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The contribution of genotype to cocoa (*Theobroma cacao* L.) flavour

John Clapperton

Mars Confectionery, Dundee Road, Slough SL1 4JX, U.K.

Stephen Yow, Joe Chan and David Lim

BAL Plantations Sdn Bhd, Tawau 91007, Sabah, Malaysia

Rob Lockwood

Commonwealth Development Corporation, One Bessborough Gardens, London SW1V 2JQ, U.K.

Lee Romanczyk and John Hammerstone

M&M/Mars, High Street, Hackettstown, New Jersey 07840, U.S.A.

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The possibility of genetic effects on cocoa (*Theobroma cacao* L.) flavour was investigated. Consistent differences in flavour attributes, especially cocoa flavour intensity, acidity, sourness, bitterness, and astringency, were found among the West African Amelonado variety (AML), four Upper Amazon clones[Iquitos Mixed Calabacillo 67(IMC67), Nanay 33 (NA33), Parinari 7(PA7), and Scavina 12 (SCA12)1, and a Nicaraguan "Criollo" (UIT1) grown in Sabah, Malaysia. The flavour of UIT1 was distinctly different from the West African standard, being characterized by intense bitterness and astringency associated with caffeine and polyphenols; it also tasted the most acid. These attributes were ameliorated by prolonged storage of the pods before processing the wet beans. The six genotypes differed also in bean size and butter fat content. The differences in flavour were independent of the differences in bean size. The results demonstrated a significant contribution of genotype to flavour in addition to effects of processing.

Keywords: Cocoa; Quality; Flavour; Sensory evaluation; Processing

Traditionally, the market has distinguished between fine grade cocoa produced from Criollo and Trinitario beans (with distinct variants such as Arriba) and bulk cocoa of Amazonian Forastero origin, for which the industrial standard is Ghana, where most of the production is from a uniform lower Amazonian Forastero type known as West African Amelonado. Varieties differ in fermentation requirements, but the relative contribution of the environment, processing techniques, and genotype to flavour development is not understood.

During the last 20 years, there has been an accelerating trend towards the planting of 'modern' varieties selected in breeding programmes. Such varieties have had the greatest impact in Cameroon, Cote divoire, and Malaysia (Kennedy et al., 1987). Most are crosses between a parent derived from Pound's (1938) collection and either an unrelated Upper Amazon or, more commonly, a lower Amazon Forastero or a Criollo/Trinitario type introduced as a clone.

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The earliest work on the quality of Upper Amazon cocoa was done in Ghana in the early fifties. Manufacturers reported that the flavour was within the West African range, although often weak (MacLean and Wickens, 1951). Subsequently, breeders in Ghana and elsewhere submitted samples of promising new varieties for manufacturers' evaluation, but the results were difficult to interpret. This arose partly because the methods used to process small quantities of wet cocoa gave inconsistent results, often introducing off-flavours, and partly because flavour evaluations were reported on the basis of individual preference rather than on an analysis of flavour attributes. In these circumstances, breeders are unable to select for flavour, and this can be potentially detrimental to both producers and consumers in the long term. Recent investigations in Malaysia, following the study by MacLean and Wickens (1951) on pod ripeness and pod storage, led to an improved understanding of processing techniques (Lewis and Lee, 1986; Duncan et al., 1989). The degree of pod ripeness, storage of pods before breaking, number of turns during fermentation, and speed of drying were shown to influence the development of off-flavours, especially acidity which masks expression of desirable flavours. During that study manufacturers refined their sensory evaluation techniques by dividing flavour into components which were reported separately. Such developments provided an opportunity to re-examine the role of genotype in flavour development. In this study the results of a series of trials are described. These trials were designed to test whether the new processing techniques and improved methods of sensory evaluation would allow identification and analysis of the contribution of genotype to flavour.

Materials and Methods

Selection and cultivation of plant materials

Six genotypes, West African Amelonado (AML), Iquitos Mixed Calabacillo 67 (IMC67), Nanay 33 (NA33), Parinari 7 (PA7), Scavina 12 (SCA12), and UIT1 [an introduced clone with strong morphological affinities to Pound's (1936) Nicaraguan Criollo selections (ICS39, 40, and 60)] were selected for preliminary trials because of their widespread use in cocoa breeding in Malaysia and elsewhere. AML is a seedling population. The other five genotypes are clones.

Fermentation and drying

Experiment 1

Ripe pods from all six genotypes were harvested on the same day and stored for five days before breaking. Spoiled pods and beans were discarded. Fifty kilograms of wet beans were fermented in heaps on trays lined with banana leaves. The wet beans were covered with banana leaves and allowed to ferment for 120 h with a single turn after 48 h. After fermentation, the beans were dried on a platform, using heat from the sun supplemented by an indirect source of heat in prolonged wet weather. Positions on the platform were rotated to ensure that each genotype received the same drying treatment. The entire procedure was replicated four times between October and December 1989.

Experiment 2

The genotype UIT1 alone was used to test the effects of 10 and 15 days pod storage, with each receiving 72 and 120 h fermentation. The trials were replicated three times over a period of six weeks. Extra pods were harvested to allow for expected losses during the longer periods of pod storage. The conditions of fermentation and drying were as described in Experiment 1.

Sample preparation

Coded samples of dried beans from each genotype and treatment of experiments 1 and 2 were sent to the United Kingdom for taste testing. Cocoa liquors and chocolates were prepared according to the recommendation of the Biscuit, Cake, Chocolate and Confectionery Alliance (1980).

Taste testing

Cocoa from each of the four replicates of Experiment 1 were tasted separately as liquors. A hidden reference of West African beans was included in each set before liquor preparation and tasting. The seven liquors (six treatments and the hidden reference) were tasted according to an incomplete block experimental test design in which each liquor was tasted three times. Low intensity red illumination was used to mask colour differences among liquors. The tasting was carried out first by an independent assessor and then by trained panellists from Mars Confectionery who used similar procedures to the one described above, and by trained panellists from other chocolate manufacturers using their own taste procedures for cocoa liquors and chocolates. The 12 liquors from Experiment 2 on Uri with 10 and 15 days pod storage together with a

composite sample of UIT1 from Experiment 1 (5 days pod storage and 5 days fermentation) were tasted by the independent assessor according to an incomplete block experimental test design in which each liquor was tasted four times.

For both experiments, intensities of flavour characteristics in Figures 1 and 2 were scored on a continuous 15-cm line scale. Higher scores denoted stronger intensities. Data analysis was by analysis of variance on each flavour attribute.

Cocoa was analysed for several constituents:

Cocoa butter

Cocoa butter contents were estimated by soxhlet extraction (International Office of Cocoa, Chocolate and Sugar Confectionery. 1972).

Caffeine and theobromine

The alkaloids caffeine and theobromine were analysed by the method described by Timbie et al. (1978) using high pressure liquid chromatography (HPLC) and estimated using a diode array detector at 280 nm.

Catechin and epicatechin

The major flavanols, (+)-catechin and (-)-epicatechin were extracted from cocoa as described by Jahal and Collin (1977) and were separated and estimated by HPLC with fluorescence detection.

Procyanidins

Procyanidins (Harbome and Mabry, 1982) were analysed qualitatively using two-dimensional chromatography as described by Jahal and Collin (1977). Fuller details of the procedures for the analyses of caffeine, theobromine, flavanols, and procyanidins are available on request from authors Romanczyk and Hammerstone.

Results

Flavour profiles of the West African reference and the six genotypes from Experiment 1, and the UIT1 cocoa varieties from Experiment 2, from tests carried out by the independent assessor are shown in Figures 1 (a and b) and 2, respectively. More detailed analysis of the scores for cocoa flavour intensity, astringency, acid taste, and (or) sourness are given in Tables, 1, 2, 4, and 6.

Sensory and instrumental analyses of cocoa

Flavour scores, cocoa butter contents, and bean counts from Experiment 1 are listed in Table 1.

The flavour scores were the averages of three tests of each liquor from the four replicate preparations. Five of the genotypes had consistent flavour profiles. The sixth, UIT1, was always the most distinct, but in the fourth replicate its profile was markedly doser to the West African reference than in the other three. To verify that this was a real difference and not the result of the taster becoming conditioned to the flavour and losing discrimination, a further series of tests were carried out in which the four samples of UIT1 and AML from the fourth replicate were compared directly with each other, again with three blind taste tests of each liquor. The results, shown in Table 2, confirmed the changes in the flavour of UIT1 in the fourth replicate. Genotypes AML and NA33 were closest to the West African flavour reference. Genotypes PA7 and SCA12 were slightly more astringent than NA33 and had slightly less cocoa flavour. Genotype IMC67 had significantly less cocoa flavour and was more astringent than the others except UIT1 which was characterized by intense bitterness (Figure lb) and astringency and also had the most acid taste.

Trained panellists from different chocolate manufacturers confirmed the flavour difference indicated by the independent assessor. Most identified AML and NA33 as being closest to the West African flavour. All identified the flavour of UIT1 as the most unlike West African cocoa. Flavour profiles of plain chocolates made from the different genotypes are shown in Table 3. The chocolates were prepared and tasted by Cadbury Ltd, Boumville, U.K. The scores were the combined results from the four preparations of the six genotypes. The chocolates made from UIT1 were significantly stronger in earthy and tobacco flavours and in astringent and metallic tastes. Although the differences in chocolate flavour were not significant, the chocolate flavour from UIT1 was signifi cantly weaker in two of the four preparations.

Genotype NA33 received the highest score for chocolate flavour and had most of the West African flavour character. Cocoa butter contents of PA7, IMC67, and UIT1 were higher than the average, whereas the levels in AML, SCA12, and especially NA33 were below the standard of 56% for West African cocoa.

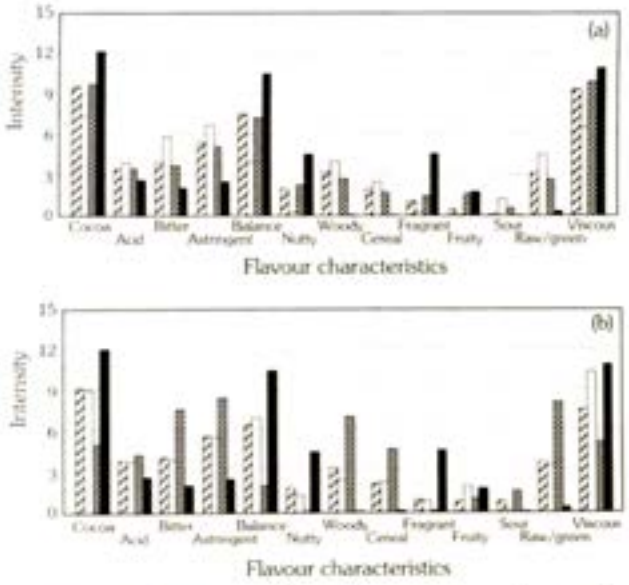

Figure 1 Flavour profiles of cocoa liquors from different genotypes. (a), ▨ AML; ❑ IMC67; ■ NA33, ■ WA ref. (b) ▨ PA7; ❑ SCA12; ■ UIT1; ■ WA ref.

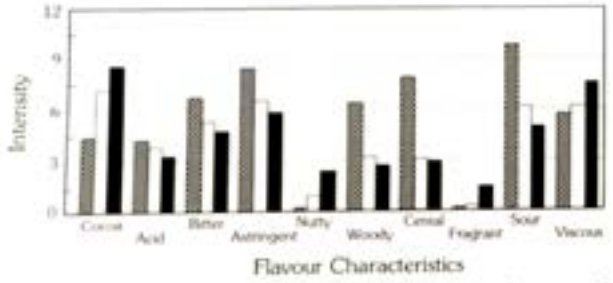

Figure 2 Effects of pod storage time on flavour of UIT1 ■, 5 days; ❑, 10 days; ■, 15 days

Table 1 Flavour scores, fat content of nibs, and bean count for six cocoa genotypes

Genotype	Flavour characteristic			Fat content of nibs (%)	Bean count 100-g^{-1}
	Cocoa	Astringent	Acid		
AML	9.5	5.4	3.4	55.6	95
IMC67	7.3	6.7	3.9	58.2	78
NA33	9.7	5.1	3.4	54.3	104
PA7	9.3	5.7	3.8	58.2	99
SCA12	9.1	5.6	3.7	54.8	124
UIT1	5.1	8.5	4.3	58.1	53
West African reference	12.3	2.4	2.1	56.1	100
SEM	0.4	0.3	0.3	0.4	3

Table 2 Confirmatory sensory evaluation of UIT1

Genotype	Replicate	Flavour characteristic		
		Cocoa	Astringent	Acid
UIT1	1	4.2	9.2	5.4
UIT1	2	4.1	9.0	5.4
UIT1	3	4.1	9.1	5.6
UIT1	4	7.9	6.7	5.5
AML	4	10.2	3.9	3.5

Differences in bean size on flavour

The possibility of a standard roasting procedure leading to under-roasting of larger beans and, thereby. developing only part of the flavours of UIT1 and IMC67 was tested by combining the beans from the first three replicates and selecting samples of AML, IMC67, NA33, and UIT1 with similar bean sizes for liquor preparation and tasting. The differences in flavour among the four genotypes were not significantly altered. The differences in flavour, therefore, are not due to differences in bean size.

Differences in bitterness and astringency levels of alkaloids (caffeine and theobromine which confer bitterness in cocoa) and polyphenols (catechin and epicatechin which confer astringency) in cocoa liquors from the six genotypes are shown in Table 5. The concentration of caffeine in UIT1 could account for its greater bitterness.

The largest differences in catechin, epicatechin, and astringency were between NA33 and UIT1.

Table 3 Flavour profiles of plain chocolates made from six different genotypes

Flavour characteristic	Mean sample scores of four replicate preparations of six genotypes					
	AML	IMC67	NA33	PA7	SCA12	UIT1
Chocolate	4.8	5.1	6.1	5.1	5.4	4.7
Sweet	4.5	5.1	5.5	5.1	5.3	5.3
Acid	1.8	1.8	1.3	2.1	2.4	2.3
Bitter	2.3	2.6	2.1	2.7	2.5	3.2
Astringent*	1.8	3.1	1.6	3.1	2.3	4.3
Metallic*	1.1	1.3	0.7	1.1	0.4	1.7
Brown fruit	2.7	1.8	2.7	2.6	2.8	1.7
Tobacco**	1.1	1.2	0.1	0.4	0.7	1.6
Mouldy*	0.3	1.6	0.2	0.9	0.8	0.7
Musty*	1.1	2.4	0.2	1.1	1.1	1.4
Earthy*	1.3	1.3	0.7	0.7	0.9	1.6
Baggy*	1.3	1.8	0.6	0.7	0.8	1.5

*, Significant difference at $P = 0.05$ between mean sample scores
**, Significant difference at $P = 0.01$ between mean sample scores
Scores here are based on a 14-cm line scale

Table 4 Flavour scores for four genotypes after selection to reduce differences in bean size

Genotype	Bean count 100·g^{-1}	Flavour characteristic			
		Cocoa	Astringent	Acid	Sour
AML	76	8.1	3.8	2.7	0.6
IMC67	68	6.6	6.5	2.3	3.1
NA33	89	9.1	3.6	2.4	0
UIT1	71	4.6	8.8	3.3	4.3
West African reference	100	9.1	3.6	2.8	0
SEM		0.5	0.4	0.2	0.3

Differences in levels of procyanidins were also detected. The highest concentrations were found in UIT1 and the greatest contrast was between NA33 and UIT1. Astringency generally decreases as the characterizing flavours develop during ripening and maturation.

Effect of pod storage and fermentation time on the flavour of UIT1

Variation in the flavour of UIT1 in Experiment 1 could have been caused by pods maturing at different rates during the five days of post-harvest storage, or by a shorter fermentation requirement because of the Criollo ancestry of UIT1. The effect of different pod storage and fermentation times on flavour characteristics were therefore studied. The flavour characteristic scores are presented in Table 6. They represent averages of eight tests of each liquor from two rounds of tasting. Differences were generally not significant and there were no consistent trends in flavour development between three and five days fermentation. It is unlikely therefore that UIT1 was over-fermented in Experiment 1. Flavour was hanged by storing pods for more than five days before breaking. In Experiment 2, replicates b and c (Table 6), there was further change between 10 and 15 days, with the flavour becoming more like West African as pod storage time increased. Changes in the condition of beans and pulp and the rates of change will vary depending on conditions of temperature and humidity during post-harvest storage, and thus are beyond experimental control. These factors are the most likely reasons for the variable effects of five days pod storage of UIT1 in the first experiment, and the differences among replicates a, b, and c in the second.

Discussion

The controlled procedures and experimental designs used in this study have revealed differences in flavour due to cocoa genotype including AML, NA33, and UIT1, which have been widely used in seed production programmes in Malaysia. The effects reported are from open pollination. Effects of specific pollen donors are currently being investigated.

Cocoa liquors from AML and NA33 were similar to typical West African liquors in colour, flavour, astringency, thickness, and viscosity. Liquors from UIT1 were distinctly different in all of these attributes.

This new information has come from the application of more analytical sensory testing procedures capable of identifying differences in specific attributes, rather than merely to express a preference for overall flavour. Previous assessments of cocoa flavour quality have been on the basis of preference tests of different chocolate manufacturers. While it is perfectly valid to differentiate between samples on the basis of preference, it is erroneous to combine the results of opposing selections. A manufacturer requiring a bitter or drying taste to balance the flavour of another cocoa or confectionery product would probably prefer UIT1 to NA33. Another manufacturer with a different requirement might prefer NA33 to UIT1. It would be misleading to average the preference data and conclude that the cocoa flavours from the two genotypes are equivalent.

Table 5 Scores for bitterness and astringency compared with concentrations of alkaloids and polyphenols in cocoa liquors from six genotypes

Taste and chemical characteristic	Genotype					
	AML	IMC67	NA33	PA7	SCA12	UIT1
Bitterness*	3.9	5.8	3.7	4.1	3.9	7.7
Caffeine (mg g^{-1})	1.1	2.1	2.2	1.1	1.8	5.2
Theobromine (mg g^{-1})	11.7	10.9	9.1	11.7	10.3	9.5
Astringency*	5.4	6.7	5.1	5.7	5.6	8.5
(+)-Catechin (mg g^{-1})	0.6	0.4	0.1	0.5	0.6	1.1
(−)-Epicatechin (mg g^{-1})	8.1	6.1	2.7	8.2	5.5	17.7

*Average scores from first experiment

Table 6 Effects of different pod storage and fermentation times on flavour of UIT1

Expt.	Rep.	Date	Pod storage (days)	Fermentation (days)	Flavour characteristic		
					Cocoa	Astringent	Sour
1		Oct-Dec. 1989	5	5	4.5	8.4	9.6
2	a	23.1.90	10	3	7.3	6.3	4.8
2	a	23.1.90	10	5	6.6	7.1	7.3
2	a	28.1.90	15	3	6.8	6.7	6.9
2	a	28.1.90	15	5	7.3	7.1	7.2
2	b	8.2.90	10	3	6.9	6.1	6.1
2	b	8.2.90	10	5	6.8	6.7	6.5
2	b	14.2.90	15	3	10.2	4.1	2.7
2	b	14.2.90	15	5	8.9	5.1	2.8
2	c	24.2.90	10	3	8.3	5.1	3.3
2	c	24.2.90	10	5	8.1	5.6	4.3
2	c	28.2.90	15	3	9.3	5.1	2.6
2	c	28.2.90	15	5	9.6	4.6	2.4
		SEM			0.4	0.4	0.6

Rep. is replicate

Although sensory evaluation must be the primary measure in any flavour study it is desirable to complement the results of sensory evaluation with more objective analytical data. Whereas an instrument can repeatedly provide the same result, the human measuring device in sensory testing may vary because of fatigue and adaptation, but, on the other hand, can reasonably be expected to learn and to develop new perceptions. During the course of the present study, small differences in acid taste observed in the first tests of the different genotypes were translated into more pronounced differences in sourness, which can result either from acetic and lactic acid formed during pulp fermentation, or be due to astringency as in under-ripe fruits. While most people consider Malaysian cocoa to be excessively acidic, some perceive it as bitter and astringent. A number of tasters, including trained and experienced panellists, equated the taste of the present samples of UIT1 with the typical acid taste of Malaysian cocoa. It is possible that part of the sharp flavour of Malaysian cocoa is derived from the choice of planting materials.

In cocoa, astringency can now be related to polyphenols, acid taste to residual organic acids (Duncan et al., 1989), and bitterness to caffeine and theobromine. Apart from differences in these characteristics in different genotypes, there are also differences in colour. The chemistry of these colour differences should be relatively easy to elucidate compared with the exceedingly complex chemistry of cocoa flavour. Cocoa liquors from UIT1 were the palest in colour and were, in this respect, in particular, typical of Southeast Asian rather than West African cocoa liquors. The colour differences most likely relate to differences in polyphenol composition.

The most important practical impact of this study is the information that it provides to planters and plant breeders to produce the cocoa qualities that manufacturers require. The results presented here support and extend the results of earlier unpublished studies on the effects of processing that have shown pod storage to be a major factor affecting cocoa flavour development.

The new evidence is that pod storage will be a major source of variation in any future trials and between different genotypes. A flavour more like the West African standard was achieved from 5 days pod storage of AML and NA33 than from15 days pod storage of UFT1. As large-scale pod storage is problematic on estates in Malaysia there is a strong argument for undertaking genotype evaluation without pod storage. Only genotypes that develop desirable flavour without pod storage would be used for breeding, or selected for large-scale cultivation.

Conclusions

Processing is still a dominant factor affecting cocoa flavour development although planting material is now shown to have a significant effect. The preparation and analytical techniques used make it possible to survey the variation in flavour characteristics of the germplasm on which cocoa breeding is based. The improvements in flavour, colour, and butter fat content could significantly impact on the future development of the cocoa and chocolate industries for the mutual benefit of producers and consumers.

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