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# Evidence for applying the concept of "Terroir" in cocoa (*Theobroma cacao* L.) flavour and quality attributes

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## ABSTRACT

Overall growth of the fine/flavour chocolate industry over the last decade has been linked to origin linked products such as dark chocolate bars. "Terroir" is concerned with the relationship between the characteristics of an agricultural product (quality, taste, style) and its geographic origin, which might influence these characteristics. This concept has been well documented in viticulture as the sensory attributes of wine have been related to the environmental conditions in which the grapes are grown. These effects are challenging to study in other crops such as cocoa where "terroir" effects similar to wines are implied in many origin specific dark chocolates but have never been systematically tested. Two experiments were conducted over three growing seasons to investigate the impact of growing environments and practices at different processing locations on the flavour attributes of selected cocoa varieties in Trinidad. The first experiment examined the influence of box fermentation at three different processing locations, with sun drying, on the flavour of six different cocoa clones each harvested from the same field. The second experiment examined the influence of four different growing environments on seven cocoa clones growing in at least two of four different sites. The sensory attributes of liquors prepared from the various samples from the two experiments were assessed using a trained sensory panel. The results showed significant processing and growing environment effects on several flavour attributes with significant clonal effects and processing and growing environment × clone interactions in the two experiments. Near infrared reflectance spectroscopic analysis of bean samples provided independent quantitative support to the sensory results obtained. The findings suggest that conditions and practices at different processing locations and growing environments may have significant impact on the flavour attributes of cocoa while the contribution of genotype to flavour could sometimes have a superseding influence with respect to certain flavour attributes. This work provides evidence and a scientific basis to support claims of "terroir" effects in cocoa for origin specific chocolates.

## INTRODUCTION

The Global Cocoa Agenda was agreed at the First World Cocoa Conference, Abidjan, Côte d'Ivoire in November, 2012 (ICCO 2012). It provides the roadmap towards achieving a sustainable world cocoa economy and outlines: The strategic challenges facing the cocoa value chain, the recommended actions to address them and the responsibilities of the stakeholders in the cocoa sector at national and regional and international levels. One key action item arising from the Global Cocoa Agenda is the need to *"Improve cocoa quality by better communication of industry needs, post-harvest processing and quality assessment"* (ICCO 2012). This frames the context for this paper and highlights the greater appreciation of the interconnectivity along the cocoa value chain affecting flavour development and ultimate quality. It recognises the roles of growing environment and care of tree in producing cocoa beans with a genetic flavour potential that is a function of pre harvest conditions as well as post-harvest processing and the role of the main operations during chocolate manufacturing in expressing the genetic flavour potential of the cocoa beans as a chocolate.

This paper reports on work done to collect evidence towards understanding *"Terroir"* effects in cocoa where the unique set of environmental factors and practices collectively impact on the flavour character of cocoa beans grown in Trinidad. Two aspects of the environment were considered; the growing environment (which would include weather patterns and soil properties) and the conditions of post-harvest processing (which would include location specific environmental conditions and the local practices for fermentation and drying). The flavour of cocoa liquors was assessed by a taste panel and a fingerprint of biochemical composition of the samples was also analysed independently using near infrared reflectance spectroscopy (NIRS).

## MATERIALS AND METHODS

Two separate studies were conducted to demonstrate the importance of Processing Location (PL) and Growing Environment (GE) on the flavour attributes of selected cacao varieties. Standardised protocols for micro-fermentations and drying small scale batches of cocoa as well as for the preparation of cocoa liquor, panellist training and sensory evaluations used in this study were adapted from those suggested by Clapperton et al. (1994b). Subsequently the sensory design and data analysis methods were refined to produce more rigorous results (Sukha et al. 2008) and have allowed sensory assessment to be used as an analytical tool in its own right to investigate the relative importance of processing location and growing environment on flavour. Details of the procedures followed in these experiments for primary processing are given in Sukha et al. (2008).

**Processing Location study**

In the PL study, three different processing locations: La Reunion Estate, Centeno (LRE), Manickchand Estate, Sangre Grande (ME) and San Juan Estate, Gran Couva (SJE) were used to process six (6) contrasting cacao varieties harvested from the same growing environments over the 3 crop years. Micro-fermentations (Clapperton et al. 1994a; Sukha 1997 and Sukha et al. 2008) were done at these three processing facilities to compare beans from the same clone growing in the same place. Four clones (ICS 1, IMC 67, CCL 200 and CCL 201) were processed at all three locations over the three crop years whilst two clones (SCA 6 and ICS 84) were processed only at estates ME and SJE, due to the availability of pods. All the samples were sun dried until reaching a final moisture content of 6 – 7%. Since only artificial drying with a diesel-fired burner is done at LRE, samples fermented at LRE were taken to ME and sun dried there using the ME sun drying practices. Samples processed at SJE were dried following their sun drying practices.

Fermentation mass temperature was measured (top, middle and bottom layers) and pH (testa and cotyledon) at all three processing locations three times during each of the three crop years. Temperature was measured continuously during fermentation using a data logger (Campbell Scientific CR10X) with PC208W 3.2 Data logger Support Software (Campbell Scientific Inc., USA) whilst pH measured using an Oakton® Acorn pH meter, featuring pH5 meter, Model no. WD-35613-70).

**Growing Environment study**

In the GE study, all the major factors affecting flavour were kept constant except for the growing environment. Samples from the same clones were taken from four growing locations with different soils and rainfall patterns in the north and east of Trinidad: LRE, Centeno (North Trinidad), UWI, St Augustine (North Trinidad), the University Cocoa Research Station (UCRS), Centeno (East of LRE) and Marper Farm, Manzanilla (East Trinidad). Micro-fermentations were all conducted in a fermentation box at one processing location and the samples were sun-dried in wooden trays until reaching 6 - 7% moisture content. The number of clones used in each location depended on the availability of verified true-to-type trees. Samples from two clones (IMC 67 and ICS 1) were taken from UCRS, UWI and LRE, those from four clones (West Africa Amelonado (WAA), GU 243/H, ICS 84 and SCA 6) were taken from UCRS and UWI and those from two clones (JA 5/5 [POU] and PA 165 [PER]) were taken from UCRS and Marper Farm.

In both studies, all fermentations were carried out simultaneously and at least three repetitions of fermentations were done within a particular crop year to provide a composite sample that covered the start, middle and end of the crop.

**Liquor preparation and sensory evaluation**

Each dry bean sample was roasted at 140ºC for 30 minutes, and milled to produce smooth liquor by the method described by Sukha et al. (2008). The cocoa liquors were stored at -6 to -8ºC prior to sensory evaluation.

Liquors were assessed by a sensory panel in the Cocoa Research Centre, UWI, Trinidad, trained with the protocol of Sukha et al. (2008). Coded liquors were tasted three times by each panellist in a random order to minimise carry-over effects and positional bias. The design also ensured that no two panellists were presented liquors in the same order in any given session. Sensory profiles were recorded for nine flavour attributes using a continuous line scale from 0 (absent) to 10 (strong).

**Near infrared reflectance spectroscopy (NIRS)**

Representative fermented and dried bean samples from clones in the processing location and growing environment experiments were sent to the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France. NIRS acquisitions were performed on a Foss-Perstorp 6500 using a

spin cell. Three grams taken from 100 g of hulled, ground and sieved (<0.5 mm) cocoa samples were analysed in diffuse reflectance from 400 nm to 2,500 nm in 2 nm steps.

**Data analysis**

Restricted maximum likelihood (REML) variance estimates were obtained with Genstat 4.24 DE (VSN International) to determine the significance of treatment effects and interactions on the sensory data. Principal component analysis (PCA) was performed on the pooled sensory data using Palaeontological statistics software (PAST) Version 1.34 (Hammer et al. 2001) and graphical representation was carried out in Microsoft® Excel and PAST.

Spectral data from NIR were collected and processed at CIRAD using Winisi 1.5 software (InfraSoft International, Port Matilda, USA). PCA was performed on the spectral data from each clone used in the processing location and growing environment experiments. A Step-by-step linear discriminant analysis (LDA) was then performed on the principal components (PC) extracted from the PCA analysis to enable classification of the cocoa genotypes according to processing location, growing environment and genotype. The choice of PCs that were introduced into the LDA was made by a stepwise procedure so as to select PCs displaying the best discriminating power (Devaux et al. 1998). At each stage, the variable (PC) maximising the between-group Mahalanobis distance was introduced into the model (Naes et al. 2002).

## RESULTS

**Sensory data – PL effects**

The sensory data for each clone processed at the three locations over the different crop years was pooled and analysed using PCA. Points in the PCA plot (Figure 1) are labelled with the numerical part of the clone name combined with the processing location code.

In Figure 1, the first two principal components (PC) accounted for 72.1% of the variation between samples. CCL 200 samples were grouped together suggesting a clonal effect, and were associated with floral flavour. Other points in Figure 1 were grouped according to the procession locations, with SJE and ME being most distinct and LRE lying between them. The clones processed at SJE were associated with cocoa and nutty flavours whilst those processed at ME were associated with fruity, acid, bitter, raw/beany/green and 'other' flavours.

REML variance estimates on the sensory data generated by each panellist over three repetitions per year revealed significant ($p \leq 0.001$ and $p \leq 0.05$) processing location effects in cocoa, acid, fruity, floral and nutty flavours. There were significant ($p \leq 0.001 - p \leq 0.05$) clonal effects in all flavours except fruity and raw/beany/green, whilst acid and fruity flavours showed significant ($p \leq 0.01$ and $p \leq 0.05$ respectively) processing location × clonal interactions.

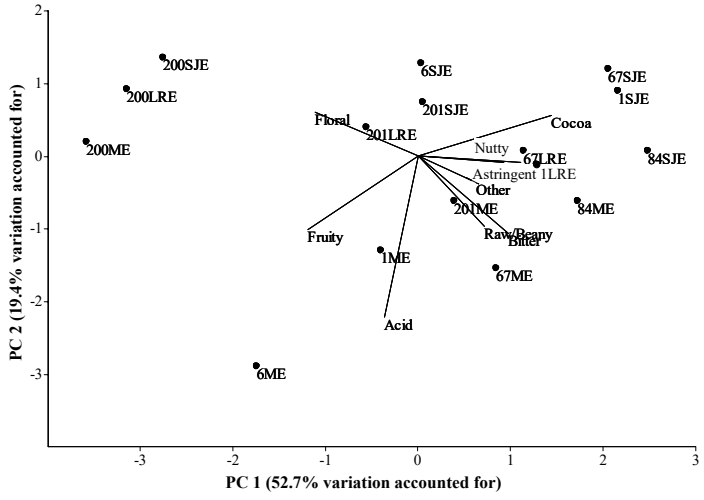



**Figure 1. PCA plot of different flavour attribute scores for cocoa samples processed at different locations in Trinidad.**

**Sensory data – GE effects**

Sensory data from each clone grown in at least two of the four different locations over at least two of the three crop years were pooled and analysed by PCA. Points in the PCA (Figure 2) are labelled with the numerical part of the clone name combined with growing location codes.

The first two principal components accounted for 62.1% of the total variation among the samples. Those from the UWI growing environment were associated with acid, astringent, bitter and raw/beany/green flavours, while those from UCRS and Marper Farm were associated with cocoa, nutty and 'other' flavours (except SCA 6). SCA 6 grown at UCRS was an outlying sample, and both the SCA 6 samples from UWI and UCRS were associated with floral and fruity flavours, suggesting a dominant clonal effect. Samples from Centeno were mainly associated with fruity, floral and acid flavours.

REML variance estimates on pooled samples gave significant differences due to growing environment for astringency, bitterness, fruity and other flavours ($p \leq 0.001 - p \leq 0.05$). There were significant ($p \leq 0.001 - p \leq 0.05$) clonal effects for all flavours except cocoa and bitterness. Acid, bitterness, fruity, floral and 'other' flavours had significant ($p \leq 0.001 - p \leq 0.05$) growing environment × clonal interactions.

Acid and fruity flavours were significantly higher ($p \leq 0.05$ and ($p \leq 0.01$ respectively) for clone WAA grown at UWI compared to UCRS (Figure 3). Scores for 'other' flavours were generally less than one and the only significant effect was a higher score at the UCRS for WAA ($p \leq 0.01$) (Figure 3). The descriptors used for 'other' flavours by panellists were for an off flavour described as "over-roasted" or "putrid".

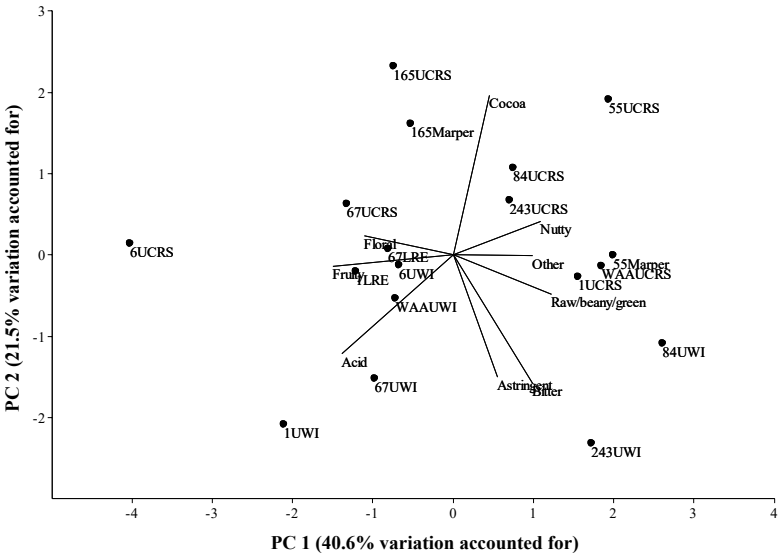
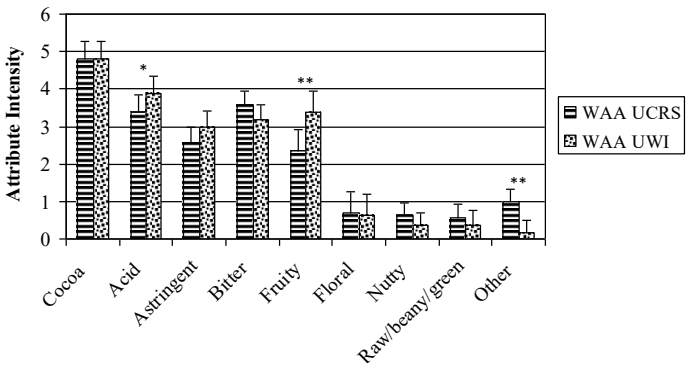



**Figure 2. PCA plot of different flavour attribute scores for cocoa samples grown at different locations in Trinidad.**




* $p \leq 0.05$ ** $p \leq 0.01$

**Figure 3. Average flavour profiles of WAA grown at UCRS and UWI over 3 crop years, and processed in a single location.**

**Near infrared reflectance spectroscopy data – PL effects**

Spectral data were generated on all samples except ICS 84 and SCA 6, for which insufficient beans were available. Linear discriminant analysis was done on the first 10 PCs extracted from the PCA analysis of spectral data from ICS 1, IMC 67, CCL 200 and CCL 201 processed at the three locations. Results from the LDA shows perfect discrimination of the four clones with a classification rate of 100%. Factors 1 and 2 accounted for 97% of the variation between the clones (data not presented).

The classification rate of the three processing locations by LDA was 87% with factors 1 and 2 accounting for 100% of the variation between processing locations (Figure 4). The LDA analysis of the spectral data indicates a stronger clonal than processing location effect in the spectral data.

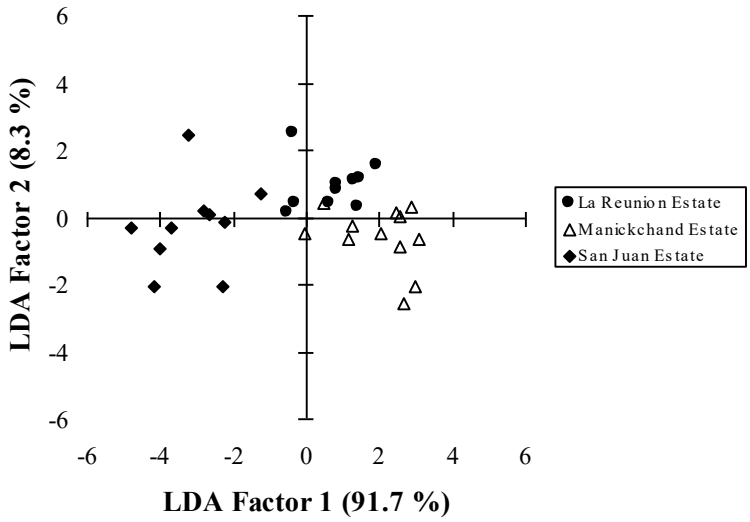



**Figure 4. Linear discriminant analysis of spectral data from cocoa beans from the same trees processed at different locations in Trinidad.**

**Growing environment effects**

Linear discriminant analysis was also done on the first 10 PCs produced from the PCA analysis of spectral data from clones taken from the four growing environments. Even though coordinates 1 and 2 accounted for 91.1% of the variation between the different samples (Figure 5a), the classification rate between the different growing environments was 62.9%. This suggests that the effect of growing environment is not a strong source of variation among the spectra, however most of the overlap between locations was with the samples from Marper Farm. The LDA analysis was repeated without the Marper samples for two clones (IMC 67 and ICS 1). The classification rate for the three growing environments was equal to 81.2% (Figure 5b). The LDA analysis was repeated with four clones (WAA, ICS 1, ICS 84 and IMC 67) taken from two growing environments (UCRS and UWI). This led to a classification rate of 90% for farms and 95% for clones. This indicates a marked growing environment effect between UWI and UCRS, almost as strong as the clonal effect.

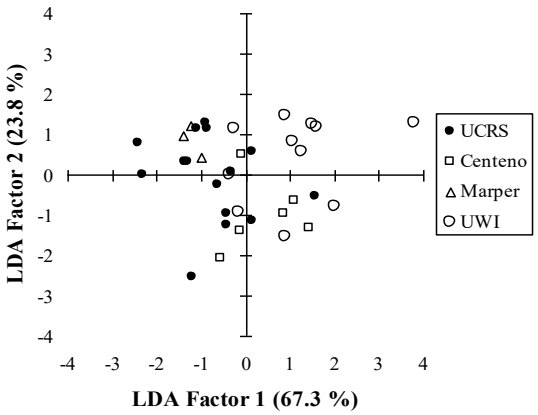



**Figure 5a. Linear discriminant analysis of factors from spectral data on cocoa beans of the same clones from for four growing environments and processed at a single location in Trinidad.**

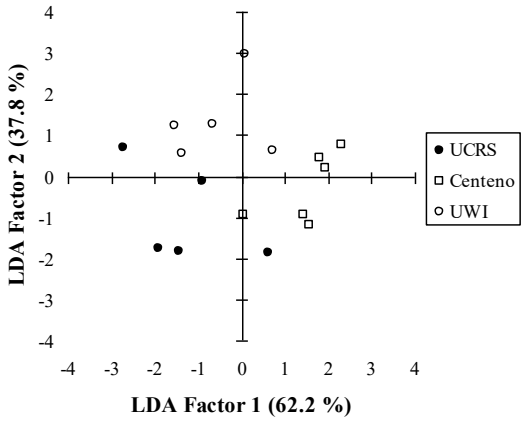



**Figure 5b. Linear discriminant analysis of factors from spectral data on cocoa beans of the same clones from three growing environments and processed in a single location in Trinidad.**

**DISCUSSION CONCLUSIONS**

The PCA plots from the pooled sensory assessment data provide clear evidence that both the processing location and growing environment affect certain flavour attributes. In the PL study the grouping of samples revealed that the ME and SJE processing locations were different from each other with the LRE lying between the two. Fruity flavour varied with processing environment agreeing with related work by Sukha et al. (2008) where fruitiness was always present in different commercial clones but its intensity varied between similar Trinitario cocoa samples processed at different estates. The GE study showed that samples from UWI were distinct from those from UCRS, but those from Marper Farm overlapped with those from UCRS.

REML variance estimates provided measures of the significance of effects for specific flavour attributes that varied between processing locations and growing environments. REML was also able to highlight significant clonal effects and processing location × clonal and growing environment × clonal interactions.

Measurement of fermentation mass temperature and pH during fermentation showed no major differences between the three processing locations (data not presented). Therefore, one can conclude that both the fermentations (neglecting any differences in micro flora) and weather during drying was similar at the processing and drying locations. The fermentation temperature and pH profiles agree with findings of previous work done in Trinidad (Knapp and Churchman, 1937; Rombouts, 1952; Forsyth and Quesnel, 1963; Ostovar and Keeney, 1973 and Sukha 1997), as well as, Rohan 1958 and 1963) in his findings for West African Amelonado fermenting beans.

There were differences in the measured drying rates between ME and SJE processing locations (data not presented). Drying rates were much faster at ME compared to SJE with the result that drying times tended to be shorter by as much as two days at ME compared to SJE. Temperature and relative humidity (RH) measurements (data not presented) revealed that the temperature at ME was higher than at SJE, but RH was similar at times when the drying rates were measured (data not presented). However, the practices on each estate revealed differences in drying protocols that explain the measured drying rates. Bean samples were dried in trays and closely followed actual estate practices at ME (samples from LRE were dried at ME) and SJE for the fermentation mass from the fermentation box. At ME, micro-fermented bean samples were spread one bean layer thick in the trays and dried. On the other hand, micro-fermented samples dried at SJE were heaped at the centre of the tray for drying after heating up the drying tray for 2 hours. The differences in bean layer thickness for drying would change the surface area available for drying and thereby strongly affect the drying rate.

The difference in drying rates observed most likely contributed to the flavour differences between ME and SJE (mainly higher acidity at ME). Previous work has linked drying rate to the acidic characteristics of cocoa (Bonaparte et al. 1998; Jinap 1994; Jinap and Thien 1994 and Jinap and Dimick 1990) and the residual acetic and lactic acid in the bean are strongly implicated as the major cause for acidic taste.

The agro-ecological characteristics across the four different growing environments revealed that rainfall, elevation and field layout were generally similar (Table 1). The four growing locations were not separated from each other by very large geographic distances or elevations since they were all located in Trinidad which is itself situated between 10°02' and 10°50' N latitude and 60°55' and 61°55' W longitude with a land area covering 5,124 sq. Km. However, the distances between the different growing environments were sufficient in all but one instance (between LRE and UCRS) to occur on different soil types and receive different amounts of rainfall. Three soil types occur over the 45.9 ha expanse of Marper Farm that was the largest growing location in this study, however, the two fields (Blocks C and D) from which pods were harvested to use in this study were adjacent to each other and on the same soil type (Brasso Clay) (Table 1).

Based on 75-year average figures, UWI typically receives the least amount of rainfall (less than 1,800 mm) among the four locations and Marper Farm the most (between 2,400- 2,600 mm). However, LRE and UCRS both lie in the same hydrometric area and receive just under 2,000 mm per annum. The actual rainfall received in the years during which this study was conducted revealed that the mean rainfall amounts differed significantly ($P \leq 0.01$) between the different growing environments over the years 2000 – 2004 only for the month of January (data not presented).

Apart from the differences highlighted above, the field layout at the different locations was similar, with respect to shade and windbreaks whilst spacing was the same (1.8 × 1.8 m) at UCRS, LRE and Marper but was different (2.1 × 2.1 m) at UWI.

Although the growing locations were not very far from each other in absolute terms and only small environmental differences are found within the island of Trinidad, different soil types occurred at three out of the four locations and could be a contributing factor to the organoleptic differences observed between the different locations. A further study is needed to confirm this.

**Table 1. Some of the agro-ecological characteristics of UCRS, UWI, LRE and Marper Farm.**

| *Growing location* | *Latitude & Longitude location* | *Soil Type* | *Rainfall (75 year averages)* | *Elevation above sea level* |
|---|---|---|---|---|
| University Cocoa Research Station, Centeno | 10°34' N Latitude 61°18' W Longitude | Cunupia Fine Sandy Clay | <2,000 mm | 15 m |
| University of the West Indies, St Augustine | 10°38' N Latitude 61°24' W Longitude | St. Augustine Clay | < 1,800 mm | 16 m |
| La Reunion Estate, Centeno | 10°35' N Latitude 61°20' W Longitude | Cunupia Fine Sandy Clay | <2,000 mm | 12 m |
| Marper Farm, Manzanilla | 10°30' N Latitude 61°05' W Longitude | Mt. Harris Sandy Loam<br>Brasso Clay<br>L' Ebranche Clay | 2,400 – 2,600 mm | not available |

There was general agreement between the organoleptic results and the LDA analysis of the spectral data generated by NIRS. Both studies indicate that: a) there is a significant processing location and clonal effect on flavour but the clonal effect is stronger and b) there is a clear effect of growing environment between UWI and the UCRS but overlap between Marper Farm and the UCRS.

The results from this study provide strong evidence to support the relative contribution of genotype, growing environment (climatic, edaphic etc.) and processing (fermentation and drying) practices on the flavour and quality attributes of different cocoa genotypes. The relative contribution of all elements of the growing and processing environment to final flavour in cocoa permits consideration of applying the concept "terroir", already well established for wines, to cocoa and also provides a scientific basis for cocoa quality certification programmes.

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