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Advances on Genetical and Naturally Induced Variations for Fine Flavors and Aromas in Theobroma cacao

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Abstract

The fine-flavor cocoa industry explores mainly six varietal chocolate sensory traits found in four traditional cocoa (*Theobroma cacao* L.) varieties. The role of cocoa pulp flavors and aromas has been ignored until recently when we showed that pulp sensory traits are correlated to fine-chocolate sensory traits. This opened the way for two important applications: 1. Selection of fine flavor cocoa varieties by fresh fruit sensory evaluations, and 2. Transformation of bulk cocoas into fine cocoas by aromatic substances added to fermenting cocoa masses ("TropMix" method). Progress for both applications is presented.

Firstly, growers of the native *Chuncho* variety in Cusco, Peru, make pulp juices from preferred trees. Pulp and raw bean evaluations of 226 farmers' preferred trees disclosed 64 *unique* and mostly *multi-trait sensory profiles*. Twenty-nine of the 40 flavors and aromas identified *mimic* those of known fruit and flower or spice species, such as *mandarin, soursop, custard apple, cranberry, peach, banana, inga, mango, mint, cinnamon, jasmine, rose, lily,* **etc.** Such large genetic diversity and mimicry is still unknown to occur in other cocoa varieties and also not in other commercial fleshy fruit species. The 14 sensory traits found so far in other cocoa varieties are all included in the Chuncho sensory trait panoply, suggesting that Chuncho is part of the "*Centre of Origin*" for cocoa flavors and aromas. Commercialization of the Chuncho sensory profiles should potentially boost the fine flavor cocoa industry, this time based on the Matsigenka and not on the Maya cocoa traditions.

Secondly, 55 "TropMix" fermentations to induce flavor and aroma variations showed that:

a. "Bulk" cocoas can be transformed into fine cocoas, even by the TropMix control treatment (*bean pre-conditioning*);
b. Bulk cocoas fermented with eight fruit pulps and/or ten spices generated innumerous "extra-fine" sensory profiles.

Finally, in view of our results we feel the necessity to propose that the expression of "*extra-fine*" is used for cocoas displaying two or more fine-flavor sensory traits. Our results warrant important cocoa quality *paradigm changes* with repercussions for the fine cocoa production chain that potentially may become a mainstream cocoa production chain.

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Introduction

Pulp vs. fine-flavor chocolate sensory trait relationship

Fine-flavor cocoa (*Theobroma cacao* L.) represents five percent of the world cocoa market and has historically been provided by the Criollo, Trinitario and Nacional varieties and, more recently, also by hybrids with the SCA6 genotype (TSH clones in Trinidad). Chocolates made with these fine-flavor varieties express variable flavor and/or aroma traits that are generally not expressed in bulk cocoa varieties (ICCO 2016).

Regular tasting of cocoa pulp by the first author in 11 cocoa producing countries led in 2006 to the assessment that "*countries that produce fine chocolates (e.g. Trinidad, Ecuador, Venezuela and Peru) grow varieties that have nice cocoa pulps*". This lead to the hypothesis of a fine-flavor pulp *vs*. fine-flavor chocolate relationship. Well-known pulp and chocolate (Presilla 2009; Sukha and Butler 2005; Afoakwa 2008; Clapperton et al. 1994) sensory traits of commercial fine-flavor varieties are presented in Table 1. The chocolate sensory profiles "caramel", "fresh fruit", "floral" and "brown fruit/raisin/floral" are apparently related to the pulp sensory profiles "very sweet", "citrus", "jasmin" and "Muscat grape/lily", respectively. Ancient Criollo pulps and chocolates both do not

Table 1. Comparison of known chocolate and pulp sensory traits for traditional fine-flavor cocoa varieties and for SCA6

Variety	Pulp flavor/aroma	Chocolate flavor/aroma
Ancient Criollo	Very sweet pulp, no flavors or aromas	Caramel/honey, Nutty
Trinitario	Citrus flavor, balanced sweet/acid pulp	Fresh fruit
Nacional	Jasmin aroma and sweet pulp	Floral
SCA6 (clone)	Muscat grape flavor, lily aroma	Brown fruit or raisin, floral

express fruity flavors nor aromas. The nutty flavor is not present in the pulp and is known to be formed only during post-harvest processing. These comparisons support the idea of the existence of a pulp *vs*. chocolate fine-

flavor relationship, be it with certain modifications such as the grape flavor of SCA6 that is transformed into the related brown fruit/raisin flavor.

The pulp vs. chocolate fine-flavor relationship has opened the way for two important applications: 1. Rapid selection of new fine-flavor varieties by pulp flavor and aroma evaluations, and 2. Transformation of bulk cocoas into fine-flavor cocoas by adding aromatic substances to the cocoa mass during fermentation (TropMix fermentations).

Selection of cocoa for fine-flavor traits

Objective breeding towards fine-flavor varieties over the last century has probably been restricted to the selection over the last 50 years of the TSH varieties in Trinidad that express high-quality multi-trait pulp and chocolate sensory profiles with strong influence of that of SCA6. The selection for yield and resistance alone has occasionally resulted in lower than average bulk cocoa quality, as is the case with the CCN51 clone selected in Ecuador in the 1980's. Lockwood and Eskes (1995) concluded that selection for cocoa quality was hampered by differences in objectives among chocolate manufacturers and by lack of efficient individual tree screening methods. Eskes *et al*. (2012) showed that pulp sensory traits are highly discriminative among nine cocoa clones. Human preference was related to sweetness, flavor and aroma which were correlated to fine-flavor chocolate traits of three well-known clones (EET62, ICS1 and CCN51). Our survey in the native Chuncho variety represents the first systematic study in *T. cacao* demonstrating the feasibility of within-variety single-tree diversity evaluations for pulp and raw bean flavors and aromas.

The native Chuncho cocoa variety

"Chuncho" cocoa is native to the La Convención province in the Cusco region in Peru. Chuncho cocoa was cultivated already in the XVI century, but occurring also spontaneously in association with the indigenous Matsigenkas (Aparicio 1999). This tribe has always had a special interest in consumption of Chuncho fruit pulp (Gade 1975, Missioneros Dominicos 2009) and of slightly fermented and roasted raw beans. Rozas (1861) cited by Aparicio (2000) describes native cocoa in the valley of "high quality, noble and healthy" while Paz Soldan (1852), cited by Gade (1975), labels Chuncho cocoa from the Echerate Estate as "the best cocoa in the world". Chuncho acreage culminated in the 1980s with 14,000 ha and a production of 10.000 tons. The acreage of Chuncho has thereafter declined due to low productivity (150-250 kg/ha) linked to aged trees and competition from CCN51 and from other replacement crops. Chuncho is still mainly used as a low-valued bulk cocoa bean source for butter and powder extraction.

However, traditional farmers consume pulp juices from selected Chuncho trees which is a practice not known to occur elsewhere in the world. Our pulp and bean survey was therefore focused on Chuncho trees used by farmers for juice production. Chuncho beans without specific sensory traits express already a certain level of undefined flavor and aroma (Condori Cruz 2015) the intensity and type of which may vary with the collecting site. Even when unfermented or little fermented, Chuncho beans are neither acid nor bitter nor astringent but may occasionally become so with fermentation duration of more than 4.5 days (Condori Cruz 2015). SNP marker studies showed a close relationship of Chuncho with accessions from the Madre de Dios and Beni river basins (Dapeng Zhang Pers. Comm.).

Transformation of bulk cocoas into fine-flavor cocoas

The first TropMix fermentation carried out in Brazil in 2007 involved additions of cupuaçu (*T. grandiflora*) and of soursop (*Annona muricata*) pulp to the fermentation mass of Amelonado cocoa beans. The raw beans and chocolates expressed strong cupuaçu and soursop flavors and aromas that were highly appreciated by French fine-chocolate consumers. This result was the basis for the European patent application *WO/2009/103137A2* that was granted in 2009 but abandoned in 2013. This method was used by Valrhona to launch two chocolates in 2016. Our TropMix fermentations carried out between 2008 and 2011 yielded variable results that might be ascribed to the complex interactions of foreign aromatic substances with the cocoa pulp environment in traditional fermentations. In the present paper we describe highly repeatable and positive results obtained between 2015 and 2017 by carrying out TropMix fermentations in association with bean pre-conditioning.

Materials and Methods

Genetic variation for fine-flavor sensory traits

Identification of pulp flavors and aromas outside La Convención Occasional single-tree pulp tasting exercises were carried out mainly by the first author between 2007 and 2017 in Trinidad, Brazil, Ecuador, Piura and by Wilbert Cruz in Satípo in Peru.

Chuncho pulp and bean sensory trait identifications The pulp and raw bean sensory trait survey was performed by 2 to 3 experienced cocoa scientists including 100 and 126 trees, respectively, from a 200 km long stretch of the La Convención valley. Tree selection was mainly based on preference by farmers for pulp juice usage. Confirmation of the Chuncho identity of 100 of these trees was obtained in 2014 with SNP markers (Dapeng Zhang Pers. Comm.) Aromas were identified upon pod opening and fruity flavors during tasting of pulp of two ripe pods per tree. For the slightly fermented bean survey bitterness, acidity, astringency, flavor and aroma intensities and general preference were scored on 0-5 point scales. Repeatability of trait identifications was assessed by blind re-evaluation of pulp and bean sensory traits from 20 different genotypes during the 2016/17 harvest.

Naturally induced variation for fine-flavor sensory traits

TropMix fermentations with CCN51 Four-day fermentations were carried out with CCN51 fresh beans pre-conditioned by overnight dripping followed by three hours of bean spreading. Aromatic substances were added at the onset of the fermentation process for spices (cumin, cinnamon, glove and ginger) and after 24 hrs for fruit pulps (banana, custard apple, soursop and mandarin peel). Chuncho beans were used as a control treatment but with only two hours of bean spreading. Ten litre perforated polystyrene boxes were used for micro-fermentations Fermentation index varied from 60 to 100%. Liquor preparation and sensory evaluations on 0-10 point scales by an eight-member panel were carried out at CIRAD, France. Simple and comparative ANOVA analyses were carried out to test the significance of the results.

TropMix fermentations in Brazil Twenty and 35 TropMix fermentations were carried out in 2015 and in 2017, respectively, in two cocoa farms nearby Linhares, Espírito Santo State. The number of aromatic fruit species and spices, used separately or in combination, were 9 and 11 in 2015 and 8 and 15 in 2017, respectively. The cocoa mass used was composed of a mixture of 10-15 commercial Brazilian cocoa clones selected for resistance to witches' broom and yield capacity. The fermentation method used was similar to the one used in Peru. Fermentation index varied from 70 to 90%. Sensorial evaluations were carried out on the raw beans using 0 to 10 point scales. The results shown here relate to six representative TropMix treatments.

Results and Discussion

Genetic variation for fine-flavor sensory traits

Pulp flavors and aromas identified outside La Convención, Peru

As described in the introduction, six pulp flavors and aromas have been identified in the traditional four fine-flavor varieties. Between 2007 and 2017 eight hitherto unknown cocoa pulp flavor and aroma traits were discovered, outside La Convención, in very different varieties in Trinidad, Peru, Ecuador and Brazil. The most commonly flavor was soursop identified in six locations. A very interesting mango/rose profile was found in the Ecuadorian clone EET397 that used to be grown in Brazil for its witches' broom resistance. The banana pulp flavor was first discovered in Trinidad in 2008 in an old Trinitario plantation. Chocolates made with beans from this tree by Ed Seguine showed strong and persistent cooked banana/banana jam flavor. Five sensory traits (banana, soursop, jasmine, citrus and annona) were identified by Wilbert Cruz in Amazon genotypes in Satipo, Junin, Peru, in 2012 as verified with molecular markers (Dapeng Zhang, Pers. Comm). In 2017 it was discovered that the "bulk" Amelonado variety in Brazil harbors the jasmine aroma, which is very surprising, and that the CEPEC 2008 clone expresses the soursop/rose sensory profile.
The discovery of eight hitherto unknown pulp cocoa flavor and aroma traits between 2007 and 2017 showed that different cocoa varieties may harbor unexpected sensory traits that should be of interest for fine-chocolate manufacturing. The surprising discovery in 2017 of three fine-flavor traits in two commercial Brazilian varieties suggests that varieties considered as bulk cocoa may in fact be fine-flavor genotypes with potential for commercial usage as fine-flavor cocoas.

Chuncho pulp and bean sensory traits survey

Pulp flavors and aromas of 100 Chuncho trees preferred by farmers for making pulp juices were evaluated in 2012. Ninety seven showed qualitative expression of pulp flavors and/or aromas representing 30 distinct pulp sensory profiles. These include 17 fruit flavors and nine aromas or spices. The most frequently encountered sensory profiles were *soursop/jasmine*, *floral, citrus/jasmine, mandarin/jasmine and jasmine* found in at least 6 trees each (Table 4).

Ninety-two Chuncho bean samples out of the 126 evaluated in 2015 received overall preference scores of three and above. A total of 39 different sensory profiles were identified involving 16 fruit flavors and eight aromas or spices. The four most frequently encountered sensory profiles were *mandarin/jasmine, soursop/floral, cranberry*, *malt, mint/floral, malt/jasmine* and *cranberry/rose,* found each in four or more trees.

The combined analysis revealed 64 unique sensory profiles that were detected in 226 Chuncho pulp and raw bean samples. Sixty-six percent is based on combinations of two or more sensory traits. Fifty-two percent contain exclusively fruit flavor and flower or spice aroma combinations. The total number of flavor and aroma traits identified so far in Chuncho is 28 plus 12, respectively. Out of these 40 traits, 29 (73%) are mimicking those of known fruit, flower and spice species. Chuncho mimics not only flavors of individual fruit species but also of varieties within species, such as observed for the four mimicked banana varieties. It also mimics flavors of related species within the citrus genus (citrus, mandarin and tangelo) all combined with the aroma of the citrus flower aroma (jasmine).

The 28 Chuncho **flavor traits** identified are: soursop, citrus, mandarin, grape, tangelo, fruity, banana, annona, guava, roseapple, banana "Cavendish", banana "Manzano", banana"Isla", banana "Chinito", mango, green apple, custard apple, inga, cranberry, peach, dried apple, ripe plum, fresh red fruit, ripe red fruit, ripe yellow fruit, honey, jackfruit and raisin. The 12 **floral or spicy aroma traits** are: floral, jasmine, lily, heavy floral, rose, vanilla, yeast, basil, mint, malt, cinnamon and spices.

The 40 sensory traits and 64 sensory profiles identified in Chuncho represent 10 and 15 times, respectively, the number of sensory traits and profiles identified in the traditional fine-flavor varieties (Table 1) indicating the enormous potential for commercial valorization of the Chuncho sensory profiles. All 14 sensory traits identified so far by us in other varieties than Chuncho belong to the Chuncho sensory trait panoply, suggesting that Chuncho is part of the centre of origin for fine-cocoa sensory traits. The high percentage of the Chuncho sensory traits that are mimicking traits from known fruit, flower and spice species appears to be unique among commercial flesh fruit species. The observed stable expression of multi-trait sensory profiles suggests simple pleiotropic inheritance which is also a unique feature of cocoa with regard to other fleshy fruit species.

Naturally induced variation for fine-flavor traits

Induced sensory trait variation in CCN51

Sensory trait intensities in the cocoa liquors that were significantly and positively influenced as compared to the control CCN51 by TropMix treatments were acidity (glove, ginger, cinnamon and mandarin peel), astringency (glove), fruitiness (cinnamon, glove, mandarin peel, ginger and custard apple), red fruit (ginger), yellow fruit (cinnamon, mandarin peel), floral (custard apple) and spicy (cumin). No significant differences were observed for the treatments with aromatic substances in comparison with the CCN51 control treatment for bitterness, nutty, chocolate and citrus. Global quality on a 0 to 10 point scale was significantly improved in comparison with the CCN51 (2.5) control treatment with additions of glove (4.5), custard apple (4.4), mandarin peel (4.1), cinnamon (4.0), soursop (3.8) and ginger (3.7). Treatments with these aromatic substances were statistically similar for global quality to the fine-flavor Chuncho control variety (4.6).

The above shows that TropMix fermentations can induce significant variations for fine-cocoa quality traits and modify the lower than bulk cocoa global quality of CCN51 into a fine-flavor cocoa type. This suggests that commercial applications to improve CCN51 with TropMix fermentations should be feasible.

Transformation of bulk cocoa into fine cocoa

The TropMix bean pre-conditioning fermentation control treatment was compared to that of the traditional farmers' fermentation method (Table 2) using commercial Brazilian cocoa varieties. Bitterness and astringency scores of raw beans varied from 2 to 4 for the farmers' method as compared to 0 to 2 for the bean pre-conditioning fermentation method. Scores for mature fruit, spicy and floral varied from 2 to 3 for the bean pre-conditioning method while the farmers' method yielded just a 0 to 2 for mature fruit. When roasted, only the accelerated method produced a nutty flavor with intensities of 2 to 5. Overall preference for raw beans of the farmers' method varied from 4 to 6 while the average score for the bean pre-conditioning control method was 7.

It is known that pre-conditioning of cocoa beans may decrease the intensity of basic flavors (Edem Kongor et al*. 2016). However, the TropMix control method decreased not only unpleasant basic flavors but also increased positive flavors and aromas and yielded in addition a nutty flavor. This shows that the TropMix control treatment has the potential to transform bulk cocoa into fine-flavor cocoa.*

Naturally induced variation for fine-flavor traits

Table 2 shows the perceived intensity of sensory traits in six representative TropMix treatments, two carried out with spices and four with fruit pulps. All TropMix treatments had very low astringency scores and low to medium scores for bitterness. The highest bitterness score of 4 obtained for treatment 6 is likely related to the bitter lemon peel that is part of this treatment. The spicy treatments 1 and 4 resulted not only in high spicy scores but also in relevant scores for fresh and mature fruit as well as for floral. The fruity TropMix treatments 2, 3, 5 and 6 induced broad spectrum sensory profiles not only with high scores for fresh and mature fruit but also with relevant scores for the spicy and floral traits. Preference scores for the TropMix raw beans, varying from 9 to 10, were substantially higher than those of the farmers' and of the TropMix control treatments.

These six examples out of a total of 55 TropMix treatments carried out between 2015 and 2017 demonstrate clearly the feasibility of transforming bulk cocoa varieties into fine and extra-fine cocoa types with sensory profiles that correspond to the type of aromatic substances added during the TropMix fermentation. The superior preferences of TropMix raw beans in relation to the control treatments suggest that the TropMix method can have important commercial applications.

Table 2. Examples of naturally induced fine-flavor traits assessed on a 0 to 10 point scale in TropMix fermentations as compared to the farmers' (F) and TropMix (C) control treatments

Treat-ment	Nº days	Acidity	Bitter-ness	Astrin-gency	Nutty	Fresh fruit	Mature fruit	Spicy	Floral	Prefe-rence
F	6	0	3	4	0	0	2	0	0	**5**
C	3	0	2	0	4	0	3	2	2	**7**
T1	3	0	2	0	-	0	2	7	3	**9**
T2	3	0	3	0	-	0	8	3	4	**9**
T3	4	2	2	0	-	6	5	2	3	**10**
T4	3	0	3	2	4	4	4	6	6	**10**
T5	5	0	3	2	2	2	5	3	2	**9**
T6	4	0	4	1	5	6	2	6	4	**10**

T1 = Glove and cinnamon, T2 = Jackfruit, T3 = Mango, T4 = Aroeira and Jamaica pepper, T5 = *T. grandiflora,* T6 = Passion fruit, sweet lime and lemon

Conclusions

The significant progress described here on genetic and naturally induced variations for fine-flavor traits in *T. cacao* is a direct consequence of the assessment made in 2006 (Eskes *et al*. 2012) that cocoa pulp sensory traits are related to fine-cocoa chocolate traits.

The disclosure of simple as well as complex genetic and induced sensory profiles makes us suggest to use the term "fine-flavor" for cocoa with simple and "extra-fine flavor" for cocoa with complex sensory profiles.

The *genetic variation* for 40 sensory traits and 64 sensory profiles disclosed in Chuncho is *enormous* as compared to the four sensory traits and profiles

present in the four traditional fine-flavor cocoa varieties. Hence, the potential of commercial valorization of the Chuncho sensory profiles, be it in Chuncho or in other cocoa varieties, is also enormous.

The *naturally induced* variation by aromatic substances in TropMix fermentations is by definition *unlimited*. Hence its commercial applications are potentially innumerous. Confronting this innovation with the chocolate sector point of view during conferences and cocoa sector development programs helped to highlight that the technical and financial aspects came out like a challenge to the cocoa industry having access to a cocoa resource that could be compensated by a high price values on the market.

Commercial exploration of fine or extra-fine sensory profiles would depend largely on the capacity of chocolate makers to reproduce the interesting raw bean sensory traits in chocolates. This remains an important challenge to the fine-chocolate making industry according to several tests carried out eg. in Peru, Vietnam, Italy and the USA. Roasting and processing tend to reduce unpleasant basic flavor traits but, even when done by fine-chocolate manufacturers, also significantly positive flavor traits such as fresh fruit, spicy and floral may cause significant deformations in the original sensory profile constitution.

The Chuncho genetic background and TropMix fermentations both display low basic flavors in raw beans making it possible for fine-chocolate makers to concentrate on valorization of the abundant positive flavors and aromas present in those cocoas rather than on the elimination of unfavorable basic traits.

The authors involved in the disclosure of the Chuncho sensory profiles concluded that eating slightly fermented raw beans is a superior tasting experience ("*The best Chuncho chocolate is a Chuncho bean*"). This suggests strongly that there is a good potential to enhance the special market for raw beans, or for raw bean nibs, of diverse genetic or naturally induced fine and extra-fine flavor cocoas.

The recent advances on the large genetic and naturally induced variations for fine and extra-fine cocoas warrant important cocoa quality paradigm changes to occur:

- The potential for fine-flavor cocoa market diversification is unlimited;
- Fine cocoa may become a mainstream cocoa because of the large potential to increase the offer of fine cocoa worldwide based on existing bulk and fine-flavor varieties;

- Fine-flavor cocoa raw beans or nibs may become an increasing segment in the fine-flavor cocoa market.

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