JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Composition and Flavor of Ecuadorian Cocoa Liquor

FABIENNE LUNA,[†] DOMINIQUE CROUZILLAT,[†] LOÏC CIROU,[§] AND PETER BUCHELI*,[‡]

Department of Plant Science, Nestlé Research Center Tours, 101 Avenue G. Eiffel, Notre Dame d'Oé, B.P. 9716, 37097 Tours Cedex 2, France; Nestlé France, Usine de Saint Menet, 41 Avenue de la gare, 13011 Saint Menet, France; and Nestlé R&D Center Shanghai Ltd., No. 13 Qiao Nan, Cao An Road, Jia Ding District, Shanghai 201812, People's Republic of China

The contribution of the chemical composition to the flavor of cocoa liquor from an Ecuadorian selfed population of clone EET 95 was investigated. Polyphenols, purine alkaloids, organic acids, and sugars were quantified, and the key sensory characteristics of cocoa were scored by a trained panel. Despite the short bean fermentation (2 days) commonly used for Arriba cocoa, acetic acid content was closely correlated to liquor pH, demonstrating its essential role in cocoa liquor acidification. Polyphenols were positively correlated to astringency, bitterness, and the green note and negatively correlated to the fruity character. Alkaloid and polyphenol levels fluctuated significantly within the selfed progeny and tended to be lower than those of the heterozygous clone EET 95 (inbreeding effect). These results support the idea that polyphenols might be essential to the overall perception of cocoa liquor characteristics and indicate that the composition and the sensory quality of cocoa liquor are the result of both a genotypic contribution and the conditions of fermentation and roasting.

KEYWORDS: Cocoa; *Theobroma cacao*; liquor; flavor; polyphenols; alkaloids; sugars and acids; sensory evaluation

INTRODUCTION

The different cocoa flavor attributes such as cocoa flavor intensity, bitterness, astringency, and acidity are thought to be the result of bean fermentation and roasting (*1*). Indeed, they may be caused by several constituents, which are modified during postharvest processing (fermentation and drying) and roasting (*2*, *3*).

The acidification of cocoa beans by acetic acid during fermentation leads to various biochemical modifications necessary for cocoa flavor development (*4*). These changes include the generation of peptides and amino acids from storage proteins by the action of cocoa seed proteases (*5*) and reducing sugars, which serve as precursors for the Maillard reactions taking place during the drying and roasting of cocoa beans (*6*, *7*). Fermentation also leads to a strong reduction of soluble polyphenols (*8*). During this stage, one-fourth of the purine alkaloids theobromine and caffeine are lost by exudation (*9*) and various volatile components are generated (alcohols, esters, and aldehydes) (*10*, *11*). Roasting leads to the development of specific cocoa aromas via the Maillard reaction, caramelization of sugars, degradation of proteins, and formation of volatile components such as pyrazines (*2*, *3*, *12*), which were described as one of the few classes of compounds with desirable flavor properties (*13*).

The extent to which the genotype of *Theobroma cacao* L. affects cocoa flavor attributes has not been unequivocally established, and very few studies have addressed this question in detail. The investigations of Clapperton et al. (*14*−*16*) on six varieties grown in Sabah (Malaysia) demonstrated a genotypic effect on sensory attributes such as astringency, bitterness, and cocoa flavor intensity. They showed a link between polyphenols, astringency, and cocoa flavor intensity and between alkaloids and bitterness intensity. Other studies pointed to the involvement of other chemical components in cocoa flavor. Holm et al. (*17*) worked on the effects of organic acids on cocoa flavor and acidity, whereas Yoo et al. (*18*) investigated the pattern of the organic acid and sugar composition in cocoa mass produced by a new processing method.

To improve our understanding of cocoa flavor and the underlying chemical basis of this flavor, we have initiated a study on an Ecuadorian cacao population to determine the relationship between genotype, the chemical constituents likely to be involved in flavor (polyphenols, alkaloids, organic acids, and sugars), and the key sensory characteristics of cocoa (bitterness, astringency, cocoa flavor intensity, acidity, fruity, floral and green note).

MATERIALS AND METHODS

Plant Material. Cacao beans were harvested tree by tree in August 2000, from a population derived from self-pollinated clone EET 95 (Nacional × Venezolano Amarillo) grown at the same farm in Quevedo (Ecuador). The trees were open-pollinated within 1 month, but due to

* Corresponding author (telephone +86 21 5913 4488; fax +86 21 5913 4819; e-mail peter.bucheli@rdsh.nestle.com).
[†] Nestlé Research Center, Tours.
[§] Nestlé France, Saint Menet.
[‡] Nestlé R&D Center Ltd., Shanghai.

10.1021/jf0116597 CCC: $22.00
Published on Web 04/30/2002

the self-compatible status of the EET 95 clone, most pollinations were in fact selfings. The pods were not harvested at the same time due to different pollination dates and differences in the duration of pod maturation within the segregating population. All pods were harvested at maturity, and each bean sample was fermented for 2 days in individual net bags in a heap of 80 kg of cacao beans and then sun-dried to obtain a minimum of 500 g of beans for each tree. Thirteen batches of beans from trees of the progeny, coded from D to P, were randomly selected. One of them was divided into two aliquots before the stage of fermentation (O1/O1′), as were four others after the drying (D1/D2, G1/G2, M1/M2, and O1/O2) to get duplicates for the assessment of the chemical and sensory reliability. Three batches of the parental clone EET 95 (A/B/C) were fermented in three different net bags but in the same heap. One of these batches was divided after the drying (B1/B2). In addition, two representative Ecuadorian cocoa bean samples (Q and R) were harvested in the same period for use as references for Arriba flavor and fermented for 2 days the same as the other samples. The effect of different fermentation times was also tested on clone EET 95. Cocoa liquor samples corresponding to 2, 3, 4, and 5 days of fermentation were prepared in duplicate.

Liquor Analysis. Samples were roasted in a laboratory roaster (10−12 min, 210 °C) at the Nestlé Saint Menet factory (France), and milled (Bioblock M20) to obtain cocoa liquor. Fat content was determined by placing 20 g of cocoa liquor in an NMR tube and heating in a fan oven at 50 °C for at least 1 h. The liquor sample was then introduced in the Oxford MQA 6005 pNMR, and fat content was determined and expressed as a percentage of weight. The pH of 2 g of cocoa liquor in solution with 18 mL of water was measured with a pH-meter according to an adapted version of the International Office of Cocoa, Chocolate and Sugar Confectionery method (*19*).

Defatting and Cocoa Powder Extraction. Cocoa liquor was defatted by extracting 20 g of cocoa liquor in a Soxhlet apparatus with 2 × 500 mL of petroleum ether (60−80 °C) during successively 4 and 3 h. The defatted residue (cocoa powder) was air-dried and stored at −20 °C before being extracted in triplicate for 1 h in boiling water at a concentration of 20 mg/mL. After cooling at room temperature, the samples were centrifuged in Eppendorf tubes (10000*g*, 10 min). The obtained supernatant was used to quantify alkaloids, organic acids, and sugars. For the determination of polyphenols, 1 g of cocoa powder was extracted for 2 h in ∼60 mL of boiling water under reflux according to the method described in the *Official Journal of the European Communities* (*20*). Samples were centrifuged in 50 mL Falcon tubes (1500*g*, 15 min), and the supernatant was filtered (Schleicher & Schuell paper $597^{1}/_{2}$).

Chemical Analyses of Extracts. The alkaloids were separated by HPLC using a Nucleosil 100-5 C_{18} column (250 × 4 mm i.d.) (Macherey-Nagel, catalog no. 720014) with a mobile phase of 0.05% phosphoric acid/acetonitrile (9:1). The injected sample volume was 20 μL, and the separation was performed at a flow rate of 1 mL/min. The detection (diode array) was monitored at 274 nm. Theobromine and caffeine were quantified by external calibration with standard solutions of theobromine (0.08 mg/mL) and caffeine (0.02 mg/mL). Organic acids were analyzed by high-performance anion exchange chromatography coupled to pulsed electrochemical detection (HPAE-PED) using a Dionex AS 11 (250 × 4 mm i.d.) column connected to an anionic membrane suppressor and separated by a gradient of 0.5−38.25 mM NaOH for 18 min. The injected sample volume was 20 μL, and the flow rate was 1.5 mL/min. Sugars were quantified by HPAE-PED, using a Dionex PA 1 (250 × 4 mm i.d.) column, with a gradient of 12−200 mM NaOH for 23 min and a flow rate of 1 mL/min (*21*). Polyphenols were quantified according to the method described in the *Official Journal of the European Communities* (*20*), which is based on the oxidation of polyphenols (0.1 mL of extract) by the Folin−Ciocalteu reagent. The absorbance of the solution was measured by a spectrophotometer at 750 nm against a reference sample. Linear standard curves were obtained for a solution of epicatechin in the concentration range of 0−8 mg/L. All results are expressed as a mean of six values in milligrams of epicatechin equivalents per gram of dry weight (DW) of defatted material.

Sensory Analysis. Liquor was tasted by a panel of Nestlé France (Saint Menet) composed of seven trained tasters. Seven attributes were scored from 1 to 5 (1 = absent, 2 = weak, 3 = moderate, 4 = strong, and 5 = very strong): cocoa flavor (flavor of well-fermented cocoa beans), bitterness (basic taste quickly perceived on the back of the palate and the throat), astringency (substances causing a contraction of mouth tissues), acidity, fruity (fruit flavor, round sweet aroma note), floral (flavor of flowers, fresh perfume), and green note (typical flavor of nonroasted beans). Two representative Ecuadorian liquor (Q and R) were used as reference for Arriba flavor. The liquor samples of clone EET 95,which underwent different fermentation times, were tasted by the internal cocoa panel of Nestlé PTC York (U.K.) composed of eight trained tasters. Cocoa flavor was scored from 0 to 10.

Statistical Analysis. One-way single-factor analysis of variance (ANOVA) was performed using NCSS 2000 software (version 2000). The F ratio was used to determine statistical significance at $p < 0.05$. A multiple-comparison test using Fisher's least significance difference (LSD) was achieved. Correlation coefficients (r) and their levels of significance were calculated to identify possible associations between chemical and sensory data. Principal component analysis (PCA) was realized to describe the variability of sensory and chemical data.

RESULTS AND DISCUSSION

Chemical Analysis of Cocoa Liquor. The results of the quantification of fat, alkaloids, organic acids, polyphenols, and sugars are shown in **Table 1**. Significant differences ($p < 0.000001$) were found for each chemical constituent between the 24 samples examined. Fat content ranged from 47.2 (P) to 54.1% (G2) within the progeny with a mean of 50.3 ± 1.8% and was 52.8 ± 1.0% for clone EET 95. Fat content determination by the Soxhlet method gave similar results (data not shown). Theobromine content varied from 17 (G1) to 26.3 mg/g of DW of defatted material (M2) within the progeny, with a mean of 21.8 ± 2.4 mg/g. The level was higher for clone EET 95 (29.8 ± 2.4 mg/g). Caffeine concentrations were 3.4−7 times lower than those for theobromine, which is in accordance with an earlier study (*16*). Levels of polyphenols were also higher for clone EET 95 (74.7 ± 7.1 mg/g) than for the progeny (48.0 ± 13.2 mg/g), which ranged from 30.3 (H and I) to 74.2 mg/g (D2). Comparison with other studies is difficult because of the different methods used for the quantification of polyphenols (*22*). However, the results concur with those found using the colorimetric Folin−Ciocalteu method with catechin as a standard (65 mg/g of DW of defatted material) (*23*). Generally, as illustrated for theobromine (**Table 1**), concentrations of chemical constituents tended to be lower for the selfed population than for the heterozygous clone EET 95, suggesting an inbreeding effect. This was in agreement with earlier data on the same population (*24*) that had shown that bean weight of clone EET 95 was higher than for most of the segregating progeny. Theobromine content was significantly correlated to polyphenol content ($r = 0.75$) (**Table 2**), a fact that cannot be explained by a direct link between their biosynthetic pathways. Polyphenols are synthesized by the shikimate and acetate pathways (*25*), both deriving from glucose metabolism, whereas alkaloids originate from the ubiquitous nucleotide pool (*9*). However, theobromine and polyphenols appear to be co-localized in storage cells of cocoa seed (*26*).

Citric acid was the main organic acid, whereas malic acid had the lowest concentrations of the acids examined (**Table 1**). A similar trend and the same range of concentrations were found in studies that reported the levels of these acids in cocoa beans from different geographic regions (*17*) and during different stages of bean maturity (*27*). Within the progeny, acetic acid content ranged from 8.6 to 13.7 mg/g of DW of defatted material and pH values were between 5.6 and 6.3 (**Table 1**). Acetic acid was found to be the only acid significantly correlated with pH ($r = -0.69$) (**Table 2**). Liquors D and J had the lowest pH

Table 1. Contents[a] of Fat, Alkaloids, Organic Acids, Polyphenols, and Sugars (in Milligrams per Gram of Dry Weight of Defatted Material) for 24 Ecuadorian Cocoa Liquor

		fat	theo		organic acids					poly-	sugars		
	sample	content[b]	bromine	caffeine	acetic	malic	oxalic	citric	pH	phenols	glucose	fructose	sucrose
EET 95	A	53.2	33.3	4.8	8.2	2.5	7.6	9.7	6.3	70.4	1.6	1.4	22.6
	B1	52.0	28.3	4.8	10.6	3.9	7.6	9.2	6.0	85.2	3.2	5.7	15.5
	B2	51.8	28.3	4.3	10.8	4.1	7.9	9.5	5.9	70.5	2.6	6.3	16.5
	C	54.0	29.3	4.9	9.7	3.1	7.4	8.1	6.2	72.8	1.8	2.5	18.1
selfing	D1	50.1	23.0	4.3	13.3	3.6	8.7	10.6	5.6	58.8	2.9	7.9	22.3
progenies	D2	49.8	21.3	4.9	13.5	4.2	7.7	9.1	5.6	74.2	3.1	10.5	20.3
	E	50.4	21.2	4.3	11.9	3.9	6.3	12.8	5.8	46.7	4.9	12.2	20.9
	F	48.1	21.1	3.1	8.7	3.5	7.2	16.6	6.1	34.1	4.2	8.8	20.3
	G1	53.2	17.0	2.8	8.9	3.6	6.2	8.8	5.8	33.7	4.9	7.8	16.7
	G2	54.1	21.4	4.0	10.0	2.5	7.6	12.3	5.7	51.8	4.9	12.9	21.1
	H	50.1	17.5	3.6	10.3	4.3	5.3	16.7	6.3	30.3	3.9	7.5	25.9
	I	47.3	23.5	3.9	12.0	3.7	7.1	12.7	6.1	30.3	2.5	5.2	15.5
	J	50.8	22.7	5.2	13.7	3.7	7.5	12.1	5.6	54.1	3.0	9.9	18.7
	K	51.8	22.1	4.1	12.9	2.8	7.1	9.6	5.7	66.9	2.7	9.2	10.2
	L	50.0	21.5	4.9	11.0	2.3	8.1	11.4	5.8	46.5	1.7	6.3	23.4
	M1	49.0	24.4	7.1	9.3	3.4	6.5	8.7	6.0	51.6	2.5	1.6	20.5
	M2	50.0	26.3	7.2	9.6	2.9	7.8	11.4	5.9	63.5	2.7	5.0	24.5
	N	49.1	24.8	4.4	10.7	3.7	7.3	12.9	6.0	60.1	2.5	5.5	16.4
	O1	50.9	20.2	5.1	8.6	3.2	7.1	10.2	6.3	33.9	3.9	8.1	24.5
	O2	51.1	21.1	5.0	11.3	3.8	7.5	13.3	6.2	38.1	3.7	7.1	25.0
	O1′	51.9	23.4	4.1	8.9	2.5	6.7	11.5	6.0	48.2	2.2	6.9	24.9
	P	47.2	19.7	4.0	11.9	2.7	7.2	11.4	6.0	42.1	3.2	7.9	22.1
Ecuadorian	Q	52.2	22.6	5.2	7.8	3.5	7.4	12.0	6.3	53.9	1.8	5.0	21.4
references	R	53.8	27.4	6.6	9.2	4.4	8.6	11.0	6.2	66.5	3.3	8.1	21.9

[a] Results are expressed as means of three values except for polyphenols (six values). [b] Percentage of weight.

Table 2. Correlation[a] between Sensory Attributes and Chemical Components of 24 Ecuadorian Cocoa Liquor

	bitter-		astrin-			theo-							
	ness	acidity	gency	fruity	green	bromine	caffeine	acetic	malic	oxalic	citric	pH	glucose
bitterness													
acidity	0.54**												
astringency	0.80***	0.61**											
fruity	–0.58**		–0.69***										
green	0.75***	0.53**	0.56**										
theobromine	0.59**				0.54**								
caffeine													
acetic													
malic													
oxalic													
citric	–0.64***				–0.52**								
pH		–0.72***						–0.69***					
glucose						–0.61**							
fructose						–0.60**						–0.54**	0.73***
sucrose			–0.58**										
polyphenols	0.84***		0.79***	–0.64**	0.78**	0.75***				0.55**	–0.60**		

[a] Only significant correlations (**, $p < 0.01$; ***, $p < 0.001$) are shown.

values and the highest acetic acid concentrations. Indeed, during the fermentation stage, acetic acid develops in the pulp through sugar degradation by microorganisms and then diffuses into the cotyledon (*4*), in which it generates a decrease of pH from ~6.5 to ≤5 (*28*). This indicates that acetic acid could be associated with acid flavor as was shown in a previous study (*17*). The influence of fermentation on pH was studied by analyzing liquor made from EET 95 beans that were subjected to different fermentation times. As expected, pH was found to decrease significantly as fermentation proceeded (**Table 3**). Despite the fact that sucrose is being hydrolyzed during fermentation to glucose and fructose (*2*), it was found to be quantitatively the most important with concentrations ranging from 10.2 to 25.9 mg/g of DW of defatted material. This is probably due to the short fermentation (2 days), which limits the acetic acid production and, thereby, the consequent drop of bean pH and sucrose hydrolysis. This would explain why the pH values found for the samples fermented for 2 days were high compared to those cited in a previous review (*4*) and why levels of sucrose were higher than those of maximum 0.1% reported by Ziegleder and Biehl (*2*) for cocoa beans fermented between 4 and 6 days.

Table 3. Effect of Fermentation Time on EET 95 Cocoa Flavor Score and pH

fermentation time (days)	cocoa flavor score[a]	pH[b]
2	4.5	6.4 a
3	4.6	5.9 b
4	5.0	5.3 c
5	5.5	5.6 d

[a] Cocoa scores are means of two samples tasted by eight panelists. [b] Results are means of four values. The same letter indicates that there is no significant difference ($p < 0.05$).

Sensory Analysis of Cocoa Liquor. Concerning bitterness and the green note, values tended to be higher for clone EET 95 than for the selfed population coming from this genotype

Table 4. Flavor Scores[a] for 24 Ecuadorian Cocoa Liquor

	sample	cocoa flavor	bitter-ness	acidity	astring-ency	fruity	floral	green
EET 95	A	3.1	3.4	1.7	2.9	2.4	3.3	2.3
	B1	2.9	4.0	3.1	3.4	1.7	1.7	2.6
	B2	3.1	3.3	2.0	2.9	2.3	2.7	2.0
	C	3.7	4.3	2.4	3.0	2.0	2.3	2.3
selfing progenies	D1	3.4	3.3	3.3	2.7	2.4	2.3	2.0
	D2	3.1	4.1	2.7	3.4	1.9	1.9	2.6
	E	3.3	2.7	2.4	2.4	2.7	2.4	2.3
	F	3.1	2.1	2.0	2.4	2.6	2.0	1.6
	G1	3.1	2.7	2.7	2.4	2.1	2.4	1.7
	G2	3.4	3.3	2.4	2.4	2.9	1.9	2.0
	H	2.9	2.4	1.3	2.1	2.4	2.3	1.4
	I	3.3	2.4	1.9	2.3	3.0	2.7	1.3
	J	3.0	3.1	2.0	2.6	2.7	2.4	1.9
	K	3.0	3.3	2.9	3.9	1.9	1.9	1.7
	L	3.0	3.0	2.4	2.9	2.3	2.6	1.4
	M1	3.3	3.9	2.3	2.9	2.7	2.1	1.9
	M2	3.3	3.4	2.4	3.0	2.0	2.3	2.3
	N	3.0	3.7	1.7	3.1	2.0	2.1	1.9
	O1	2.9	2.3	1.3	2.0	2.7	2.3	1.7
	O2	3.1	2.4	1.4	2.1	3.1	2.3	1.6
	O1′	3.1	2.6	2.1	2.3	2.6	1.9	1.9
	P	3.1	2.4	1.4	2.1	3.4	2.9	1.6
Ecuadorian references	Q	3.1	2.4	1.6	2.0	2.0	2.0	1.6
	R	3.4	3.3	1.6	3.0	2.4	2.9	1.9

[a] Results are means of the scores of seven panelists (1 = absent, 2 = weak, 3 = moderate, 4 = strong, 5 = very strong).

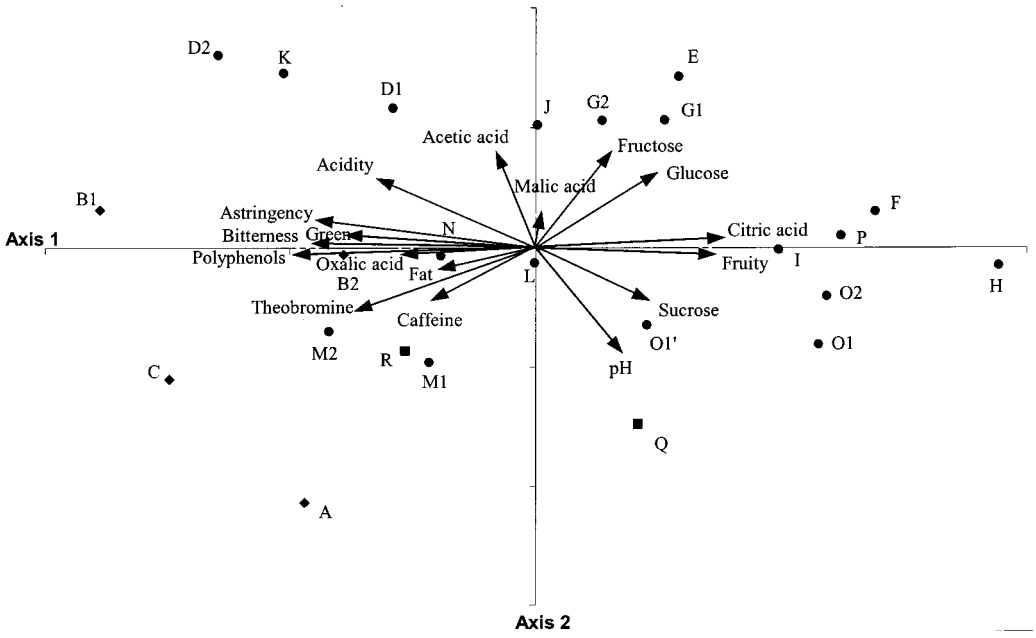

Figure 1. Principal component loading plots from chemical and sensory data of Ecuadorian cocoa liquor: (◆) EET 95 (A, B1, B2, C); (■) two reference samples; (●) samples of the progeny. Axes 1 and 2 represent 36.3 and 19.1% of the variability, respectively. Each duplicate is annotated with the same letter.

(**Table 4**). Liquor D2 had the highest scores for bitterness, astringency, and green note and the weakest score for fruity, and it had increased acidity. The correlation analysis performed on the sensory data indicated that bitterness was correlated positively with astringency ($r = 0.80$), green note ($r = 0.75$) and acidity ($r = 0.54$) and negatively with fruity ($r = -0.58$). Astringency was correlated negatively with fruity ($r = -0.69$) and positively with acidity ($r = 0.61$) and green note ($r = 0.56$) (**Table 2**). An ANOVA was performed to quantify the extent of the origin of the variability that was due to the panel and due to the liquor. There were no significant differences at $p < 0.05$ between the liquor concerning cocoa flavor and the floral note, and ~30% of the total variance of these two attributes was explained by the panel (**Table 5**). The nonsignificant variability of cocoa flavor was probably because of a weak development of cocoa flavor upon 2 days of fermentation and the difficulty for the panel to detect it. The effect of the fermentation time on the development of the cocoa flavor was confirmed by tasting liquor made from EET 95 beans (**Table 3**). This experience showed that samples with longer fermentation had higher cocoa flavor. This result is in agreement with the recent finding of Cros et al. (*3*). With regard to bitterness, significant differences were detected only for liquor (**Table 5**). For acidity and the green note, significant differences between

Table 5. Origin of the Variability of the Sensory Evaluation on 24 Ecuadorian Liquor

variable	liquor variance[a]	panel variance[a]
cocoa flavor	NS[b]	30.0******
bitterness	38.0******	NS
acidity	35.9*****	17.3****
astringency	22.6*	36.2******
fruity	20.7*	23.8******
floral	NS	27.6*****
green	24.7**	12.2**

[a] Expressed as percentage of the total variance. ******, significant at $p < 0.000001$; *****, significant at $p < 0.00001$; ****, significant at $p < 0.0001$; **, significant at $p < 0.01$; *, significant at $p < 0.05$. [b] Not significant.

the samples originated for the most part from the liquor. Liquor and panel variances were approximately equal for the fruity character.

Composition and Flavor Relationship. The results of the PCA of the chemical and sensory data, reporting the distribution of the variables and cocoa liquor samples (55.4% of the total variance on the first two axes), are presented in **Figure 1**. The two nonsignificant variables, cocoa flavor and floral note, were taken out of the data processed. Correlation coefficients calculated between each of the flavor attributes and the level of chemical constituents (**Table 2**) indicated that polyphenols were correlated positively with bitterness ($r = 0.84$), astringency ($r = 0.79$), and green note ($r = 0.78$) and negatively with fruity ($r = -0.64$). There were also positive correlations between theobromine and bitterness ($r = 0.59$) and green note ($r = 0.54$). Citric acid was correlated negatively with bitterness ($r = -0.64$) and green note ($r = -0.52$), and pH was corrrelated negatively with acidity ($r = -0.72$). Cocoa liquor samples were plotted according to their chemical and sensory composition. Liquor D and K were bitter, astringent, and acid and had high polyphenol levels. They were opposed to liquor P, F, O, H, and I, which had high contents of citric acid and high fruity scores. The two Ecuadorian reference liquors were located among the progeny samples, whereas the parental clone (A/B/C) seemed to be off-center, but linked to polyphenol, alkaloid, and oxalic content.

Assessing duplicate cocoa samples provided some information about the reliability of chemical and sensory analysis. Among the sensory attributes tested, acidity showed significant differences ($p < 0.00001$) for three of the duplicates (A/B, B/C, and O1/O1′). In contrast, for chemical parameters such as acids and polyphenols most samples were found to be significantly different ($p < 0.000001$) from their duplicate (data not shown). These findings indicate that some variations, independent of the genotype, existed despite the standardized cocoa bean sample treatment. Fermentation or roasting could be the source of these variations. It is known that fermentation is not homogeneous between the center and the edges of the mass of cocoa beans (*29*), and polyphenols appear to be particulary sensitive because fermentation leads to a reduction of soluble polyphenols due to the diffusion, browning, and oxidative polymerization phenomenon (*8*). Degradation of the phenolic compounds also occurs during the roasting stage (*3*). However, the strong correlations between polyphenols and some sensory attributes suggest that they are the principal source of astringency, confirming the studies of Clapperton et al. (*16*, *30*). Interestingly, they were also the main components involved in the bitterness and green note (**Table 2**), meaning that bitterness may not be related only to alkaloids as previously shown by Clapperton et al. (*16*) but could also be explained by the inherent bitterness of astringent polyphenolic compounds (*31*). In addition, the results show that the higher the level of polyphenols, the less fruity were the cocoa liquor. It is conceivable that bitterness and astringency, both related to polyphenols, mask the fruity flavor.

The notable diminution of alkaloids and polyphenols levels found in the selfed progeny samples compared to the heterozygous clone EET 95 point to a possible influence of the genotype on alkaloid and polyphenol content with a consequent impact on the bitter, green, and astringent notes (*16*). However, a physiological effect on the chemical and sensorial differences between the parental clone and the progeny cannot be excluded, as illustrated by the fact that clone EET 95 used in the study was ~5 years older than the segregating population.

ACKNOWLEDGMENT

We thank M. Alvarez (Orecao S.A., Quito, Ecuador) for the field and postharvest management of cocoa samples, K. Monnier and the panel of Nestlé France for the sensory evaluation of the Ecuadorian cocoa liquor, and A. Touché and S. Michaux for their contribution in the analysis of alkaloids, sugars, and organic acids.

LITERATURE CITED

(1) Rohan, T. A. The flavor of chocolate, its precursors and a study of their reaction. *Gordian* **1969**, *69*, 443−447, 500−501, 542−544, 587−590.

(2) Ziegleder, G.; Biehl, B. Analysis of cocoa flavor components and flavor precurseurs. In *Analysis of Nonalcoholic Beverages*; Linskens, H. F., Jackson, J. F., Eds.; Springer: Berlin, Germany, 1988; pp 321−393.

(3) Cros, E.; Chanliau, S.; Jeanjean, N. Post harvest processing: a key step in cocoa. In *Confectionary Science II. Proceeding of an International Symposium*; Ziegler, G. R., Ed.; Pennsylvania State University: State College, PA, 1999; pp 80−95.

(4) Jinap, S. Organic acids in cocoa beans—a review. *ASEAN Food J.* **1994**, *9*, 3−12.

(5) Biehl, B.; Voigt, J.; Heinrichs, H.; Senjuk, V.; Bytof, G. pH-dependent enzymatic formation of oligopeptides and amino acids, the aroma precursors in raw cocoa beans. In *XIth International Cocoa Research Conference*; Lafforest, J., Ed.; Cocoa Producers' Alliance: Yamassoukro, Ivory Coast, 1993; pp 717−722.

(6) Rohan, T. A.; Stewart, T. The precursors of chocolate aroma: changes in the free amino acids during the roasting of cocoa beans. *J. Food Sci.* **1966**, *31*, 202−205.

(7) Rohan, T. A.; Stewart, T. The precursors of chocolate aroma: changes in the sugars during the roasting of cocoa beans. *J. Food Sci.* **1966**, *31*, 206−209.

(8) Villeneuve, F.; Cros, E.; Macheix, J. J. Recherche d'un indice de fermentation du cacao. III. Evolution des flavan-3-ols de la fève. *Café Cacao Thé* **1989**, *33*, 165−170.

(9) Timbie, D. J.; Sechrist, L.; Keeney, P. G. Application of high-pressure liquid chromatography to the study of variables affecting theobromine and caffeine concentrations in cocoa beans. *J. Food Sci.* **1978**, *43*, 560−565.

(10) Gill, M. S.; McLeod, A. J.; Moreau, M. Aroma components of cocoa beans. Proceedings of the 4th Weurman Flavour Research Symposuim, Dourdan, France; *Development in Food Science 10*; Adda, J., Ed.; Elsevier: Amsterdam, The Netherlands, 1985; pp 261−266.

(11) Ziegleder, G. Composition of flavor extracts of raw and roasted cocoas. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 521−525.

(12) Cros, E.; Mermet, G.; Jeanjean, N.; Georges G. Relation précurseurs−développement de l'arôme cacao. In *XIth International Cocoa Research Conference*; Lafforest, J., Ed.; Cocoa Producers' Alliance: Yamassoukro, Ivory Coast, 1993; pp 723−726.

(13) Maga, J. A.; Sizer, C. E. Pyrazines in food. *J. Agric. Food Chem*. **1973**, *21*, 22−30.

(14) Clapperton, J. F.; Yow, S. T. K.; Chan, J.; Lim, D. H. K.; Lockwood, G.; Romanczyk, L.; Hammerstone, J. F. The effect of cocoa genotype on flavour. In *Proceedings of the International Cocoa Conference, Challenges in the 1990s*; Tay, E. B., Lee, M. T., Yap, T. N., Zulkarnain, B. I., Thong, F. T., Bong, S. L., Tee, S. K., Eds.; Malaysian Cocoa Board: Kuala Lumpur, Malaysia, 1991; pp 468−478.

(15) Clapperton, J. F.; Yow, S. T. K.; Lim, D. H. K.; Lockwood, G. Genetic variation in cocoa flavour. In *XIth International Cocoa Research Conference*; Lafforest, J., Ed.; Cocoa Producers' Alliance: Yamassoukro, Ivory Coast, 1993; pp 749−754.

(16) Clapperton, J.; Yow, S.; Chan, J.; Lim, D.; Lockood, R.; Romanczyk, L.; Hammerstone, J. The contribution of genotype to cocoa (*Theobroma cacao* L.) flavour. *Trop. Agric. (Trinidad*) **1994**, *71*, 303−308.

(17) Holm, C. S.; Aston, J. W.; Douglas, K. The effects of the organic acids in cocoa on the flavour of chocolate. *J. Sci. Food Agric*. **1993**, *61*, 65−71.

(18) Yoo, S. S.; Kim, K.; Lee, S. Y.; Hong, S. K.; Lee, M. C.; Chang, Y. Y.; Kwon, I. B.; Pyun, Y. R. Multivariante analysis of organic acids and sugars in cocoa mass produced from better and color treatment by high performance ion chromatography. *Food Sci. Biotechnol*. **1998**, *7*, 165−177.

(19) International Office of Cocoa, Chocolate and Sugar Confectionery (CAOBISCO, Brussels, Belgium). *Analytical Methods*; 1972; p 9-E.

(20) *Off. J. Eur. Communities* **1990**, Chapter 41, 178−179.

(21) Locher, R.; Bucheli, P. Comparison of soluble sugar degradation in soybean seed under simulated tropical storage conditions. *Crop Sci*. **1998**, *40*, 189−201.

(22) Wollgast, J.; Anklam, E. Polyphenols in chocolate: is there a contribution to human health? *Food Res. Int.* **2000**, *33*, 449−459.

(23) Vinson, J. A.; Proch, J.; Zubik, L. Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate and milk chocolate. *J. Agric. Food Chem*. **1999**, *47*, 4821−4824.

(24) Crouzillat, D.; Rigoreau, M.; Cabigliera, M.; Alvarez, M.; Bucheli, P.; Pétiard, V. QTL studies carried out for agronomic, technological and quality traits of cocoa in Ecuador. In *Proceeding of the International Workshop on New Technologies and Cocoa Breeding*; INGENIC: Kota Kinabalu, Malaysia, 2000; pp 120−126.

(25) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev*. **1998**, *56* (11), 317−333.

(26) Brown, H. B. Separation of pigmented cells of cacao. *Nature* **1954**, *173*, 492.

(27) Bucheli, P.; Rousseau, G.; Alvarez, M.; Laloi, M.; McCarthy, J. Developmental variation of sugars, carboxylic acids, purine alkaloids, fatty acids and endoproteinase activity during maturation of *Theobroma cacao* L. seeds. *J. Agric. Food Chem.* **2001**, *49*, 5046−5051.

(28) Howat, G. R.; Powell, B. D.; Wood, G. A. R. Experiments on cocoa fermentation. *J. Sci. Food Agric*. **1957**, 8−65.

(29) Barel, M. Le traitement post-récolte en Afrique et en Amerique latine. Son influence sur la qualité. In *Proceeding of Cocoa Meetings*; CIRAD-CD: Montpellier, France, 1995; pp 91−98.

(30) Clapperton, J. F.; Romansczyk, L.; Hammerstone, J. F.; Yow, S. T. K.; Lim, D. H. K.; Lockwood, G. Polyphenols and cocoa flavour. In *Proceedings of Meeting of XVIth International Conference of Groupe Polyphenols*; Lisbon, Portugal, 1992; pp 112−115.

(31) Lea, A. G. H.; Arnold, G. M. The phenolics of ciders: bitterness and astringency. *J. Sci. Food Agric*. **1978**, *29*, 478−483.

Received for review December 17, 2001. Revised manuscript received March 21, 2002. Accepted March 21, 2002.

JF0116597