- After 24 hours, when the split seeds have hardened off, open the lid of the propagator and cover the emerging radicle with the same cotton that was used to cover the half seed. Wet the cotton on top of the half seeds slightly (0.5 ml of distilled water for each half seed).
- Afterwards, if necessary, wet the cotton on top of the seeds with 5-10 drops every day until at least ten days after sowing.

Transplanting

- Leaf expansion of the split seedlings begins in the propagator between the 10th and 15th day.
- The plantlets should best be transplanted into polythene bags containing high quality humid organic soil placed in the same propagator 10 days after the start of germination.
- The polybags should be lined up in double rows and labelled to identify the twin seedlings.
- The lids of the propagator (or plastic covering the tunnel) should then best stay partly opened. However, it is important to make sure that the seedlings are protected from heavy rain or insect damage.
- Four to six weeks after planting, the polybags with the plants can be taken out of the propagator and placed into a normal nursery bed.

Final considerations

⊙ This method when applied on a large number of seeds showed high success rates and uniformity in vigour between twin seedlings. However, twin-seedlings are less vigorous than normal seedlings. The differences between twin and normal seedlings tend to decrease with time in the nursery.

⊙ Important factors for success seem to be the use of a sterile substrate (cotton) for germination with strict control of humidity (not too high or too low). The droplet placed on the radicle stimulates the germination (outgrowth of the root). The importance of eliminating the seed coat before splitting the seed is yet to be studied.

Figure 1 : Laterally cut, peeled cocoa seed one day after placing on wetted cotton wool. The germination has been stimulated by placing a droplet of water on the radicles on the day of sowing, and the half seeds are then covered with cotton wool.

Is Genetic Variation for Sensory Traits of Cocoa Pulp Related to Fine Flavour Cocoa Traits?

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Summary

A first attempt is made to link some of the known fine flavour attributes found in cocoa liquor or in dark chocolate (excluding basic cocoa flavour) with the sensory traits of the pulp of ripe pods of cocoa varieties representing different genetic cocoa types. Overall pulp preference expressed by the sensory panel appeared related to the known fine flavour potential of the cocoa varieties tested, and specific pulp flavour attributes appeared related to specific types of fine flavour cocoa types. EET 62 (Nacional x Trinitario type) displayed a characteristic odour when the fruit was opened and had sweet pulp with an intense flavour identified as floral and fruity. This flavour may possibly be related to the typical Arriba flavour. The ICS 1 clone, known for its typical Trinitario fresh fruit flavour, showed a combination of relatively high acidity and sweetness of its pulp and also an intense fruity flavour. The pulp of the CCN 51 clone and of two Huallaga clones was rated as astringent and acid, with low flavour quality. This could be related to the low potential for fine flavour cocoa of many Forastero types (which may otherwise however have a potential for strong cocoa flavour). The two Ucayali clones tested displayed sweet pulp with a medium intensity of fruity flavour that resembles the pulp of the SCA 6 clone known to produce cocoa with a dry fruit or floral flavour. The pulp of the Pandora 1 clone from Colombia was characterised by a typical flavour associated with that of soursop (guanabana) and may therefore represent a new type of fine flavour cocoa. The results suggest that a significant part of fine flavour attributes of cocoa products (excluding basic cocoa flavour) can be related to sensory traits in the pulp of ripe cocoa pods. More research is required to further verify and possibly explain this relationship. However, the results suggest that rapid screening of germplasm

and of breeding populations for pulp characteristics may be useful for selecting cocoa varieties with known as well as with new fine flavour traits. Such would be of great value to producers and other stakeholders in the rapidly growing and increasingly more diverse "gourmet" cocoa and chocolate market. Methods for rapid pulp flavour assessments to meet different needs in cocoa breeding for high cocoa quality are proposed.

Introduction

Objectives of cocoa breeding broadly include high yield, resistance to diseases and pests and good quality. Quality of fine flavour cocoas is known to be affected by genetic and geographic origin, and thus breeding programmes have aimed at maintaining the cocoa flavour associated with the country of origin (Lockwood and Eskes 1996). Research on cocoa flavour has been receiving increasing attention over the last 15 years and has broadened the spectrum of recognised flavour traits (*e.g.* Clapperton *et al.* 1994; Eskes 2006; Sukha and Butler 2005). Besides variation in the intensity of basic cocoa flavour, presence of specific aromas such as "fruity" (*e.g.* as found in Trinitarios), different types of "floral" (*e.g.* as Nacional and Scavina types), and nutty and caramel flavours (*e.g.* in Criollo types) have been demonstrated (Sukha and Butler 2005). The market for fine flavour cocoa (mainly as dark chocolate with high cocoa content) is rapidly expanding and diversifying, providing new opportunities for the producers to obtain premium prices for high quality cocoa beans. Besides selecting for traditionally recognised fine flavour cocoa types, the challenge for breeders is also to look for new fine flavour types that may be of interest for the cocoa market. Cocoa breeding is complex, as many traits need to be selected for simultaneously. Selection for special flavour attributes is a laborious and time-consuming process that requires collaboration between breeders, specialists in post-harvest technologies, sensory specialists and chocolate manufacturers. Most cocoa selection programmes do not have the capacity to carry out routine sensory evaluations of genotypes in their collections or in the breeding populations. These factors reduce the efficiency and speed of progress in the selection of fine flavour cocoa types, especially if this trait is to be combined with good yield and resistance to diseases. Therefore, the use of a quick method to identify special flavour traits of cocoa genotypes would be very useful in cocoa breeding. Regular visitors to cocoa plantations are familiar with the broad variation that exists in the taste of cocoa pulp. The most conspicuous variations that can be observed are related to the level of acidity and sweetness. This is especially noticeable when pulp of cocoa pods of different genetic origins is tasted. For example, it is well known that Criollo and Scavina genotypes have very sweet pulp whereas the pulp of Trinitario and Forastero clones are generally more acid. Sensory traits of the pulp and of the beans (degree and type of bitterness) have been used over the last 10 years in Ecuador as criteria for pre-selection of trees that might produce the "Arriba" flavour profile associated with the Nacional cocoa variety (Gilles Roche, CIRAD, personal communication), but no published studies exist on the possible relationship between pulp traits and fine flavour cocoa.

The objective of the current study is to assess if variation in sensory traits of the pulp in ripe cocoa pods from different genetic origins can possibly be related to the known variation of fine flavour traits of cocoa beans.

Materials and methods

In September 2007, an experiment on flavour attributes of ripe pulp of different cocoa types from the collection of the Universidad Nacional Agraria de la Selva in Tingo Maria, Peru, was carried out. The varieties chosen included genotypes that are known to vary for fine flavour attributes (Table 1). EET 62 is associated with the Arriba floral flavour and ICS1 with the typical Trinitario fruity flavour. The U and H clones represent sub-spontaneous and cultivated origins collected in the Huallaga and Ucayali river basins, respectively (Garcia Carrion 2000; Dapeng Zhang *et al.* 2006). The Huallaga accessions appear to be mainly true Forasteros while some are of hybrid origin.

Two ripe pods of each of nine clones were collected in the morning, numbered 1 to 9, and placed in the laboratory for sensory evaluation in the afternoon. The panel consisted of six persons, who scored the following flavour attributes on a 0 to 5 point scale: intensity of volatile aroma (odour) when opening the pod, pulp acidity, pulp sweetness, pulp astringency, bean bitterness, presence of a special flavour in the pulp after tasting it, the type of flavour of the pulp and overall preference of the panellists for the taste of the pulp.

Classical statistical analyses using linear models (ANOVA, linear correlation) were performed to calculate the treatment effects, and Principal Component Analysis (PCA, using the XLSTAT 2007 programme version 8.01) was done to visualise the associations between traits and treatments.

Results

Tables 2 and 3 show the results obtained for the sensory evaluation of the pulp and beans of the nine varieties (clones) tested. Differences between varieties were significant for all traits at $p=0.05$. Discrimination between varieties was highest for pulp astringency ($F=13.8$) and lowest for pulp sweetness ($F=3.5$). Differences between panellists were not significant for overall preference, pulp acidity and pulp sweetness suggesting that these traits were evaluated in a uniform manner by the panellists. For ease of reading, the varieties in Tables 2 and 3 have been ordered according to the mean overall preference scores for the pulp.

The panel expressed highest overall preference for EET 62 and lowest preference for CCN 51 and H 56 (Table 2). The odour on opening the pods and the flavour intensity in the pulp were also perceived strongest for EET 62. The flavour intensity was intermediate for ICS 1, PAN 1, U 43 and U 45 and lowest for U 53, H 60, H 56 and CCN 51. The pulp acidity was highest for ICS 1, H 60, H 56 and CCN 51, and lowest for the three U clones and for EET 62 (Table 3). Pulp sweetness was high for six clones (EET 62, the three Ucayali clones, PAN 1, and ICS 1), intermediate for the two Huallaga clones and low for CCN 51. Pulp astringency was high for CCN 51, intermediate for the two Huallaga clones, low for PAN 1 and ICS 1, and very low for EET 62 and the three Ucayali clones. The bitterness of the beans was high for CCN 51 and average for the other varieties.

The predominant type of flavour identified in the pulp was "fruity" (including mainly fresh fruit flavour). The pulp of EET 62 was also considered to be "floral", whereas the PAN 1 variety displayed a very characteristic flavour identified as that of soursop (*guanabana* in Spanish and *graviola* in Portuguese). The intense flavour of ICS 1 was considered to be a fresh fruit flavour. The pulp of the CCN 51 variety did not contain a specific flavour according to three of the panellists whereas two panellists identified a low intensity of fruity flavour.

Linear correlation coefficients are presented in Table 4. The odour, flavour intensity and sweetness of the pulp were all positively correlated with overall preference, and pulp flavour intensity was positively correlated with odour and pulp sweetness. Pulp acidity, astringency and bean bitterness were positively correlated with each other and negatively correlated with pulp sweetness. Pulp acidity, pulp astringency and bean bitterness were not significantly correlated with pulp preference, odour and pulp flavour.

The associations between the genotypes and sensory attributes, as analysed in the PCA, are depicted in Figure 1 and described in Table 5. The most contrasting varieties are EET 62 (sweet pulp with strong flavour and odour), Ucayali clones (sweet pulp with low acidity and low astringency), CCN 51 (high pulp acidity and astringency, and very bitter beans) and the Huallaga clones (acid and quite astringent pulp).

Discussion

The analyses show strong effects of genotype on the sensory traits of ripe pulp. The most preferred pulps (EET 62, PAN 1, Ucayali clones and ICS 1) were sweet and rich in flavour. The EET 62 and ICS 1 clones are well-known for their floral and fresh-fruit, fine flavour cocoa traits, respectively, which might be related to the "fruity-floral/sweet" and "fruity/acid/sweet" flavours in the pulp, respectively (Table 3, Figure 4). The sweet and fruity pulp of the Ucayali clones resembles the taste of the well-known pulp of SCA 6, and might be related to the special cocoa flavour associated with the SCA 6 clone. The two Huallaga clones had acid pulp and did not show any special pulp flavour. These pulps are similar to that of many known Forastero types (such as IMC 67), which may have a good potential for strong cocoa flavour, but do often not have special flavour attributes. Interestingly, the H 60 had an odour on opening the pod that was not observed for H 56. This might be due to the likely hybrid origin of this clone (Table 1), including possibly SCA 6 as one of the parents (Dapeng Zhang, personal information). The least preferred pulp (CCN 51) was acid, very astringent and presented little or no special pulp flavour. This might be related to the reported presence of acidity and astringency in cocoa liquors made of CCN 51 beans that have not undergone special fermentation treatments to remove these unfavourable traits.

Interestingly, while the sweet and acid traits were generally opposed traits, the pulp of ICS 1 appeared to have considerable levels of both acidity and sweetness. This is why this clone is located in the middle of Figure 1. The combination of sweet and acid pulp could well be related to the origin of ICS 1 (a Trinitario clone), descending from hybridisation between Criollo (sweet pulp) and Forastero (acid pulp). The not well known PAN 1 also has a similar acid/sweet pulp, but it has a very typical soursop flavour. Therefore, the cocoa made with beans of this clone may also display a soursop related flavour that could be a new flavour to the current spectrum of fine flavour cocoa traits.

The relationship between cocoa pulp flavour traits and traits of cocoa products (liquor or dark chocolate) could possibly be due to the presence of aromatic substances or aroma precursors in the pulp that may migrate into the cocoa beans during the fermentation process. As indicated by Rohsius *et al.* (2006), the mycropyle of the cocoa bean becomes permeable during the fermentation process allowing for the entry of acetic acid and other soluble compounds into the cotyledon. It may, however, also be that some of the aromatic substances that are present in the beans are also present in the pulp. Our findings do not suggest that the strength of the basic cocoa flavour in cocoa products is related to any characteristic of the pulp.

More research is certainly required to further verify and possibly explain the relationship between pulp flavour traits and flavour of cocoa products. These studies could involve comparisons between pulp flavours and flavours of cocoa liquor of diverse genotypes, and fermentation of de-pulped beans with low fine flavour potential in fermentation boxes that contain a pulp of rich flavour, and vice-versa.

Although the potential for fine flavour cocoa is largely determined by the cocoa genotype, it is recognised that the environment, post-harvest handling and chocolate preparation techniques may equally affect the flavour of cocoa products. Our results suggest, however, that rapid screening of germplasm and of breeding populations for pulp characteristics can be attempted to select cocoa varieties that display good potential for known as well as for new fine flavour traits.

Possible applications in screening and evaluation of fine flavour cocoa genotypes

Rapid selection of fine flavour cocoa genotypes in diverse populations

According to our results, the genotypes with most preferred cocoa pulps appear to be of direct interest for pre-selection of fine flavour cocoa. Preferred pulps tend to be sweet and show quite intense, diverse types of floral and/or fruity flavours. If the interest is to carry out screening of fine flavour genotypes in diverse populations (germplasm collections, double crosses, farm populations, *etc.*) a start can be made by rapid screening of the genotypes for pulp preference. Field screening can be done by one or two persons opening at least two ripe pods of each genotype in the field and by subjective scoring of preference, indicating presence of special flavours. The most preferred genotypes can subsequently be evaluated by a more detailed method (see below).

Rapid selection of known fine flavour cocoa genotypes in segregating populations

Complex crosses are required in cocoa breeding to try to select for genotypes with known fine flavour attributes (fruity, floral), high yield and resistance to diseases or pests. Pre-selection for the specific quality traits can be carried out by field screening for pulp flavour attributes of individual trees (as above). However, the observers need to have a good idea of the flavour attributes of the type of quality which is being selected. Therefore, the flavour attributes of the control varieties that have the required quality traits need to be well known to the observer. For example, based on the results of our study, if the objective is to select for the typical Trinitario "fresh fruit" flavour, the pulp that one would select for should have medium to high acidity and sweetness, associated with the fresh-fruit flavour of ICS 1 pulp.

Detailed evaluation of pulp flavour traits

Detailed evaluation of pulp flavour traits may be necessary for special studies, such as to relate different pulp flavour traits with fine flavour attributes in fermented and dried cocoa beans and in roasted cocoa products. The method could be similar to the one used in our study, with possible adaptations as required for specific objectives. In general, it will be necessary to have a trained panel of four to six members and to apply two to four replicates depending on the importance of the study. The flavour traits are scored on a 1 to 5 point scale, where 1 = absence of the trait, 3 = medium intensity of the trait and 5 is high intensity of the trait. Each replicate would include the following steps:

1. Collection of ripe pods of the test genotypes.
2. Simultaneous collection of ripe pods of control genotypes, which can be representatives of known fine-flavour types (*e.g.* ICS 1, SCA 6, Nacional) and of commercial varieties grown locally with or without fine flavour traits (Amelonado, IMC 67, CCN 51, *etc.*).
3. Opening of the pods in the laboratory and placing of the pulp in numbered plastic boxes that are closed to contain the pulp odour. The pulp is best left for one or two hours to adapt to the environment and to attain uniform temperatures.
4. Observation of the intensity and type of odour of the pulp is done alter opening the lid of the plastic box by each member of the panel.
5. Tasting of the pulp of two or three beans is followed by scoring of the degree of sweetness, acidity, astringency and intensity and type of flavour (different types of fruity and floral, using the control varieties as standards for known flavours).
6. The overall preference of the pulp based on the above traits is scored afterwards.
7. Alter peeling of two beans, the cotyledons are scored for the intensity of bitterness.

Data analysis may involve linear statistics as applied in our study (ANOVA, correlation studies, PCA) to establish associations between traits and between control and test genotypes.

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Table 1: Cocoa varieties from the UNAS collection that were tested for sensory traits of pulp and beans

Variety	Country of Origin	Type of clone	Genetic origin
EET 62	Ecuador	Commercial clone	Nacional x Trinitario
CCN 51	Ecuador	Commercial clone	Three-way cross
Pandora 1 (PAN1)	Colombia	Luker farm	Unknown
ICS 1	Trinidad	Commercial clone	Trinitario
U 43, U 45, U 53	Peru (Ucayali river basin)	Sub-spontaneous cocoa types	Forastero
H 56	Peru (Huallaga river basin)	Farm selection	Forastero (1)
H 60	Peru (Huallaga river basin)	Farm selection	Forastero x Scavina (1)

(1) According to ancestry inference analysis of molecular data (Dapeng Zhang, personal communication)

Table 2: Overall preference and aroma perception of cocoa pulp from nine cocoa varieties

Variety	Overall preference	Odour on opening the pod	Pulp flavour intensity	Predominant pulp flavours (2)
EET 62	4.3 a (1)	3.9 a	4.0 a	Fruity,floral
PAN 1	3.2 b	1.7 d	2.5 bc	Soursop
U 53	2.8 bc	1.9 cd	1.8 cd	Fruity
U 43	2.8 bc	2.6 bc	2.3 bc	Fruity
ICS 1	2.7 bcd	2.3 bcd	3.0 b	Fruity
H 60	2.3 bcd	3.0 b	1.8 cd	Fruity
U 45	2.3 bcd	2.2 bcd	2.2 bcd	Fruity
CCN 51	2.0 cd	1.5 d	1.3 d	No aroma, fruity
H 56	1.8 d	1.5 d	1.7 cd	Fruity
Mean	2.7	2.3	2.3	
F-value panelists	2.07 ns	2.6*	4.7*	

(1) Different letters identify significant differences between means according to Duncan's test at 5% probability
(2) Identified by at least two out of the six panellists

Table 3: Perception of acidity, sweetness and astringency of cocoa pulp and bitterness of the beans (cotyledons) from nine cocoa varieties

Variety	Pulp acidity		Pulp sweetness		Pulp astringency		Bean bitterness	
EET 62	1.5	cd (1)	3.2	a	0.7	d	2.2	bc
PAN 1	2.3	bc	2.8	ab	1.3	cd	2.8	b
U 53	1.2	d	3.0	ab	0.7	d	1.8	c
U 43	1.2	d	3.0	ab	1.2	d	2.5	bc
ICS 1	2.8	ab	2.8	ab	1.3	cd	2.5	bc
H 60	2.8	ab	2.0	bcd	2.2	b	2.3	bc
U 45	1.0	d	2.7	abc	0.7	d	2.0	bc
CCN 51	3.5	a	1.5	d	3.3	a	4.2	a
H 56	3.0	ab	1.7	cd	2.0	b	2.5	bc
Mean	**2.2**		**2.5**		**1.5**		**2.5**	
F-value panelists	**2.4**	**ns**	**1.7**	**ns**	**5.8****		**8.5 ****	

(1) Different letters identify significant differences between means according to Duncan's test at 5% probability

Table 4: Coefficients of linear correlation between pulp and bean flavour attributes of nine cocoa varieties

	Odour at opening the pod	**Pulp flavour intensity**	**Pulp acidity**	**Pulp sweetness**	**Pulp astringency**	**Bean Bitterness**
Overall preference	0.70 * (1)	0.88 **-	0.48	0.78 *	- 0.61	- 0.32
Odour at opening the pod		0.73 *	- 0.38	0.50	- 0.40	- 0.42
Pulp flavour intensity			- 0.36	0.72 *	- 0.62	- 0.36
Pulp acidity				- 0.81 **	0.87 **	0.71 *
Pulp sweetness					- 0.91 **	- 0.63
Pulp astringency						0.84 **

*(1) The level of significance is indicated by *(=0.05) and by ** (=0.01)*

Table 5: Description of typical pulp and bean flavour attributes of nine cocoa varieties

Variety	Description
EET 62	Intensive odour and fruity and floral sweet pulp
CCN 51	Astringent and acid low flavour pulp, very bitter beans
Pandora 1 (PAN1)	Sweet pulp with characteristic soursop flavour
ICS 1	Acid and sweet, intensive fresh fruit flavour
U 43, U 45, U 53	Sweet pulp with medium flavour intensity
H 56, H 60	Acid, medium astringent, low sweetness and low fruity flavour

Figure 1: Principal Component Analysis plot for five sensory traits of the pulp and for bean bitterness of nine cocoa varieties. The percentages between brackets indicate the percentage of variation explained by axis 1 (F1) and axis 2 (F2)