Annex 8

Heirloom Cocoa Preservation Fund - Bean Submission directions and requirements for processing and evaluation
PROTOCOLS FOR SUBMISSION OF BEANS THROUGH SITE VISIT FOR GENETIC TESTING
HEIRLOOM CACAO PRESERVATION

BEAN SUBMISSION DIRECTIONS AND REQUIREMENTS FOR PROCESSING AND EVALUATION

HCP IDENTIFICATION NUMBER
Upon paying the application fee, registering on the USDA site, and completing the application, the Heirloom Cacao Preservation Initiative (HCP) Applicant receives an HCP Identification Number. This number and bean information will be the ONLY information the HCP Lab sees when performing the blind processing and evaluation procedures for the Tasting Panel.

QUANTITY OF BEANS NEEDED FOR EVALUATION
The HCP requires three (3) kilograms of cocoa beans – cleaned and dried weight – representing the population of trees and commercial shipment quantity proposed Heirloom designation. The HCP defines “cleaned” as having all broken beans and foreign material removed. For those Applicants who normally wash and polish beans after drying, the HCP considers washing and polishing part of the cleaning process.

WHAT KIND OF BEANS SHOULD BE SUBMITTED
Fully mature, ripe, un-diseased beans harvested during the normal crop cycle so as to be fully representative of long-term production. Three kilograms of clean, dried beans will require beans from 20-60 pods (depending on bean weight and bean count per pod) from 20-60 bearing trees representing the population being assessed. Trees should be marked or tagged so they can be assessed for genetic diversity at a later time. (Genetic evaluation is done after the HCP designates the flavor of the beans as Heirloom.) If less than 3kg of clean, dried beans are available, the Applicant must receive agreement in advance from the HCP.

WHY WE NEED THREE KILOGRAMS OF BEANS
We ask for 3 kg of beans to ensure sufficient beans for the primary Lab tasks, provide spare beans in case of preparation or shipment problems, and allow for retained samples and returning liquor and chocolate samples to the Applicants. We assume the beans will be clean with no cleaning losses, and the yield of cleaned, roasted nibs from raw beans will be 65%. Thus, 3kg of beans are needed to cover the following HCP Lab tasks for evaluation:
<table>
<thead>
<tr>
<th>Physical tests</th>
<th>175 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquor for liquor evaluations</td>
<td>875 g</td>
</tr>
<tr>
<td>Liquor for chocolate evaluation</td>
<td>900 g</td>
</tr>
<tr>
<td><strong>Total beans needed</strong></td>
<td><strong>1950 g</strong></td>
</tr>
<tr>
<td>1.95kg (65% of 3kg)</td>
<td></td>
</tr>
</tbody>
</table>

**FERMENTATION & DRYING REQUIREMENTS**

- Fermentation and drying must be done in a manner that is consistent with the larger scale (commercial) production of this bean type. The HCP does not specify fermentation or drying practices.
- **NO** fruit, fruit pulps, juices, spices, flavors, or any substance may be used to alter, enhance, add, or “spice up” the flavor of the beans during fermentation.
- Drying should be completed until the moisture content of the beans is 6.5 to 7.9%. The ideal moisture content of the beans is 7.0 to 7.5%.
- Following the completion of drying, samples must be stored for a minimum of six (6) weeks to allow the flavor to equilibrate and be representative of commercial shipments.

**STORAGE REQUIREMENTS**

It is recommended that Applicants store a minimum of 6kg of beans in the following ideal storage conditions, retaining 3kg as an insurance against possible loss of sample during shipment or problems with the initial shipment.

- Beans should be stored in a breathable bag such as new, clean, odor-free burlap, jute, or cotton. Any material used should be smelled prior to its use as a storage bag for the beans to ensure that it is free from any odor taint that would impart an off odor or flavor to the beans as a result of storage. Care should be taken to ensure this does not happen.
- Storage should be at ambient conditions but protected from excessive moisture or any possible off odors in the storage area. Care must be taken to avoid exposure to any conditions that will cause re-wetting or re-humidification of the beans and resulting mold growth on the beans. Mold present in a cut test above United States FDA standards (4% internal mold) will be grounds for immediate rejection of the sample. Care should be taken to ensure this does not happen.
- Bagged samples should be stored in screened but breathable containers that will protect them from insect infestation. The mesh size of the screen

*HCP Protocols p 3*
should be small enough (like mosquito netting) to prevent the entry of moths and larvae. The presence of any insect infestation in the cut test will be grounds for immediate rejection of the sample. Care should be taken to ensure this does not happen.

PRE-SHIPPING REQUIREMENTS
Applicants will need to confirm the details of the farm from the first part of the HCP Application and email the following additional information to the HCP prior to shipping:

- Date of harvest
- Date of Drying Completion
- Bean Type/Tree/Clone Information (necessary to determine the proper roasting conditions for each sample without un-blinding the application)

Applicants will also need to agree in that email that they utilized commercial practices for the fermenting and drying of the beans and all other Submission Protocol conditions.

Applicants MUST ensure that all necessary paperwork including bill of lading, commercial invoices, customs declarations, and any required United States FDA Prior Notice requirements are met. If you do not have an account for Prior Notice you can create an account in less than ten minutes on the FDA Site: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/PriorNoticeImportedFoods/default.htm

Prior to shipment of the cocoa beans, the Applicant should assemble and then enclose all this paperwork as well as a copy of the application information provided at the end of the Submission portion of the HCP Application with the Applicant’s HCP Identification Number.

SHIPPING REQUIREMENTS
Beans should be shipped in the same breathable bags that they were stored in and not any other bag, like plastic Ziploc bags – applicants accustomed to shipping samples in plastic Ziploc bags should take care to note this point.

Bags should NOT have any markings aside from the HCP Identification Number. Multiple samples must be shipped separately and require individual applications for each sample being submitted.

HCP Protocols p 4
Samples will be sent to the FCIA, which will immediately remove the bags of beans from Applicant's box, log them in, place them in a new, anonymous shipping box, and send them to the HCP Lab for processing. This ensures the Applicant's HCP number and bean type are the only information the Lab sees when performing the blind processing and evaluation procedures for the Tasting Panel.

**SHIPPING INFORMATION**

Paperwork and unmarked bags of beans should be sent to the HCP LAB. ADDRESS IS DISCLOSED WHEN USDA APPLICATION IS COMPLETED.

The HCP Lab will log the receipt of the beans by their HCP Identification Number. Applicant and HCP Tasting Panel will receive notification when this shipment is logged as received.

While at the HCP Lab, prior to evaluations, beans will be stored in a temperature and humidity controlled environment to ensure their stability. Beans will be stored separately from all other cocoa beans to minimize the opportunity for any infestation.

Following receipt at the HCP Lab, beans will be scheduled for bean counting, cut tests, and raw bean moisture content test and prepared for processing into liquor and chocolate covered in the next protocols.

You will soon be able to track your application in the HCP Database once it is received by the FCIA.

*HCP Protocols p 5*
PROTOCOLS FOR HCP LAB TESTS & RAW BEAN CHARACTERIZATION PRE-LIQUOR PREPARATION & ANALYSIS

The following protocol covers what happens after the HCP Lab logs the receipt of the beans by their HCP Identification Number and bean information that will allow determination of the proper roasting conditions. While at the HCP Lab, prior to these tests, beans will be stored in a temperature and humidity controlled environment to ensure their stability.

Raw Bean Characterization Tests

Upon receipt, the HCP Lab will conduct the following tests on the Applicant’s beans as they are prepared for processing into liquor and chocolate (covered in the following set of protocols):

• Bean Count (Beans/100g)
• Cut Test (2x 50 beans)
• Raw Bean Moisture by Mettler loss in weight moisture balance calibrated to vacuum oven moistures

There is no a priori requirement for the Cut Test evaluation with the exception of the internal mold and infested categories. The Cut Test simply documents the characteristics of the Applicant’s beans. Mold and insect infestation must comply with the Proposed ISO Standard ISO/TC 34/SC “Cocoa Beans – Specification” (01/12/2012): maximum 3% moldy; maximum 3% infested.

The HCP Lab will also photograph the cut tests. Photos will include a (MacBeth) ColorChecker or equivalent to allow standardization of the colors due to lighting differences.

In the Unlikely Event Beans Fail Cut Tests

If all tests are passed, the HCP Lab will mark the tests as passed. Should a sample fail a Cut Test in the HCP Lab, the HCP Lab will mark the test as failed, and the Applicant and Tasting panel will be notified. 110 beans will then be sent to two HCP Tasting Panel members who have labs and can perform additional Cut Tests of 2 x 50 beans and photograph them. The new Cut Test information will be entered into the HCP Database.

• If the result of the Cut Tests on the combined 6 x 50 beans passes the standard, the HCP Lab will mark the Cut Test as passed in the HCP Database and continue with the processing.

HCP Protocols p 6
• If the result of the Cut Test still fails the standard, the HCP Lab will mark that bean as rejected in the HCP Database, which will email the Applicant to resubmit the beans at the Applicant’s cost.

Once the beans are resubmitted following the standard HCP Submission Protocols, all tests will be performed again by the HCP Lab and if necessary the two additional Tasting Panel labs.

• If the results of the Cut Tests on the 2 x 50 or combined 6 x 50 beans pass the standard at any point, the HCP Lab will mark the Cut Test as passed in the HCP Database and continue with the processing.

• If the result of the Cut Test fails the standard a second time, the HCP Lab will again mark that bean as rejected in the HCP Database.

If rejected a second time, the HCP Tasting Panel will review the data of all the tests performed and provide their final recommendation. If the consensus of the panel agrees with the Cut Test determinations then the HCP Lab will mark the beans as rejected. The HCP will then follow up with the Applicant to discuss the failure of the sample and any next steps.

Beans that pass the Cut Test are now processed into Liquor and Chocolate using the following Protocol.
PROTOCOLS FOR HCP LAB
LIQUOR AND CHOCOLATE PREPARATION AND ANALYSIS

Processing of beans by the HCP has been standardized to ensure consistency for all submissions for Roasting, Liquor Milling, Chocolate Making, and Analyses of Liquor and Chocolate. Bean type information from the Applicant is essential to avoid delays in this protocol.

A. ROASTING, CRACKING, AND WINNOWING

Oven Specification
High efficiency convection ovens are required: Binder laboratory convection oven Model 111G-06-01 (800 gm full load of beans) or FD 23-UL (200 gm full load of beans), ThermoScientific LabLine Imperial series laboratory convection oven, or equivalent.

Ovens are loaded with a single, wide mesh screen tray. Beans are loaded single bean depth across the loading area. (Filler beans will be used as necessary to ensure the same loading for all roasts.)

Roasting Conditions
Specific roasting conditions for the beans are designed to maximize the flavor potential for each type of cocoa bean. Conditions are consistent with the Cocoa of Excellence roasting conditions used by CIRAD and Mars and international project evaluation conditions across a wide range of clones, geographical locations, and bean types:

- Trinitario Type (expected for most samples): 120°C for 25 minutes
- Forastero Type (typical of Amelonado types): 130°C for 25 minutes
- Ancient Criollo Types (e.g., Porcelana, Guasare, etc.): 112°C for 25 minutes

All times are measured from -2°C of set point on oven recovery after insertion of the tray of beans into the oven. (Note: Binder ovens have a recovery time of 4.5 minutes for first model above and 2.5 min for the second model, which has a smaller cavity.)

In most cases, beans will follow the Trinitario protocol, as most beans will fall into the fruity/floral category. Modern Criollo types will primarily be roasted at Trinitario conditions as they are generally much closer genetically and processing wise to traditional Trinitario beans. Ancient Criollos are distinguished from the needs of the Modern Criollos (i.e., Criollo leaning Trinitarios) by the requirement for much lower temperatures to best express

HCP Protocols p 8
the nutty/caramel notes. The Forastero protocol is specified to bring out the maximum chocolate intensity in this type of sample. While referred to as “bulk” or “base” beans, the Forastero contribution to the chocolate flavor profile is critical and we encourage the work of the Cocoa of Excellence program, which awards this category of bean.

If necessary, based on the Lab raw bean tests and information available from the Applicant, the HCP Lab and Tasting Panel Chair may discuss the sample beans and what they know of them before roasting the quantity needed for liquor and chocolate evaluation. Then, if necessary, the Lab and Chair may elect to do a quick, small pilot roast of 30-50g to make liquor for the Lab and Panel Chair to taste if need be to determine the proper roasting conditions.

Bean type information from the Applicant is essential to avoid delays in this protocol. If necessary, in Applications in which the bean type is not provided or is unknown, the HCP Lab will consult with an HCP Tasting Panel member with access to a lab who will receive a 150g sample of the beans for cut test evaluation and roast recommendation. If that cut test is not sufficient in the judgments of the HCP Lab, the HCP will allocate an additional 175g of beans and do small scale roasting and liquor milling on 50g samples at all recommended roasting conditions in this protocol to determine the proper roasting condition based on flavor of the samples. The HCP Lab will then use the selected condition to produce the liquor for liquor and chocolate evaluations by the Panel.

Roasting Needs

Amounts needed are based on supplying liquor to the HCP Tasting Panel for liquor flavor evaluation and the USDA for analytical flavor profiling, returning a sample to the Applicant, retaining a sample by the HCP Lab, and providing sufficient nibs and therefore liquor for the preparation of the chocolate samples.

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total liquor required for Panelists</td>
<td>260g</td>
</tr>
<tr>
<td>Liquor Retained Sample</td>
<td>150g</td>
</tr>
<tr>
<td>Liquor for returning to Applicant</td>
<td>50g</td>
</tr>
<tr>
<td>Liquor loss in preparation (milling)</td>
<td>85g</td>
</tr>
<tr>
<td>Total nib clean, shell free required</td>
<td>505g</td>
</tr>
<tr>
<td>Raw beans roasted at 65% yield</td>
<td>775g</td>
</tr>
<tr>
<td>Total chocolate required for Panelists</td>
<td>910g</td>
</tr>
<tr>
<td>Chocolate making loss</td>
<td>50g</td>
</tr>
<tr>
<td>Chocolate tempering loss</td>
<td>50g</td>
</tr>
<tr>
<td>Liquor needs at 61% liquor recipe</td>
<td>540g</td>
</tr>
<tr>
<td>(liquor losses included in liquor milling above)</td>
<td></td>
</tr>
<tr>
<td>Raw beans roasted at 65% yield</td>
<td>835g</td>
</tr>
</tbody>
</table>

Unless absolutely necessary, roasting and liquor preparation will be done in several batches run at the same time to create a uniform batch of liquor. This

HCP Protocols p.9
would entail roasting 1.8 kg of raw beans. Depending on the roaster used, this will entail 3-5 roasting batches.

**Winnowing**

Following roasting, beans are cracked and winnowed. Cracking can be accomplished in any suitable device (e.g., Limpirma breaker by Capco Test Equipment, UK) or by hand. Following cracking, beans are winnowed using typical winnowing equipment such as a John Gordon or Capco Test Equipment Winnower or equivalent.

Following winnowing, all nibs are combined and well mixed. All nibs will be handpicked to remove all traces of shell—both free shell and shell still stuck to the nibs. Winnowing and handpicking will be performed in an area governed by GMP practices and with an HACCP program in place to ensure the wholesomeness of the product.

After winnowing, nibs will be stored in a sealed bag. Every effort will be made to convert nibs into liquor within 48 hours of roasting. If the nibs cannot be liquor milled within 24 hours of roasting, they will be stored in a tightly sealed bag, preferably a multi-layer, barrier film vacuum seal type to provide barrier film protection without vacuuming. Nibs will not be stored longer than seven (7) calendar days (even in a sealed bag) prior to liquor milling.

Storage temperature should be 10-24°C (50-75°F). If nibs are stored at temperatures less than 18°C (64°F), they must be allowed to warm to room temperature prior to opening the bag.

The expected yield of cleaned roasted nibs from uniformly fermented and dried cocoa beans will be 70%. The HCP has calculated its needs based on 65% to provide added insurance against loss.

**B. LIQUOR MILLING**

Liquor milling may be accomplished in any suitable slow rotating stone or porcelain grinding mill. Metal milling (e.g., ball mills) or high-speed mills are not to be used. Milling will be performed in an area free of other odors and protected from environmental influences. GMP practices will be in place as well as an active HACCP program to insure wholesomeness of the product.

During milling, the mill will be held at warm room conditions to insure that the liquor will not solidify during the milling process. The mill may be pre-warmed to operating conditions to facilitate milling.

Milling temperature will not exceed 55°C (130°F).

*HCP Protocols p 10*
Exact milling times CANNOT be specified as this is dependent on a number of factors such as fat content of the nibs, degree of fermentation of the beans, specific mill used, condition of the stones in the mill, etc. But milling will be accomplished gently and without the addition of significant external mechanical pressure. The objective is to produce liquor that will have no discernible grit to the HCP Tasting Panel in their evaluation without being excessive. The balance between fineness and time will be determined by the HCP Lab, which has extensive experience in this process.

C. CHOCOLATE MAKING

The HCP Lab will use a standard 68% cacao, semisweet chocolate recipe for all evaluations:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate Liquor</td>
<td>65.10%</td>
</tr>
<tr>
<td>Deodorized Cocoa Butter¹</td>
<td>3.00%</td>
</tr>
<tr>
<td>Sugar²</td>
<td>31.55%</td>
</tr>
<tr>
<td>Soya Lecithin³</td>
<td>0.35%</td>
</tr>
</tbody>
</table>

¹ Cocoa butter used in this formulation will be neutral tasting so as to not shift the flavor inherent in the liquor. The HCP Lab will verify by taste the use of neutral butter.
² Prior to use, the sugar must be assessed to ensure that it is neutral in taste and smell by placing 2-4 ounces of sugar in a jar twice that size, securely capping the jar, and holding for at least one hour. The sugar will then be uncapped and immediately smelled to determine that it has no inherent odor.
³ Soya lecithin used should be double bleached and also verified to ensure that it will not alter the flavor of the chocolate.

The same protocol steps for liquor milling then apply to chocolate making:

- Chocolate milling may be accomplished in any suitable slow rotating stone or porcelain grinding mill. Metal milling (e.g., ball mills) or high-speed mills are not to be used.
- Milling will be performed in an area free of other odors and protected from environmental influences. GMP practices will be in place as well as an active HACCP program to insure wholesomeness of the product.
- During milling, the mill will be held at warm room conditions to insure that the liquor will not solidify during the milling process. The mill may be pre-warmed to operating conditions to facilitate milling.
- Milling temperature will not exceed 55°C (130°F).

HCP Protocols p 11
Like liquor milling, exact chocolate milling times CANNOT be specified. However, in the case of chocolate, finished fineness is critically important so priority is given to achieving the fineness. The required fineness is less than 17 microns (6.7 10,000ths inches). This will be verified by micrometer (AACT method or equivalent) as an average of five independent measurements of a sample of the mass being milled.

Once the requisite fineness is reached, milling is concluded.

D. ANALYSES OF LIQUOR AND CHOCOLATE AND HOLDING OF SAMPLES

Following liquor milling, liquor will be checked either by PNMR or by NIR for total fat content. This data and the fineness of the chocolate will be provided to the HCP Tasting Panel with their evaluation samples.

Following all analytical tests on the beans and processing into liquor and chocolate, the remainder of the beans will be stored in a temperature and humidity controlled environment until the HCP completes all its analyses, including genetic sampling and ensure sufficient time for all parties, including the Applicant, to review the HCP results. Once it is determined that no further sampling of these beans is needed, the beans may be discarded or the HCP will provide the HCP Lab with other directions.

NOTE: The HCP IS aware that chocolate and in particular semisweet chocolate will change flavor profile—particularly mellowing—with long term storage. While this is understood, it is not practical to hold chocolate 2-4 months to provide a response the Applicant within a suitable time frame. Thus, HCP Tasting Panel samples will be stored one (1) week prior to flavor evaluation, which is covered in the following protocol.
PROTOCOLS FOR HCP LAB
SAMPLING, STORAGE, & SHIPPING OF SAMPLES AND PANELIST RECEIVING & STORAGE OF SAMPLES

LIQUOR AND CHOCOLATE SAMPLING AND STORAGE

Samples - Liquor
The HCP Lab will pour melted and homogenized liquor into sample containers (VWR Polypropylene Wide Mouth Bottle, 30 ml (Cat No. 414004-122) or equivalent and tightly capped. Each sample bottle will be evaluated to insure they are free of any off odors.

Liquor samples will be prepared in the following amounts for the HCP Tasting Panel, USDA Applicant, which can change based on the needs of the HCP Tasting Panelists and the USDA:

HCP Tasting Panel and USDA
• 6 (FOUR) 20g containers 120g total
• 3 (THREE) 30g containers 90g total
• 2 (TWO) 25g containers 50g total

To Return to Applicant
• 2 (TWO) 25g containers 50g total

Retained by HCP Lab
• 2 (TWO) 75g samples in 4oz non-sterile polypropylene specimen jars 150g total

All samples will be labeled with the HCP Application Number and the date of liquor milling.

Storage – Liquor (Pre-Shipping)
Liquor will be stored at chocolate warehouse temperatures (17-21°C, 62-70°F) until shipped to the HCP Tasting Panel or returned to the Applicant.

Samples – Chocolate
Following milling, all chocolate for evaluation will be homogenized, hand tempered, and molded into the HCP Lab’s standard molds of approximately 10g each. Tempered bars will be allowed to equilibrate over night and will then be vacuum-sealed in multi-layer, barrier film vacuum seal bags (e.g., FoodSaver or equivalent) allocated as follows:

HCP Protocols p 13
Chocolate for HCP Tasting Panel (60g x 9 Panelists) 540g
USDA 20g
Chocolate for returning to Applicant 100g
Chocolate for retained sample 250g

All samples will be labeled with the HCP Blind Code and the date of chocolate milling and molding.

Storage – Chocolate (Pre-Shipping)
At all times, chocolate will be stored at chocolate warehouse temperatures (17-21°C, 62-70°F) until shipped to the HCP Tasting Panel or returned to the Applicant. Storage will be at least two days but is not expected to be more than four days from date of molding.

LIQUOR AND CHOCOLATE SHIPPING AND LONG TERM STORAGE

Liquor and Chocolate Shipping for Evaluation
The HCP Lab will use overnight shipping with heat protection, frozen packs, and/or any other methods deemed appropriate by the Lab to send samples to the Tasting panel and the USDA. (The HCP Lab based on the location of the Panelists will determine the best carrier. For shipments to Venezuela and Trinidad, FedEx is the preferred carrier due to delivery logistics within those countries.) For international shipments, packages will be labeled “research samples for evaluation” or something similar to avoid being held at customs or charged any duties.

Prior to shipping the HCP Lab or the chair of the HCP Tasting will verify that Panelists are available to receive the sample shipment and to conduct the sensory evaluations in a timely manner.

Storage of Liquor and Chocolate (Following Shipping of Samples)
Following the shipment of liquor and chocolate samples to the HCP Tasting Panel and the USDA, all liquor and chocolate (for returning to Applicant and the retained sample) will be placed at refrigerator temperatures in an odor-free cooler at less than 13°C (50°F) until the HCP Tasting Panel completes its evaluation and samples are returned to the Applicant. (Retained liquor and chocolate samples may be disposed of following the same steps as beans in the previous protocol.)

If storage longer than 2 (TWO) months from date of milling is expected, samples will be transferred to odor-free frozen storage for long term holding. Any sample stored under these long-term conditions will be equilibrated to room temperature prior to opening the container/vacuum-sealed bag.

HCP Protocols p 14
HCP TASTING PANEL RECEIVING AND STORAGE OF SAMPLES

Upon receipt of samples, if samples have been shipped with frozen packs, the HCP Panelist will opened the package and remove the samples BUT keep them in their sealed containers and allowed to equilibrate to room temperature. No sample will be opened when cold temperatures would allow any moisture condensation.

Panelists will store samples during this time at ambient conditions (air conditioned room temperature). If ambient conditions are too warm for the chocolate and pose risk of melting or bloom, then an odor-free refrigerator or wine cooler will be used to store the chocolate.

Panelist evaluation MUST BEGIN AT LEAST ONE WEEK from the completion of chocolate milling. Panelists determine their own schedule for the evaluation of the samples but will attempt to provide turn around of the evaluations within four weeks of receipt of the samples.

HCP Tasting Panel Evaluation Procedures are covered in the next protocol.
HCP TASTING PANEL EVALUATION PROTOCOLS
FOR EVALUATION AND HEIRLOOM DESIGNATION

The international HCP Tasting Panel is currently made up of nine experts from six countries with a minimum of 15 years' experience in chocolate—all of whom have all served as professional evaluators of cacao bean flavor and give a wide field view of the cacaos of the world, the cocoa supply, and fine chocolate production across the globe. Since these Panelists have established approaches to evaluating liquor and chocolate made from that liquor, the HCP Tasting Panel evaluation protocol initially retains the uniqueness of these approaches.

EVALUATION

The HCP Tasting Panel's initial sensory evaluations of liquor and chocolate samples will be in the format they currently use. Panelists will then translate their evaluations into HCP global scores for flavor, write short written evaluations of the liquor and chocolate IN ENGLISH, and make a Yes/No vote for Heirloom designation based on this scoring and evaluation.

HCP Panelists will enter their scores, written evaluations, and recommendations in the HCP Database. (If the Database is unavailable or offline, the Chair of the HCP Tasting Panel will compile the results into a single review and circulate it to the Panel.)

Panelists will conduct all evaluations independently and only discuss each other's assessments after the entire Panel's evaluations are complete. While the names of the HCP Tasting Panel are public, Panelists' scores, evaluations, and recommendations will be blinded; Applicants will only be able to see unattributed individual scores, chocolate and liquor flavor and evaluations, and recommendations.

GLOBAL SCORING

In addition to a written Sensory Evaluation of Liquor and Chocolate, Panelists will make two standard attribute evaluations from 1-10 (10=maximum) for:

- Overall Flavor (Quality and Balance); and
- Unique Flavor (distinctive or unusual flavor profile of long term value to the community of cacao worthy of preservation).

HCP Protocols p 16
HCP RECOMMENDATION – YES/NO

Based on scoring and evaluation, each Panelist will cast a Yes/No vote for Heirloom designation.

While individual scores should play a part in making that designation, Panelists are NOT required to correlate their recommendations to a score (i.e., one Panelist could score a sample a “5” and another a “9” and both could vote yes, no, or split on Heirloom designation).

DETERMINATION OF HCP STATUS/NOTIFICATION

AFTER the evaluations are received, the Panel Chair will schedule a conference call to review the results with the Panel and prepare a final report. Full Panel participation in this call is preferable but not mandatory. Upon completion of this call and report, the Panel Chair will notify the HCP and the HCP office will notify the Applicant.

Supermajority Vote FOR Heirloom Designation
If a supermajority (70% or more) of the HCP Tasting Panelists vote yes, the sample will receive HCP designation as Heirloom flavor.

Majority but not Supermajority Vote FOR Heirloom Designation
If a majority but not a supermajority recommendation is made for Heirloom designation or the Panel is split, the HCP Tasting Panel Chair will take one or both of the following steps:

- If any Panelists were unavailable for the initial evaluation but are now available in a reasonable time frame to make an evaluation, the Panel Chair can hold the final result until one or more of those Panelists make an evaluation. If the recommendation(s) create(s) a supermajority or minority vote for Heirloom designation, the Panel Chair will follow the steps outlined above.
- If no Panelist is missing or missing Panelists are unavailable, AFTER the evaluations are received, the Panel Chair will schedule a conference call to review the results with the Panel and prepare a final report. The Panel Chair during the Panel discussion will see if any Panelist wants to re-taste the beans based on the discussion. If a re-tasting results in a Panelist vote for designation that creates a supermajority, the Panel Chair will follow the steps outlined for the supermajority. (Only the final consensus of the Panel will be made public.) If the Panel remains unchanged, the Panel Chair will take the steps in that follow.

HCP Protocols p 17
Simple Majority, Tie, or Minority Vote Against Heirloom Designation
If a simple majority of the HCP Tasting Panel votes yes, the Panel is tied, or a minority vote for Heirloom designation, the sample will NOT receive HCP designation as Heirloom flavor but will receive a score from the Panel.

If the Panel perceives that the beans display the POTENTIAL for heirloom, regardless of whether there are any processing issues, the Panel may vote to allow the Applicant to re-submit the beans for re-evaluation under the rules under “Evaluation Troubleshooting.”

Upon completion of this call and report, the Panel will notify the HCP and the HCP office will notify the Applicant.

OFFICIAL DESIGNATION OF HCP STATUS/NOTIFICATION
While Heirloom designation by the HCP is not contingent on genetics (unless a problem with the beans is detected – see “Troubleshooting” section that follows), official designation as Heirloom flavor IS CONTINGENT on a field visit by the USDA or its representative to gather leaf material from the marked trees and verification/review of the fermentation process. Ideally, this will be done during production but always in a reasonable amount of time to not unnecessarily delay the announcement of the designation or Applicant’s production and marketing of those beans.

If upon site visit, any beans are found or suspected to be in violation of any of the HCP Submission Protocols at any time during or after this field visit, the HCP will withhold HCP Heirloom designation pending further discussion by the Tasting Panel, Lab, and Board.

EVALUATION TROUBLESHOOTING
Perceived Postharvest Processing Problem/Vote for Resubmission
If the HCP Lab or Panelists perceive a failure in the sample due to postharvest processing AND feel that the liquor and chocolate display some desired attributes, the Panel will recommend the beans be resubmitted for re-evaluation by the Applicant as soon as new beans are available.

The HCP will allow for ONE resubmission per Application – provided the Applicant wants to have its beans re-evaluated by the HCP. Regardless of the Applicant’s decision, it will still receive a full report of the original evaluation.

Applicant will be responsible for submitting the beans for re-evaluation, but the HCP will NOT require an additional application fee. Re-submitted beans

HCP Protocols p 18
must come from the same trees as the original submission. If the Applicant decides not to re-submit, the evaluation of the beans by the Panel will be submitted to the HCP as the final evaluation.

Perceived HCP Lab Processing Problem
IF in the unlikely event Panelists perceive a failure in the sample due to the processing of the beans into liquor and chocolate by the HCP Lab AND feel that the liquor and chocolate have reasonable potential for displaying HCP desired attributes, those Panelists will immediately inform the Panel Chair and may request another sample of liquor and chocolate along with the beans be re-sent for evaluation, if needed, to make a final recommendation. IF after re-evaluation Panelist(s) detect the same problems, the Chair will review the comments and rationale and convene a Panel discussion as appropriate.

IF a Panelist perceives a failure in the sample due to processing of the beans into liquor and chocolate BUT feels that the sample DOES NOT have Heirloom potential, no action will be taken and the Panelist will vote NO.

Perceived Fermentation Alteration
IF the HCP Lab or any Panelist perceives a sample has been altered in any way during fermentation – a direct violation of the HCP Submission protocols – AND feels that the liquor and chocolate display HCP desired attributes, the Panelist will immediately inform the Chair and the HCP Lab and the Chair will convene a Panel discussion as appropriate and decide what, if any, action to take. The HCP Tasting Panel Chair may recommend Heirloom designation be withheld pending a site visit AND genetic testing.

IF the Lab or a Panelist perceives the sample has been altered in any way AND feels that the sample DOES NOT have Heirloom potential, no action will be taken and the Panelist will vote NO.

HCP Panelist Unavailable
The HCP strives to have all Panelists provide evaluation input but recognizes there may be times when, due to travel, holidays, or emergencies, Panelists may not be available for an extended period of time.

The HCP Tasting Panel Chair will be responsible for determining whether a panel will proceed at these times or whether it will wait to send out samples. IF the decision is made to wait, all samples whether at the HCP Lab or in the hands of Panelists will be frozen.

In no case will the panel proceed with fewer than five Panelists.

HCP Protocols p 19
HCP PROTOCOLS FOR FIELD SITE VISIT & COLLECTION OF SAMPLES FOR GENETIC ANALYSIS

Official designation as Heirloom or fine flavor by the HCP IS CONTINGENT on a visit to the Applicant’s field site by a USDA/ARS representative to gather leaf material from the marked trees that produced the HCP sample to determine their genetic makeup, supplemental data on those trees, and verification/review of the fermentation process. The HCP will coordinate this visit as soon as designation is final. Ideally, this visit will be done during production but most importantly in a reasonable amount of time to not unnecessarily delay the announcement of the HCP designation. The USDA will provide all materials for sampling and send them to the representative prior to his/her visit.

IF at any point during the site visit, the Applicant is found or suspected to be in violation of any of the HCP Submission Protocols or the representative has any concerns about the sampled trees, the representative will document them and inform the USDA/ARS and HCP immediately. If a violation is suspected that would affect designation and cannot be resolved during the site visit, the HCP will terminate the visit and withhold Heirloom designation pending further discussion by the Tasting Panel, Lab, and Board.

FIELD SITE VISIT PROCESS FOR TREE SAMPLING

When the Applicant takes the representative to the trees used for the HCP bean samples, a sample will be collected for genetic analysis from the most recent fully expanded leaf from no more than 46 trees. Data will be taken for each sampled tree and if trees are not marked or clearly marked, the Applicant will mark them with the number 1-46 corresponding to the number of each leaf sample.

- Only leaves that appear to have no browning or any signs of disease or pests will be taken.
- Only half of one leaf from each tree will be harvested and that leaf will be placed into a Ziploc type plastic bag with a desiccant. (Leaf samples will be completely dry in less than 24 hours and will remain green.)
- Trees sampled will be assigned a code and the sample bags will be labeled to indicate the tree’s code.

The representative will also gather supplemental data about each tree (tree height, pod characteristics, bean color, yield, tree age, tree origin, disease resistance/susceptibility, etc.) and submit this information along with the leaf

HCP Protocols p 20
samples using the HCP data sheet.

FIELD SITE VISIT PROCESS – POSTHARVEST PROCESSING

The Applicant will show the representative all aspects of the postharvest processing used to process the beans submitted to the HCP.

The representative will gather basic information regarding the processing (fermentation times/temperatures, drying methods, etc.), as well as production and agronomic data (fertilizer use, soil characteristics, topography, climate, etc.). A list of basic information to collect in addition to other observations will be provided to the representative.

Photos of the process, unless proprietary, should be taken. FIELD REPRESENTATIVE WILL ASK IF ANY PART OF THE PROCESS IS PROPRIETARY BEFORE TAKING PICTURES. GPS of the farm (not the cooperative) must be taken.

SHIPPING OF SAMPLES

United States Animal and Plant Health Inspection Services (APHIS) guidelines will be followed to prevent the importation and release of plant pathogens.

The healthy dried leaf samples will be prepared for shipment to the USDA by the representative, including the APHIS permit (provided by the USDA) that will be placed in the package. The Applicant will then send the package to the USDA and submit the shipping receipt to the HCP for reimbursement.

Samples should be sent to:
Lyndel W. Meinhardt
USDA/ARS Sustainable Perennial Crops Lab
Building 001 Rm 222, BARC-WEST
Beltsville, MD, 20705-2350
Tel 301 504 1995 Fax 301 504 1998

Photos and information gathered should be emailed to Dr. Lyndel Meinhardt:
lyndel.meinhardt@ars.usda.gov

All submitted plant samples will be subject to quarantine and inspection upon arrival in the United States. If APHIS inspectors identify any signs of plant disease on the samples, the samples will be destroyed at the port of entry.

HCP Protocols p 21
PROCESSING OF SAMPLES & NOTIFICATION

Once the samples arrive at the USDA, they will be processed and sent to the DNA testing facilities for analysis.

DNA will be extracted and analyzed with standard markers and compared to all known reference types. Parentage and sibling analysis will be done to determine what groups, hybrids, or clones are involved in the genetic makeup of the sampled trees.

The results of the genetic analysis will be sent to the Applicant and placed into a secure part of the HCP database for a period of five (5) years. This database will be the repository for genetic diversity population analysis, GIS population locations, bean quality traits, and flavor analysis. After that period it will be incorporated into the HCP public database. Until then, the public database will be the storage area for all of the international reference types, and after the designated time period, for all cacao types designated as Heirloom.

Once the samples are received and tested, the DNA matches (within reason) the DNA of the originally submitted beans, and Lyndel Meinhardt signs off on the report from the USDA representative, the HCP will provide the Applicant with the “Permission to Disclose” form in order to proceed with the announcement of Heirloom designation.
Annex 9

Protocol for use of HCP mark and Logos
PROTOCOL FOR USE OF THE HCP MARK & LOGOS

PLEASE READ THE FOLLOWING PAGES CAREFULLY TO ENSURE COMPLIANCE WITH THIS PROTOCOL

Long-term and increased commercial success is part of the Heirloom Cacao Preservation Initiative (HCP) mission and paramount to its success (which is why the HCP only designates trees currently producing cacao for sale). Growers are in control of the HCP Mark, not manufacturers or intermediaries, even if those manufacturers/intermediaries have sponsored a grower and must be approved by the grower, or sponsoring manufacturers or intermediaries of those growers, of the designated beans. Use of the HCP Designation Mark (center, above), HCP logo (left), and FCIA logo (right) for promotional and sales purposes requires strict compliance to the following protocol before any mark can be used for the sale of beans and manufacture of chocolate.

The HCP is not a certifying agency per se; the HCP Mark (middle, above) is recognition of valued flavor via a designation of a specific stand/orchard/farm of trees from which the beans were submitted and leaf samples were taken for genetic analysis. Heirloom designation is transferrable to trees propagated immediately adjacent to the HCP designated stand in and in rough proportion to the individual genetics of that stand provided it is owned or operated by the grower at the same location (no more than 20-meter separation – more requires prior approval by the HCP. Heirloom designation does not apply to similar trees grown in the vicinity or any region not owned and operated by the grower no matter how close-by they are and no matter how similar their genetics.

Improper use of the HCP Mark may result in suspension of Heirloom designation pending investigation. While the HCP will make every endeavor to monitor Heirloom designations after future harvests, this protocol is designed to prevent improper use of, and false claims about, HCP Heirloom designations.

USE OF PRESS RELEASE & INFO POSTED ON THE HCP/FCIA WEBSITE

The HCP Mark and Logo as well as the FCIA logo are approved for immediate use by growers, as well as sponsoring manufacturers or intermediaries of those growers,
in conjunction with the approved press release and links to any information posted on the FOIA/HCP web site, including Tasting Panel notes and USDA genetic profile as they relate to the specific trees named in the designation certificate. A digital version of the certificate may also be placed on the web site of the grower. The HCP allows designees to adapt the release for local/regional/international purposes with written permission from the HCP. All members of the HCP will be available for comment on those adapted releases.

**THE FOLLOWING PERMISSIONS MUST BE RENEWED ANNUALLY**

**DIRECT GROWER/APPLICANT USE OF THE HCP MARK**

All beans used directly by the applicant – for direct sale or for use in direct manufacturing – with traceability to and from the EXACT farms/orchards/stands designated Heirloom by the HCP AND that have NOT been mixed with any other non-Heirloom designated cacao may carry the HCP logo.

If transferring the designation to additional stands, the HCP may require a signed letter IN ADVANCE OF ANY SALE OR MARKETING from the grower (hard copy by mail or scanned copy via email okay) requesting the use of the HCP Mark and Logo that states approximately how many kilos of that transferred cacao will be made available for commercial sale that year and that the cacao being sold under that mark.

**INDIRECT USE OF THE HCP MARK FOR SALES & MARKETING**

The HCP requires a signed letter from brokers, traders, reps, associations, cooperatives, and manufacturers NOT directly controlled by the applicant IN ADVANCE OF ANY USE (hard copy by mail or scanned copy via okay, an email is not) requesting the use of the HCP Mark and Logo. This letter must state and verify that the cocoa mass of the chocolate or products being sold under that mark is exclusively from Heirloom beans and how many tons are being used in production. (The HCP takes no stand with manufacturers on roasting, sugar or other formulation parameters, inclusions, or percentage.)

The HCP will then authorize in writing the use of its marks and logos for this Heirloom designation and kilos stated and provide high-resolution images of each. (Note: A manufacturer that wants to blend Heirloom beans with non-Heirloom beans must receive clearance from the HCP to do so and percentages must be clearly noted on the packaging.) If beans are not directly traded, a statement must also be received by the HCP from any intermediaries (brokers, traders, reps, cooperatives) that no mixing was done and that the beans passed directly from the grower to the intermediaries.
Annex 10

The use of an optimised organoleptic assessment protocol to describe and quantify different flavour attributes of cocoa liquors made from Ghana and Trinitario beans

Darin A. Sukha · David R. Butler · Pushmanarthan Umaharan · Emma Boul

Received: 29 May 2006 / Revised: 12 October 2006 / Accepted: 8 December 2006
© Springer-Verlag 2007

Abstract An optimised protocol for organoleptic assessment of cocoa liquors was developed and used to determine what were the flavour attributes and sensory differences between samples of Ghana beans (normally classified as ‘bulk’) and bean samples from local commercial clones and estates in Trinidad (normally classified as ‘fine or flavour’ cocoa). The optimised protocol was validated by independent sensory assessment carried out at Masterfoods, UK. Trinitario samples from four local commercial clones and five commercial estates from Trinidad were investigated for their liquor quality, over three crop years using the optimised sensory panel and an appropriate sensory design with replications. The optimised protocol was not only able to consistently differentiate between the ‘bulk’ and ‘fine or flavour’ cocoa types but also able to consistently quantify the level of each attribute in genotypes, over replication and season. This method was used to delineate quantitative differences in flavour attributes among cocoa genotypes as well as to determine the influence of environment on cocoa flavour profiles. The optimised organoleptic protocol involves a detailed description of primary and secondary processing of beans, preparation of liquor, panelist selection, training, sensory design and data analysis methods. The optimised organoleptic protocol provides a robust methodology to improve cocoa quality by selection of genotype and environment.

Keywords Fermentation · Local clones · Commercial estates · Sensory evaluation · Panelists

Introduction

Cocoa (Theobroma cacao L.; family: Malvaceae) [1] originated as an understory tree species in the tropical rainforests of the upper Amazonian region of South America. Three different morpho-geographical groups (Criollo, Trinitario and Forastero) have been recognised within the species, T. cacao, based on genetic origin, pod morphology and size, colour and flavour of beans [2].

From an international marketing perspective, cocoa beans are categorised as ‘bulk’ or ‘ordinary’ and ‘fine or flavour’ cocoa types. Bulk cocoa beans originate from Amazonian Forastero varieties (the industry standard is cocoa beans from Ghana), and are usually used in making main stream milk chocolates and chocolate confectioneries with other fillings. Fine or flavour cocoa beans, which fetch a premium over terminal prices paid for bulk cocoa [3], normally originate from Criollo and Trinitario cacao, each having its own characteristic flavour. These are mainly used to make premium plain dark chocolates and couvertures.
There is a need within the cocoa industry for protocols and standards that can be used to adequately differentiate ‘bulk’ from ‘fine or flavour’ cocoa at the point of marketing. This is particularly important in countries where mixed production of both types occurs. It is difficult to define flavour properties until the product has been processed, since certain flavour properties develop during processing; and can be assessed only by sensory evaluation.

The lack of an optimised standard protocol for general cocoa flavour assessment and profiling by trained sensory panels has hindered effective collaboration between research institutions and meaningful input from chocolate manufacturers. The protocols currently used by chocolate manufacturers only identify specific defects in cocoa beans and liquors, mainly smoky and mouldy, but broad flavour profiling is not usually done [4, 5]. Additionally, the protocols and vocabulary used in sensory evaluation differ greatly between chocolate manufacturers and research institutions. This has been an impediment to effective research aimed at improving cocoa quality and flavour. A standardised protocol for fermenting, drying and preparing cocoa liquors from small cocoa bean samples as well as a general procedure for organoleptic evaluation was first developed by Clapperton et al. [6]. This was done in collaboration with the chocolate manufacturing industry, and was adopted for local conditions by the Cocoa Research Unit, Trinidad [7]. Key elements pertaining to sensory panel training and experimental design, as well as, data analysis techniques to produce statistically rigorous results had, however, still not been fully optimised at the time of the passing of Clapperton. The application and repeatability of the organoleptic method in distinguishing between ‘bulk’ and ‘fine or flavour’ cocoa types had also not been tested.

Using the protocol of Clapperton et al. [6] for small-scale sample processing and liquor preparation as a starting point, this study aimed to develop an optimised method of sensory evaluation. There were three main objectives: (1) To optimise a method for sensory evaluation, (2) use the method to distinguish between liquors made from Ghana beans (considered a ‘bulk’ type) and Trinitario beans from Trinidad (considered ‘fine or flavour’ type) and (3) to detect differences in flavour between crop years. The study was conducted as part of the CFC/ICCO/INIA project to establish physical, chemical and organoleptic parameters to differentiate between bulk and fine or flavour cocoa.

Materials and methods

Genotypes

Four locally developed commercial clones [Trinidad selected hybrids (TSH)] identified with CCL accession codes, were used in the study and are referred to as ‘local clones’. The identities of every tree used in the study were verified using random amplification of polymorphic DNA or simple sequence repeats. Cocoa beans were collected from identified trees of each clone, over 3 years, and processed using the standardised protocol of Clapperton et al. [6], at one processing location. A homogenous batch of fermented and dried seeds from West African Amelonado beans from Ghana was used over the three crop years as a reference.

Commercial estates

Random samples of fermented, dried cocoa beans were taken from five different commercial estates spread over a wide geographic area in Trinidad, over three crop years. Mixed Trinitario germplasm [TSH and Imperial College Selections (ICS)] are typically grown on these estates. Fermentation on the different commercial estates followed the standard practice for Trinidad of 168 h with turning after 48 and 120 h. The drying method and duration varied between the different commercial estates from sun drying to artificial drying using a diesel fired dryer. However for each estate the same drying method was used over the three crop years. Samples taken from the different commercial estates were alphanumerically coded.

Primary processing

Three kilograms of beans were extracted from healthy, fully mature pods of each selected local clone and placed in labelled 24 cm × 80 cm nylon net bags of 10 mm mesh and 0.7 mm thread diameter for micro-fermentations. The bags were buried 30 cm deep into ca 2,000 kg of wet cocoa, contained in a sweat box. The fermentation mass consisted of beans from commercial Trinitario trees. The sweat box was constructed with wood, with dimensions of 155 cm (W) × 150 cm (L) × 102 cm (D), and a slatted floor to provide aeration and drainage for optimal fermentation. The beans were covered with banana leaves and jute sacks and left to ferment for 168 h with turning after 48 and 120 h. The buried sample was turned by holding the ends of the
nylon net bags and shaking the bag to mix the beans inside, the nylon net bags were then buried again 30 cm deep in the sweat box. After fermentation the beans were removed from the nylon bags and spread out in individually labelled wooden drying trays (60 cm × 60 cm × 10 cm) for sun drying. During drying the beans were turned every 2 h and dried for a maximum of 8 h per day if the weather permitted. If the moisture content of the beans had not reached 6-7% after 120 h of sun drying, drying was completed in a mechanical convection oven set at 35 °C (Shell Lab L300 FX, Sheldon Manufacturing Inc., USA). The same drying method was used in each of the crop years.

After drying, the beans were stored in quarter sized jute sacks, made from similar material to those used for commercial bean shipments. These were treated with vegetable oils. To avoid problems with moths, mould and other infestations the jute sacks were placed in plastic bags and sealed in plastic buckets with airtight lids. These buckets were then stored in an air-conditioned room at 19 °C.

Secondary processing

Secondary processing includes roasting, breaking and winnowing, coarse grinding and milling of the bean samples into cocoa liquors in preparation for organoleptic evaluation. For each sample, 330 g of dry beans from a coned and quartered sub-sample were roasted in a mechanical convection oven at 140 °C for 30 min. The roasted beans were broken into ca 0.2-0.5 cm sized pieces by passing them twice through a cocoa breaker (John Gordon International, UK). Nibs were separated from the shell using a cocoa winnower (John Gordon International, UK). Coarse milling of the nibs was achieved by placing 36 g at a time in a Toastmaster Coffee Mill (Toastmaster Inc., USA) and milling until the sample was coarsely ground. These nibs were then stored in sealed food grade 2 L capacity plastic containers. To prepare the cocoa liquor, the ground nibs were gradually seeded into a mortar and pestle mill (Model 0, Pascal Engineering Co., UK) which was initially heated to approximately 40 °C with a heat gun (Black and Decker Inc., USA). The mill was run for 90 min from the last addition of nibs, which allowed a smooth liquor to be formed. During milling the heat gun was turned on for 2 min at 10-min intervals to maintain the mortar temperature at approximately 40 °C. After milling the cocoa liquor was stored in 120 ml capacity sterile specimen containers at -6 to -8 °C prior to organoleptic evaluations.

Organoleptic evaluation

Panel training and sample evaluations were conducted in a quiet, air-conditioned room with the experimental designs, test methods and statistical analyses outlined below.

Panellist selection and training

Potential sensory panellists were initially screened to assess their availability and general attitude towards taste testing through a written questionnaire, evaluation continued via a series of tests that increased in complexity. These included firstly: identification of basic tastes using aqueous solutions such as sweet (sucrose at 5.0 g/500 mL), bitter (quinine chloride at 0.072 g/500 mL), salt (sodium chloride at 0.8 g/500 mL), acid (citric acid at 0.25 g/500 mL), astringent (maleic acid at 0.25 g/500 mL) as well as flavour attributes associated with cocoa liquor (fruity and floral at a concentration of 2 mL/500 mL of kola flavour and orange blossom water, respectively). This was followed by identification of bitter, acid and astringent tastes at threshold level concentration using quinine chloride, citric acid and maleic acid (at 0.009, 0.1 and 0.15 g/500 mL, respectively) in solutions to gauge the sensitivity of individuals to these attributes. Panellists who were successful in the identification of basic tastes and threshold level concentration tests were selected for continued training with an introduction to flavour attributes of cocoa liquors and vocabulary generation exercises.

The selected panellists were trained to associate specific flavour descriptors for cocoa liquors with either previous taste experiences or with flavour references that were provided so that all panellists agreed on the same sensory language. Eight flavour attributes were considered in the training exercise viz. cocoa, acid, astringent, bitter, fruity, floral, nutty and raw/beany/green flavours. In addition identifiable off-flavours such as smoky, hammy, mouldy and unfermented were included. Panellists were also encouraged to identify any other ancillary flavours or defects that were apparent in the cocoa liquors in a section for ‘other’ flavours. The intensity of each flavour was scored initially through paired comparison tests combined with ranking of samples according to different intensities of a particular flavour attribute. This was followed by blind profiling with hidden reference liquors [8-10]. Reference liquors were used to check panellist consistency between repetitions during training and evaluation sessions.

© Springer
Liquor evaluation

Cocoa liquor samples were removed from frozen storage and melted in a water bath (Labline Instruments Inc., USA) set at 45 °C. The liquors were mixed thoroughly to correct any fat separation before evaluation.

Liquors were assessed by a panel of at least six trained individuals using a factorial statistical design that incorporated hidden reference liquors. Liquors were coded with three-digit numbers and randomised over three repetitions to minimise carry-over effects. No two panellists received liquors in the same order for any given evaluation session and a maximum of six liquors were tasted in any one session to prevent panellist fatigue. The coded samples were presented in 20 mL glasses placed in a hollowed-out aluminium block, which had been pre-heated to 55 °C to keep the samples warm during tasting. Panellists were asked to place about 1 mL of cocoa liquor on a teaspoon in their mouth and keep it there for 20 s. During this time the different attributes making up the flavour profile become apparent. Panellists were instructed to look for different flavour attributes at three contiguous time intervals viz. initial front flavour notes, middle flavour notes and residual end flavour notes since some flavours either appeared and disappeared very quickly or were easily masked whilst other flavours could linger for a longer time.

Sensory profiles were recorded for the nine cocoa flavour attributes using 10-cm line scales with a possible range of scores from 0 to 10 where the higher numbers denoted stronger flavour intensities. Panellists had the option to expectorate or swallow the sample but in all cases they were instructed to rinse their mouths out with water and cleanse their palate with a biscuit cracker between each sample. The performance of the sensory panel was optimised during liquor evaluations by including a hidden reference sample in the sensory design made with beans from Ghana. The Ghana reference also served as a “bulk” flavour reference sample.

Independent assessment

Representative cocoa bean samples from the second and third crop years were assigned random three-digit codes and sent to Masterfoods, UK for independent organoleptic assessments. Liquor preparation at Masterfoods, UK involved roasting the beans at 145 °C for 30 min on the centre shelf of a forced air oven. The beans were broken and winnowed and the cocoa liquor was made using a mortar and pestle mill with the mortar pre-heated to 60 °C and a milling time of 75 min.

Quantitative descriptive analysis profiling techniques were used for organoleptic assessment of the liquors [11] with open line scales for scores ranging from 0 to 15 for the following flavour attributes: cocoa, acid (acetic), bitter, burnt, winey, brown fruit, floral, nutty, acid (fruity/citrus), astringent and other flavours that included coconut and farmyard. The ‘acid’ flavour descriptors used in the Trinidad panel incorporated both ‘acetic’ and ‘fruity/citrus’ descriptors used by the Masterfoods, UK panel whilst ‘fruity’ incorporated both ‘winey’ and ‘brown fruit’ notes identified by the panel at Masterfoods, UK. The liquors were evaluated in either duplicate or triplicate (where quantities allowed) by a panel of eight or nine screened and trained panellists.

Data analysis

Individual flavour attribute scores from the profiling forms were entered into a data template in Microsoft Excel. Mean flavour profiles and the standard errors (SE) of the mean were calculated. Variance components were investigated with Genstat 4.24 DE (VSN International) using restricted maximum likelihood (REML) variance estimation to determine the significance of treatment effects and interactions. Principal component analysis (PCA) was performed on the pooled data using GenStat 7.0 (VSN International) and the freeware programme PAST version 1.39 [12], graphical representation was carried out in Microsoft Excel and PAST.

If significant panellist x flavour attribute interactions occurred, these were reduced or eliminated where possible by removing scores for individual panellists for specific traits that varied by more than three points on the sensory scale between repetitions. Results from the sensory panel were further optimised by using REML variance estimation where the possible random effects caused by different panellists present on the sensory panel over the three crop years were not weighted in the analysis.

Results

The effectiveness of the optimised protocol for sensory evaluation was demonstrated by the ability of the panel to differentiate between various cocoa liquor samples. These included samples considered to be ‘bulk’ and ‘fine or flavour’, those from different genotypes and samples from different crop years.
Panel differentiation of ‘bulk’ versus ‘fine or flavour’ cocoa

Restricted maximum likelihood variance analysis of the organoleptic data from the Trinitario local clones and the Ghana sample revealed that astringency and ‘other’ flavours were the only flavour attributes that did not vary significantly (P > 0.05) among these samples. The magnitude of the significance values for the remaining flavour attributes are presented in Fig. 1.

It is evident from Fig. 1 that the panel was able to consistently differentiate between the local clones from the Ghana sample. The Ghana sample was characterised by having the highest cocoa and nutty flavours (roasted nut) with moderate bitterness and astringency and very little acidity, fruity, floral and raw/beany green flavours. Over the three crop years, acidity and bitterness were the only two flavour attributes in the Ghana sample that showed any significant (P ≤ 0.05) panelist × flavour attribute interactions (data not presented). Figure 1 also showed that in all instances the local clones had consistently higher fruity and acid scores. The very low SE values (maximum of 0.29) highlight the consistency of the sensory panel over the duration of this study.

The PCA indicated that two principal components explained 67.9% of the variation in the data. The PCA plot of average flavour profile data over three crop years (Fig. 2) also indicates that cocoa flavour, bitterness, nutty and raw/beany/green were consistently associated with the Ghana sample, while fruity, acid and astringent attributes were mainly associated with CCL 202 and 217 and floral flavour with CCL 200 and sometimes with CCL 201. It was possible to infer from the factor scores that cocoa, nutty, raw/beany/green and bitter flavour attributes were inversely related to fruity, acid and floral flavours, respectively.

Effect of genotype and crop years on flavour attributes

Table 1 summarises the significance of crop year, clone and crop year × clone interactions on flavour attributes of the local clones using REML variance estimation. The effect of crop year was significant (P ≤ 0.001–0.05) for all flavour attributes except raw/beany/green.

Cocoa and floral flavours varied significantly (P ≤ 0.001) between the different local clones with CCL 201 and CCL 200 having the most cocoa and floral flavours, respectively (Fig. 1). Astringency, fruitiness and raw/beany/green flavour attributes did not vary significantly between the four local clones.

There were significant local clone × crop year interactions for cocoa, nutty, raw/beany/green (P ≤ 0.01–0.05) and acid (P ≤ 0.001) flavours. Although the crop year effects were significant for fruity and floral flavour attributes there were no crop year × clone interaction for these two attributes.

Commercial estates and flavour attributes

The flavour profiles of samples from individual commercial estates averaged over three crop years are compared to the Ghana sample in Fig. 3a. The average flavour profiles for each crop year from all the commercial estates pooled together are shown in Fig. 3b.
Table 1 Restricted maximum likelihood variance estimation of flavour profiles for local clones over all three crop years combined and local clone × crop year interactions

<table>
<thead>
<tr>
<th>Flavour attribute</th>
<th>Significance</th>
<th>Crop year</th>
<th>Local clone</th>
<th>Local clone × crop year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Acidity</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Astringency</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Bitterness</td>
<td>***</td>
<td>**</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fruity</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Floral</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Notty</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Raw/beany flavour</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS not significant (P > 0.05)

*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

Restricted maximum likelihood analysis showed that all nine flavour attributes varied significantly (P ≤ 0.001–0.05) among the individual commercial estates (Fig. 3a) whilst all the flavour attributes except cocoa differed significantly (P ≤ 0.001–0.05) between the three crop years (Fig. 3b). There were significant estate × crop year interactions (data not presented) for cocoa, acid, fruity, floral, nutty (P ≤ 0.05–0.001), fruity and ‘other’ flavour attributes (P ≤ 0.001).

Commercial estate E3 consistently had the highest floral score while E2 and E3 had the highest fruity score over the three crop years. The acidity was consistently the highest in cocoa liquors prepared from E2, and consistently the lowest in E1 and E5. Commercial estate E4 had a dominant smoky (‘other’) off-flavour note. All the commercial estates had significantly (P ≤ 0.001) larger values for acidity and fruitiness than the Ghana sample.

Acidity, fruity and floral notes were heightened at the expense of nutty, raw bean and other off-flavours in the 2003 samples compared to 2002 and 2004. Astringency and bitterness were highest in 2002 and lowest in 2004.

Independent assessment of flavour

Results from the independent assessment of flavour attributes done at Masterfoods, UK are presented in Fig. 4 as a PCA plot of panel means. Two principal components accounted for 84.8% of the total variation between different flavour attributes of the samples. There was a clear separation of the Ghana sample from the local clones and most of the commercial estates. The flavour attributes associated with the local clones were mainly winey, floral, acid (fruity/citrus) and acid (acetic) flavours with CCL 200 and CCL 202 being associated with floral flavours. The Ghana sample was associated with woody and burnt flavours and there were low panel mean scores for winey and acid-fruity/citrus flavours in the Ghana sample. There was also a greater spread of different flavour attributes across the commercial estates compared to the CCL results with estate E3 being associated with aq, and floral flavours. Estate E4 was associated with woody and burnt flavors whilst E2 was associated with bitter, astringent, farmyard and acetic acid flavour attributes. Estates E1 and E5 were both associated with coconut and nutty flavours.
flavour' cocoa at a premium price in the international market. The flavour profiles for these local clones show that they consistently had higher fruity and acid scores than the Ghana sample which was mainly dominant in cocoa and nutty scores. There were however significant differences among the local clones in the extent to which the floral flavour in particular was expressed. The floral note was pronounced in CCL 200. The profile trends in the local clones have been observed consistently over the different crop years [13–15] and highlight strong organoleptic differences that differentiate the Ghana sample ('bulk') from the Trinitario samples ('fine or flavour').

The contribution of genotype to flavour first highlighted by the work of Clapperton et al. [6, 16–18] was demonstrated again in this study. The fruity, sometimes floral with moderately acid flavour attributes of the local clones and commercial estates are in sharp contrast to the cocoa and nutty flavour attributes of the Ghana sample. The strong contribution of genotype to flavour is demonstrated also in Figs. 2 and 4 where both taste panels at the Cocoa Research Unit, Trinidad and at Masterfoods, UK were in agreement that local clone CCL 200 displays very strong floral characteristics over and above any crop year effects. Indeed there were no significant local clone x crop year effects for astringent, bitter, fruity and floral flavour attributes in Table 1. The local clones were harvested from the same field each year and processed at the same location to minimise the possible effects processing and tree location on the final flavour of the selected genotypes.

Fermentation in cocoa arises by the successive action of various micro-flora viz. yeasts, lactic acid bacteria and acetic acid forming bacteria. The yeasts ferment the sugars of the pulp to produce ethanol and carbon dioxide which makes the fermentation matrix anaerobic and encourages the proliferation of lactic acid bacteria. Ethanol and acetic acid infiltrate into the cotyledon and together with the concurrent rise in the temperature of the fermenting mass above 44 °C cause death of the cells in the beans. Once the cells die they lyse and the drainage of their aqueous contents facilitates a series of enzymatic and biochemical reactions resulting in proteolysis and subsequent flavour precursor formation of the typical flavours associated with well fermented cocoa [19]. These enzymatic and biochemical reactions reduce astringency and bitterness and promote flavour precursors that give rise to cocoa flavour and other ancillary flavours typical of different cacao genotypes. Cocoa flavour is a basal flavour that is considered to be always present in well-fermented and dried samples. Although, it is easily masked by other
dominant ancillary flavour attributes such as fruity, floral and acid flavours, it commonly re-emerges as a residual flavour at the end of the tasting experience during organoleptic evaluation. This could account for the fact that cocoa flavour was the only flavour attribute that did not vary significantly between the different crop years for the commercial estates and its variation was only mildly significant (P ≤ 0.05) for the local clones. CCL 200 had the lowest score for cocoa flavour, probably due to masking by the dominant floral character of this liquor.

The relative mixture of Trinitario germplasm fermented on the different commercial estates ranged from only mixed TSH varieties (estate E2), to predominantly ICS (E1 and E5) or mixtures of the two types of cocoa germplasm (E3 and E4). The germplasm composition from each estate remained the same every crop year, the fermentation and turning regime remained uniform and the drying methods were similar.

Even though fermentation practices were generally uniform among the commercial estates, drying practices tended to vary more widely and ranged from purely sun drying to a mixture of initial sun drying followed by artificial drying to purely artificial drying. As a result the temperature during drying and the drying time would vary among commercial estates and this may have directly affected the acid flavour attributes of samples dried at the different estates. Drying rate has been clearly linked to the acidic characteristics of cocoa [20–23]. From the drying rates measured at estate E1 and E2 (data not presented) it was observed that the drying rate at estate E2 was on average twice as fast as at estate E1. Estate E5 also has a sun-drying regime similar to estate E1. The results suggest that the drying regime possibly contributed to the noticeably lower scores for acidity observed over the three crop years in E1 and E5 as opposed to E2 (Fig. 3a). Quesnel [24] found that acid acid accumulates inside the bean at the end of fermentation and the internal pH was directly correlated to the acetic acid concentration inside the beans. The more rapid drying rate at estate E2 most likely resulted in the accumulation of acetic acid, trapped inside the beans, via case hardening and internal crusting [25]. Both taste panels were consistently able to identify higher scores for acidity. The estates E1 and E5 were also associated with nutty scores as opposed to estate E2 which is associated with bitter, astringent, farmyard and acetic acid flavour attributes.

The smoky, woody and burnt off-flavour noted detected at estate E4 by both the Trinidad and Masterfoods, UK taste panels (Figs. 3a, 4) is a direct result of a mal-adjusted diesel fired artificial dryer, operated without a heat exchanger. Attempts were made to rectify this problem in the 2003 crop year but it worsened again in the 2004 crop year, increasing the average score for this off-flavour assigned by both sensory panels. This demonstrates the ability of the standardised organoleptic assessment protocol to identify and quantify commercial flavour defects in samples.

Lockwood and Eskes [26], in their study of the relationship between cocoa variety and quality found strong environmental and/or seasonal effects on bean weight, fat content and shell content but concluded that flavour potential was largely a heritable function of genotype. The concept of a crop year or environmental effect on flavour has therefore never been clearly identified and is poorly understood in cocoa. In addition to the sensory data from this study, recent research findings [14] have demonstrated strong environmental and/or crop year effects in the presence and concentration of pyrazine compounds that correlate well to sensory attributes, especially ‘nutty’ and/or ‘cocoa’ flavours.

Having identified the impact of crop year or environmental influences within a particular crop year on flavour, it would be of interest to identify the factors which can either positively or adversely affect flavour. Such considerations were outside the purview of this study but could include those factors that cause subtle variations in the fermentation processes such as the micro-flora in the fermentation mass, changes in micro-flora during fermentation, effects of ambient temperature, water content and amount of mucilage surrounding the beans and the fermentation requirements of different genotypes as they affect the production of flavour precursors. These variations raise a number of questions concerning the relative importance and weighting of genotype, growing environment (climatic, edaphic and xenia effects) and how they interact during fermentation and drying to affect the flavour and quality attributes of cocoa. This study has demonstrated that it is possible to investigate these effects with the optimised organoleptic assessment protocol.

Conclusions

The results of this study highlight the successful application of an optimised organoleptic assessment protocol to identify the genotypic contribution to flavour attributes in liquor samples made from Ghana (normally classified as ‘bulk’ cocoa) and Trinitario beans from Trinidad (normally classified as ‘fine or flavour’ cocoa). The optimised protocol was not only able to identify the major flavour attributes but was
also to consistently quantify the levels of the attributes. The protocol has been used as an effective research tool to identify commercial defects and crop year or environmental effects on the flavour of cocoa liquor. The optimised organoleptic assessment protocol will allow effective collaboration between different sensory panels using similar versions of the protocol. Furthermore it provides the means to generate improved understanding of the relative importance of cocoa varieties and the environment to flavour characteristics.

Acknowledgments The financial support, assistance and collaboration of the United Nations Common Fund for Commodities, Guittard Chocolate Co., USA, Lindt & Sprüngli Switzerland, Masterfoods, UK, The Ministry of Agriculture, Land and Marine Resources (Trinidad), Nestlé Product Technology Centre, UK, Paul Masickand Fruites Ltd (Trinidad), Produe Marketing Associates Ltd (Trinidad), Mr Bruce Lauckner of the Caribbean Agricultural Research and Development Institute, Trinidad and all sensory panels are gratefully acknowledged in this study. The pioneering contribution of the late Dr John Clapperston to this area of organoleptic evaluation and his initial collaboration in this body of work is also acknowledged.

References
Annex 11
The impact of Processing Location and Growing Environment on flavor in cocoa (Theobroma cacao L.) – implications for “Terroir” and Certification – Processing Location study

D.A. Sukha1; D.R. Butler1; E.A. Comissiong2 and P. Umaharan1

1Cocoa Research Centre, the University of the West Indies, St. Augustine, Trinidad and Tobago.
2 Food Science & Technology Unit, Department of Chemical Engineering, the University of the West Indies, St. Augustine, Trinidad and Tobago.
Email: Darin.Sukha@sta.uwi.edu

ABSTRACT

The influence of different processing locations on the flavour and other quality attributes of cocoa investigated over three growing seasons is presented in this paper. Experiments were set up to examine the possible influence of three different processing locations for box fermentations with sun drying, on the flavour of six different cocoa accessions, each harvested from the same field. Processing location effects on the flavour attributes of selected cacao genotypes were also demonstrated with supporting near infrared reflectance spectroscopy (NIRS) results. Additionally, the strong contribution of cacao genotype to flavour, especially in floral flavour attributes, was demonstrated. This superseded the effects of the processing environments in some instances. The sensory evaluation results further supported the successful application of an optimised assessment protocol for training a sensory panel to systematically investigate how processing location can affect final flavour and quality in cocoa. 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.

Keywords: processing location, sensory evaluation, cocoa flavour, terroir

Sub Theme category: Enhancing Quality for Food Security

INTRODUCTION

The genotypic influence on the flavour of cocoa has been well established (Fowler 1994); and it is generally accepted that different cocoa varieties differ in their specific fermentation requirements. Fermentation and drying of beans have traditionally been considered to be of critical importance to flavour and are factors that have received extensive research attention. However, the relative contribution of processing techniques to flavour development is poorly understood and progress in this area has been restricted since we lacked an objective and standardised method of evaluating flavour (Figueira et al. 1997).

According to Enriquez (1993) environmental effects (mainly soil and climate) and more so post-harvest processing were responsible for the ‘Arriba’ flavour of Ecuadorian cocoa beans. Indeed Wintgens (1991) concluded that unlike tea and coffee, neither soil nor climate had a marked influence on cocoa flavour. Clapperton et al. (1994a-c) and Figueira et al. (1997) also concluded that the same genetic material planted in different environments exhibits similar flavour characteristics when processed in a similar manner.

Research in Trinidad (Sukha et al. 2008) showed significant differences between crop years for seven out of eight flavour attributes for cocoa liquor made from individual cocoa clones. Differences between clones (within a season and averaged over seasons) were also significant, but only for five flavour attributes. For example, liquors made from all the clones were fruity and this attribute showed no significant variation among clones. There were also significant differences in the flavour of liquor made from samples from commercial estates from a range of locations in Trinidad and significant crop year effects were also found with these (Sukha et al. 2008). These findings suggested that some aspects of the environment are likely to be influencing the flavour of cocoa liquor, even though flavour is thought to be determined mainly by genetic differences between varieties.
The work reported in this paper was undertaken to investigate more thoroughly possible influences of post-harvest practices on the flavour of cocoa liquor made from beans grown in Trinidad. The conditions of post-harvest processing were considered and included 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 spectral fingerprint of biochemical composition of the samples was also analysed independently using near infrared reflectance spectroscopy (NIRS).

**MATERIALS AND METHODS**

The 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 on flavour. Details of the procedures followed in these experiments for primary processing are given in Sukha et al. (2008).

**Processing location experiment**

In each estate where cocoa is fermented and dried in Trinidad, there are subtle differences in procedures that vary according the tradition of the estate. The environmental conditions during these post-harvest processes are also location dependent. In referring to the "processing location experiment", the effect of the combination of all these factors on flavour is being considered.

This experiment was designed to keep all the major factors affecting flavour constant except for the processing location. Three commercial processing facilities were chosen in distinct geographic locations in Trinidad. These were La Reunion Estate (LRE), Centeno (North Trinidad), Manickchand Estate (ME), Sangre Grande (East Trinidad) and the San Juan Estate (SJE), Gran Couva (Central Trinidad). Micro-
fermentations (Clapperton et al. 1994 a; 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. Drying rate measurements were done at ME and SJE.

The temperature of the fermentation mass was measured at all three processing locations three times during each of the three crop years. Temperature was measured continuously during fermentation and stored every five minutes using a data logger (Campbell Scientific CR10X) with PC208W 3.2 Data logger Support Software (Campbell Scientific Inc., USA). Three thermocouple probes were inserted at the start of fermentation at depths corresponding to the top, middle and bottom layers of the fermentation mass.

Measurements of pH were done at all three processing locations in three repetitions over the three crop years on testa and cotyledons of selected beans at the start of and during fermentation

**Sample preparation and sensory assessment**

Each dry bean sample was roasted at 140°C 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 -8°C 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 this 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**

**Sensory data**

Restricted maximum likelihood (REML) variance estimates were obtained with Genstat 4.24 DE (VSN International) to determine the significance of treatment effects and interactions. Principal component analysis (PCA) was performed on the pooled 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**

Spectral data 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 experiment. 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 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

Processing location 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.

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

**Near infrared reflectance spectroscopy data**

**Processing location 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 (Figure 2a).

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 2b). The labelled samples in Figure 2b refer to individual samples that have been misclassified in the LDA analysis as being processed in one location when in fact they were processed a different location. The LDA analysis of the spectral data indicates a stronger clonal than processing location effect in the spectral data.
Figure 2a. Linear discriminant analysis of the spectral data for clones used in the processing location experiment.

Figure 2b. Linear discriminant analysis of spectral data from cocoa beans from the same trees processed at different locations in Trinidad.
DISCUSSION AND CONCLUSIONS

The PCA plots from the pooled sensory assessment data provide clear evidence that the processing location affected certain flavour attributes. 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 and agrees 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.

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

Measurement of fermentation mass temperature and pH during fermentation showed no major differences between the three processing locations (data not presented). The 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.

Microbial identification, succession and enumeration during fermentation were outside the purview of this study and there may have been micro floral induced effects at the three different processing locations that were not identified or quantified. Ostovar and Keeney (1973) reported work conducted in Trinidad at different processing locations (including LRE) that showed different sources of inoculum (surface of the pods, hands of employees, implements used, soil etc.) that vary from estate to estate and that different species of micro organisms within a genus may be found in particular estates. There may be a potentially important role of micro flora in the fermentation mass on the flavour of the micro-fermented samples.
Schwan (1998) acknowledged the effect of fermentation micro flora on flavour and work by Lagunes et al. (2007) correlated specific flavour attributes (mainly dried fruit) and the presence of esters from chemical analyses associated with the presence of yeasts and acetic acid forming bacteria.

There were marked 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 at these two estates (data not presented) revealed that the temperature at ME was marginally higher than at SJE, but RH was similar at times when the drying rates were measured (data not presented). However, examination of the practices on each estate revealed differences that can help explain the measured drying rates. Bean samples for this study were all 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.

One can conclude that both the box fermentations (neglecting any differences in micro flora) and weather during drying was similar at the processing and drying locations. The difference in drying rates observed is likely to contribute to the sensory 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.
There was general agreement between the sensory results and the LDA analysis of the spectral data generated by NIRS. There is a significant processing location and clonal effect on flavour but the clonal effect is stronger.

The results from this study provide strong evidence to support the relative contribution of genotype 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.

The contribution of the genetic component to expression of flavour potential in certain clones with strong aromatic flavours (such as floral flavour) superseded the combined effects of growing and processing environments in some instances. This agrees with previous work done by Clapperton et al. (1994a). However, we have been able to identify marked influences on processing (mainly during drying) to organoleptic attributes.

The results from this experiment represented the first systematic study using two independent methods of analysis to provide clear evidence that the processing environment affects cocoa liquor quality. The general agreement between sensory and NIRS results highlight the effectiveness and robustness of both techniques in expanding our knowledge base about the relative impact and importance of the different factors affecting flavour in cocoa. NIRS could be a helpful and efficient non destructive tool for high throughput characterization of cocoas according to quality and/or genotypes. Knowledge of the relative importance of processing and genetic effects on flavour could allow us to manipulate these factors to better meet the needs of the industry. There are also great implications for cocoa origin certification via Geographical Indication and niche origin marketing programmes with this knowledge.
ACKNOWLEDGEMENTS

The financial support, assistance and collaboration of the United Nations Common Fund for Commodities, Guittard Chocolate Co., California, USA, Lindt & Sprüngli, Switzerland, The Ministry of Food Production of the Government of the Republic of Trinidad and Tobago, the management and staff of the La Reunion Estate, Paul Manickchand Estates Ltd. San Juan Estate (Trinidad), Mr. Bruce Lauckner (CARDI) and all sensory panellists are gratefully acknowledged in this study.

REFERENCES


Annex 12

Cocoa Liquor Evaluation form used by the Cocoa Research Centre, University of the West Indies – 10 attributes
# Sensory Assessment of Cocoa Liquors

**Name:**  
**Date:**  
**Session:**  
**Sample Code:**  

Taste sample and mark off the point on the line that corresponds to the intensity of each attribute.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Absent</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa flavour</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Acidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astringency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitterness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruity flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floral flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutty flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw/beany/green</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet/Caramel/Malt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other flavours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global Quality</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

**Comments:**
Annex 14

Master Cocoa Liquor and Chocolate Evaluation for Scientific Use - 50 Attribute Scale
Master Cocoa Liquor and Chocolate Evaluation for Scientific Use

50 Attribute Scale

This document summarizes the Creative Commons Copyrighted Excel spreadsheet:

zz3 ESSeguine-DASukha Master Sample Evaluation Liquor and Chocolate 20151001

Creative Commons Attribution-ShareAlike 4.0 International License

Available through Seguine Cacao Cocoa & Chocolate Advisors, LLC Dropbox:

Please contact Ed Seguine, ed@seguinecacao.com, for the link.

History of the Master Evaluation

Developed in its original form in the early 1990s by Ed Seguine while at Guittard Chocolate for use in fully characterizing all cocoas of the world. Used to evaluate germplasm, breeding projects, and correlations with other attributes.

The evaluation form was further evolved in a collaboration with Dr. Darin A. Sukha, Cocoa Research Centre, University of the West Indies, starting about 1997. Further evolution and scale use validation and flavor attribute and intensity alignment and calibration was conducted between Dr. Sukha and Ed Seguine in 2010-2011.

Connection to the Cocoa of Excellence Flavor Evaluation Form

The Master Evaluation format was merged with the original Cocoa of Excellence form from the CIRAD Cocoa and Chocolate flavor panel evaluation format in the 2013 edition of the Cocoa of Excellence and is now the foundational base, including attribute definitions and scaling for all Cocoa of Excellence panels. See the Cocoa of Excellence discussion for details.
Spreadsheet

Tab 1: Sample List Qual Eval (Qualitative Evaluation)

This is used to:

a) Provide details of the samples after flavor evaluation and
b) Provide a text description of the liquor and the chocolate flavor profiles.

Tab 2: Quantitative Evals

This is a very wide spreadsheet. Each sample is a row. This will be discussed in parts.

Explanations--

Col B is for the paneling date
Cols B-F are used for tracking flavor evaluations in multiple, replicate blind tastings.
Cols B-F are normally hidden for convenience in using the spreadsheet in actual tasting
Viewing is best at about 80% depending on screen width and resolution
Col G is a unique assigned code
Cols H--J is sample information entered after tasting for long term tracking.
Col K is for other blind code assignment in replicate tasting--random numbers
Cols L - BI provide the detailed evaluations for either liquor or chocolate samples

50 Attributes are designated and are grouped by flavor category

These attributes should be regarded as primary and orthogonal except for Total Acidity (Col. M) which is the sum of the individual acidities inCols O-R.

Many of the attributes are pre-filled with zeroes for convenience.

These are over written during flavor evaluation.
<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>O</th>
<th>P</th>
<th>Q</th>
<th>R</th>
<th>S</th>
<th>T</th>
<th>U</th>
<th>V</th>
<th>W</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>AA</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>AB</th>
<th>AC</th>
<th>AD</th>
<th>AE</th>
<th>AF</th>
<th>AG</th>
<th>AH</th>
<th>AI</th>
<th>AJ</th>
<th>AK</th>
<th>AL</th>
<th>AM</th>
<th>AN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floral-Woodsy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Floral-Grassy (green)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Floral-Earthy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Floral-Mushroom</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Floral-Orange blossom</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Floral-Flowers</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Woody-Light wood</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Woody-Dark wood</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Woody-Resin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

176 | Page
Columns BJ-CD provide a summary of the primary attributes grouped by category.

Note this summary is the basis for translating scores into the Cocoa of Excellence categories.
History of Use and Publications (Projects and references)

ICCO/CFC Fine Flavor Cocoa project

Original use, predecessor format and attribute scale

CATIE Breeding for Monilia Resistance

Used to provide breeding feedback to Dr. Wilbert Phillips for breeding and selection of genotypes with advanced phenotypic traits including preservation of traditional Mesoamerica cacao type flavor. Clones submitted to Cocoa of Excellence 2009, blind, received awards (CATIE R-4 and CATIE R-6) for their fruit flavor profiles. Detailed data at CATIE and results summarized in Catálogo de clones de cacao (available in pdf format from Dr. Wilbert Phillips, CATIE (wphillip@catie.ac.cr).

USDA-Mayaguez germplasm collection

Collaboration between Dr. Brian Irish, Curator USDA-Mayaguez, Puerto Rico, agricultural collection to evaluate the UDA-Mayaguez cacao clone collection. All pods / all trees harvested each year for 5 consecutive years, fermented, dried, and evaluated by Ed Seguine for flavor profile. Data in process of being prepared for publication.

ICGD-Reading data incorporation

Following publication of the USDA-Mayaguez five year study data, the spreadsheet data will be uploaded using the current attribute scale into the International Cocoa Germplasm Database.

CIRAD-USDA-Guittard project to map flavor to microsatellite markers in Ecuador population

CIRAD-Guittard study conducted by Claire Lanaud (CIRAD) and Ray Loor (INIAP) to screen and evaluate a flavor collection subset of Ecuador Nacional type clones at Pichilingue, Ecuador. Flavor attributes were connected to 25 microsatellite markers in the collection. Private Publication: CIRAD to USDA

Dominican Republic country survey

Survey of varieties and flavours of Dominican Cocoa and genetic study of Dominican Republic populations.

Caracterización organoléptica y molecular de los clones locales e introducidos” E. Boza et. al. 2012 Charts published in The Essential Ingredient of the Best Chocolates, Rizek Cacao S.A.S.
*Charts: Rizek Cacao


Used as source flavor data for the following patent applications:

WO 2013/025621 Al Micro-fermentation of cocoa Seguine, Mills, Marelli, Motamayar, Coelho 13 August 2012

WO2014130539 A1 Methods for processing unfermented fruit seeds such as cocoa beans or cupuacu beans Schnell, Seguine, Dias, Bizzotto, Marelli, Mills, Motamayar 19 Feb 2014
Analytical Standard Error

Using this attribute scale and testing among 10 clones—15 replicate samples of each clone—and 5 blind replicate tastings of each sample, the following standard errors were obtained.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa intensity</td>
<td>0.157 flavor units (10 point, 0-10 scale)</td>
</tr>
<tr>
<td>Astringency</td>
<td>0.178</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.124</td>
</tr>
</tbody>
</table>

All samples had this present and so can be analyzed by ANOVA.