0	93.9	478.2
7*	2-methylbutanol	1					1.5			11.8	6.5
8*	1,1-dimethylcyclopropane	1				0.5					
9	isobutyl acetate	1, 3	0.4		3.4	25.0	10.0				
10*	2,3-butanediol	1, 3, 4				2.6	5.6			63.2	85.6
11*	1-methoxy-2-propanol	1, 3	3.9			0.9	2.2			74.7	67.3
12*	hexanal	1					41.4			10.8	2.9
13	butyl acetate	1		0.5	1.4	0.7					
14	2-pentyl acetate	1, 3		1.6	16.3	11.9	19.9				
15	isobutanoic acid ethyl ester	1, 3		0.5	12.4	0.7	6.5				
16	unidentified compound	1					6.8				
17*	hexanol	1, 4					56.2				
18	2-heptanone	1, 2, 3, 4			3.4	3.1	9.5			191.3	61.3
19*	2-heptanol	1, 2, 3, 4				1.1	16.3			390.8	41.7
20	thujene	1, 3		0.4	1.1	0.3					
21	unidentified compound								4.7		
22	α-pinene	1, 2, 3	0.9	18.6	21.7	8.7	2.9		4.7		
23	camphene	1, 3	2.1	3.4	3.9	1.1					
24	sabinene	1, 3		1.2	1.3	0.7					
25	*β*-pinene	1, 2, 3		11.3	7.3	3.1	0.9		3.8		
26	myrcene	1, 2, 3		14.6	12.9	5.1			5.2		
27	cymene	1		1.6	2.1	0.4	7.7				
28	limonene	1, 2, 3, 4	0.8	275.6	220.7	100.7	38.4	5.7	93.8	19.4	22.7
29	unidentified compound						13.0				
30	2-heptyl acetate	1, 3			4.4	3.4	2.7				
31	4,7-dimethyl-4-octanol + ?	1							7.0		
32*	*cis*-linalool oxide	1								14.7	48.1
33*	*trans*-linalool oxide	1								12.3	48.7
34*	linalool	1, 2, 3, 4							62.2	54.6	32.3
35*	methyl salicylate	1, 2					661.0				
36*	ethyl salicylate	1					3.7				
37	oxygenated terpene	1					29.8				
38	copaene	1, 3		1.0	2.2	0.1					
39	bourbonene	1, 3		2.6	4.9	0.9					
40	caryophyllene	1, 2, 3, 4	4.3	25.0	81.3	4.9	8.4				
41	*β*-gurjunene	1, 3		0.4	0.8						
42	humulene	1, 2, 3, 4	1.5	21.5	29.4	3.8	7.3				
43*	*γ*-muurolene	1, 3		2.5		0.5					
44	D-germacrene	1, 3		0.3	5.3	0.7					
45	sesquiterpene	1, 3		0.4	1.0	0.4					

[a] Compound numbers according to the text. *, Newly identified in fresh coffee berries. [b] Method of identification: **1**, EI analysis [mass spectra compared with mass spectra in EPA/NIH library; spectra and elution order of terpenes and sesquiterpenes also compared to those quoted in Adams, (1989)]; **2**, mass spectra compared with mass spectra of pure synthetic compounds; **3**, CI-NH_3; **4**, coinjection with pure synthetic compounds. [c] **G**, green berry; **R**, red berry; **D**, dry berry. See text for further explanations. [d] Quantities of each compound in ng per berry calibrated with 2-pentanone (**1**−**11**), 2-hexanone (**12**−**17**), 2-heptanone (**18**−**19**), limonene (**20**−**29**), linalool (**30**−**37**), and caryophyllene (**38**−**45**).

peared to be one of the few compounds released by both species throughout the berry maturation, but the amounts differed greatly with the stage of ripeness. In red berries, only α-pinene, *β*-pinene, and limonene were shared by both species. Terpene and sesquiterpene compounds derived from the secondary metabolism (Bernays and Chapman, 1994) and appeared to be highly related to the physiological stage of the berries. The increase of terpene groups and in particular limonene and α- and *β*-pinene seemed to be associated with the red stage in both varieties. α- and *β*-pinene are already known as semiochemicals, important, for example, in the selection of the oviposition sites by the pine beauty moth (Leather, 1987) and in attracting and eliciting oviposition in the eastern spruce budworm (Städler, 1992). In contrast, limonene is found to be repellent for a pyralid moth (Peterson et al., 1994). Within Coleoptera and more particularly scolytids, α-pinene is reported as a semiochemical produced by a coniferous host tree and acting as a kairomone for *Dendroctonus* sp. (Borden, 1985).

In both *Coffea* species, the maturation of red berries to dry berries appeared to be related with the increase of oxygenated compounds (Figure 3). Within the red berries of Robusta R2 and R3 that were sampled some days later than R1, and thus that were a little more mature than R1, differences in blends of volatile chemicals were seen. R2 and R3 produced oxygenated compounds found in dry berries and higher amounts of the compounds already identified in R1, whereas terpenes and sesquiterpenes remained similar to the R1 sample (Table 1; Figure 2). Though comparisons were hard to make between R1, R2, and R3 due to visual determination of ripeness stages that could introduce a bias,

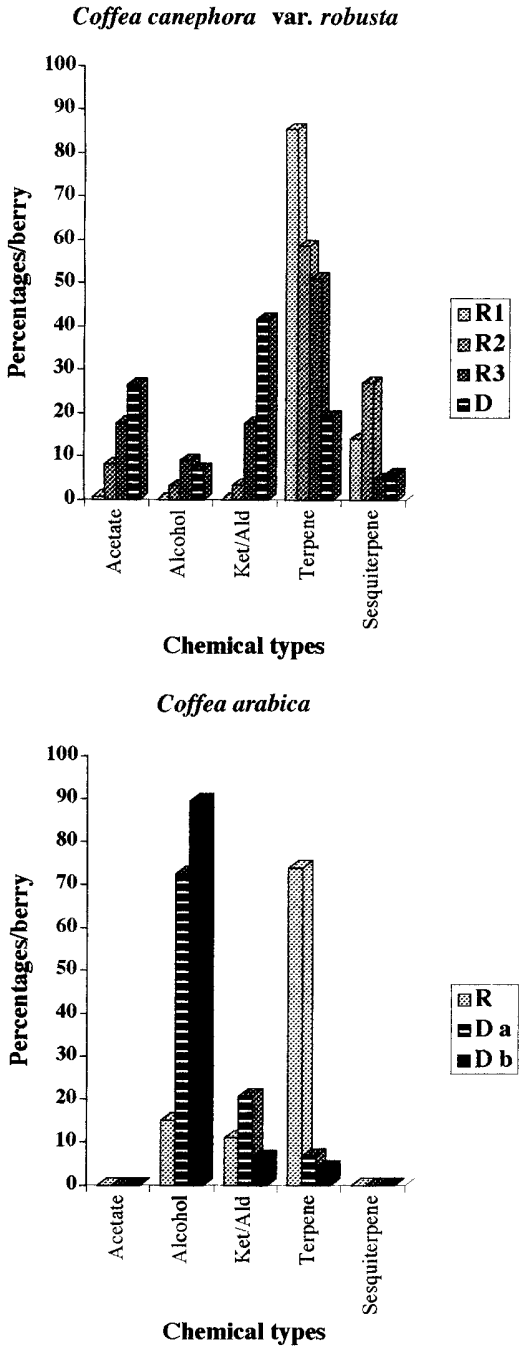

Figure 2. Variations in percentages of the volatile chemicals released by red (R1, R2, R3, R) and dry (Da, Db, D) berries of two *Coffea* species.

these results showed that aging red berries tended to produce more oxygenated compounds than younger red berries, corroborating the fact that maturation of red berries to dry berries was found to be characterized by oxygenated compounds.

The same Robusta and three different *C. arabica* varieties (unknown, Leroy, and Catimore) have been previously analyzed at the red stage (Mathieu et al., 1996). For Robusta, the results obtained here confirm the previous identifications, but for *C. arabica*, results departed from already published data. More components were detected in this study than before, whereas two previously identified components, caryophyllene and humulene, were missing, possibly due to difference

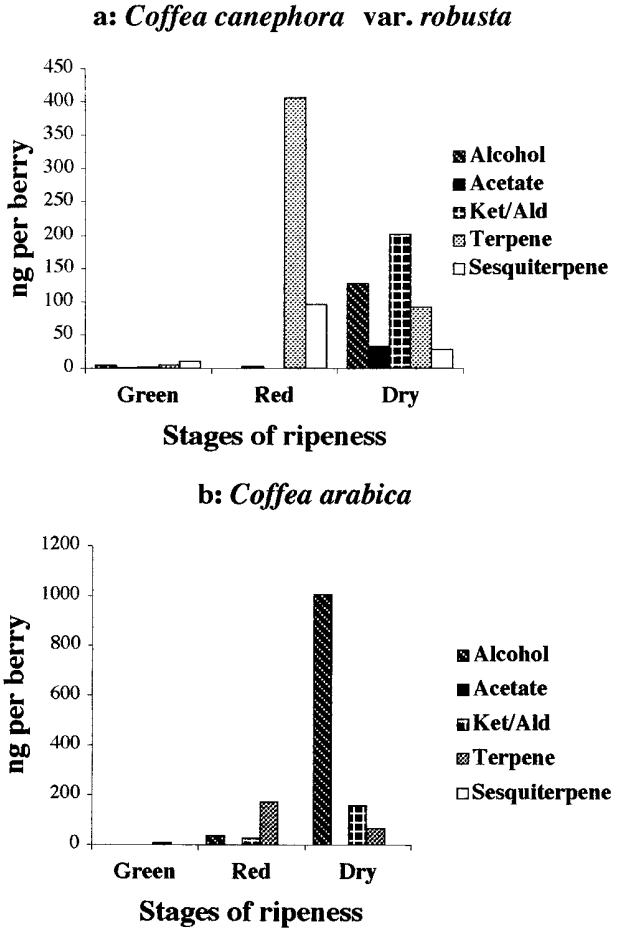

Figure 3. Variation of quantities of the different chemical types at different stages of ripeness.

either in varieties of *C. arabica* studied or in experimental conditions. The *C. arabica* previously studied belonged to other varieties grown in the field, whereas in this study we used a selected variety grown in a greenhouse. Both circumstances could account for the different results.

However, on the basis of the identifications and differences evidenced by chemical analyses between stages of maturation, further experiments will be undertaken to determine whether the common compounds and especially terpenes are involved in the host plant selection process in *H. hampei* females.

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