Microfermentation of Cacao: The CTAHR Bag System

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SUMMARY. Standardized and repeatable techniques for microfermentation and drying small samples (<100 g) of cacao (*Theobroma cacao*), also known as cocoa, are necessary to identify new varieties having high yield and quality. Sensory analyses of the processed cacao seed (bean) are a critical component to develop varieties for Hawaii's cacao to chocolate industry. A microfermentation and drying system capable of processing multiple samples of mucilage-covered cacao beans ranging from 60 to 6000 g was developed. The effects of fermentation variables, genetic background, management, site, and season on quantitative and qualitative attributes can be studied using this protocol. Beans processed using the College of Tropical Agriculture and Human Resources (CTAHR) bag system (CBS) are inoculated with microorganisms on their fruit (pod) surface. This enables a better assessment of the *terroir* of sites when the dried fermented beans are roasted and processed. Clean, inexpensive, disposable polyethylene bags serve as fermentation vessels. The fermentery has a temperature controller that follows a fermentation temperature profile. Sun drying is replaced by drying in the laboratory. Two-month storage in ambient outdoor humidity and temperature completes the protocol. The CBS is an improvement to existing cacao microfermentation methods because beans from single pods can be fermented. No microbial isolates, inoculums, or foreign pulp from other trees and sites are used. Less labor is required to maintain the fermentation. In laboratory drying is less variable than sun drying. The CBS is a flexible and reliable method to microferment cacao for scientists, small growers, and hobbyists.

Fermentation is a necessary step in the processing of cacao for chocolate (Sukha and Seguíne, 2015). Fermentation of the mucilage-covered beans is initiated by naturally present microorganisms such as yeasts, acetic and lactic acid forming bacteria on the pods and in the environment

(Moreira et al., 2013). Typically microbial isolates are not used. During fermentation, beans begin the process of germination resulting in the conversion of storage compounds in the beans to fuel and structural components for growth. Heat and acids produced by microorganisms kill the embryo before the radical emerges. When the fermented beans are roasted, the flavor compounds associated with chocolate are produced (Schwan and Wheals, 2004).

Breeding and variety evaluation programs should ferment beans for sensory analysis of the qualitative

attributes (Clapperton et al., 1994). Frequently, only small quantities of beans are available from such plots. Rohan (1963) developed the standard method for microfermentation of cacao. It involves removing fresh beans from 1 to 15 pods, which are placed in mesh bags. The mesh bags are then embedded into 100–1000 kg of beans in wooden commercial fermentation boxes. The fermenting beans are mixed (turned) aiding homogenization and aeration that encourage aerobic microorganisms. The embedded bags of beans are removed and manipulated to mix the beans inside, then re-embedded into the fermenting beans. Fermentation takes 6–7 d. The beans in the mesh bags are then dried for further processing and evaluation (Sukha et al., 2008).

Nonembedded microfermentation methods were developed when embedding the beans of interest into a commercial-sized, fermenting batch was not feasible or appropriate (Kuman and Hollywood, 2010; MacLean, 1950; Quesnel and Lopez, 1975; Seguíne et al., 2013). These nonembedded methods are particularly useful for investigations of fermentation variables, such as degree of pod maturity, different locations, temperature, and microorganisms; embedding beans into a large fermentation batch confounds or prevents this.

Our initial microfermentation efforts were not satisfactory. Microfermentation was first attempted with a bread yeast inoculum and an incubator set at 35 °C for 3 d, followed by 45 °C for 3 d then stopped by removing the samples from the incubator (Bittenbender and Kling, 2009). The bread yeast produced an unacceptable bread aroma in the dried beans and resulting chocolate. Later, modular fermenters (10-cm-diameter × 23-cm-long acrylic tubes

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
1	micron(s)	μm	1
0.0254	mil	mm	39.3701
0.4470	mph	m·s ⁻¹	2.2369
28.3495	oz	g	0.0353
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Table 1. Steps in the College of Tropical Agriculture and Human Resources (CTAHR) bag system (CBS) for microfermentation of cacao.

1.	Harvest pods from one site, place in woven sacks, store in sacks until pod opening, 0.5–2 d.
2.	Open pods, remove seeds (beans), and roll pods over beans to inoculate.
3.	Place beans in polyethylene bags and close. Place bags in fermentery and begin temperature program (see Fig. 2) at 25 °C (77.0 °F), this is 0 h.
4.	After 24 h pierce bottom of bags to drain liquid if present.
5.	At 48 h open bags, remove and mix beans, return beans to bag. Leave small opening for air exchange. Begin aeration and humidification of fermentery.
6.	At 96 h open bags, remove and mix beans, return beans to bag. Leave opening in bag for air exchange.
7.	At 144 h remove and open bag, place beans on drying racks.
8.	Begin drying, 0 h. Turn on fan for 12- to 14-h interval at indoor ambient temperature and humidity. Turn off fan, stir beans. At 24 h stir beans, turn on fan for 12- to 14-h interval. Turn off fan, stir beans.
9.	At 48 h, place drying racks in forced air oven at 35 °C (95.0 °F) for 12- to 14-h interval. Turn off oven and open door, stir beans.
10.	At 72 h stir beans, turn on fan for 12- to 14-h interval. Turn off fan, stir beans.
11.	At 96 h complete drying at 45 °C (113.0 °F) in 2-h increments until bean moisture is 6% to 7%.
12.	Store beans in paper or net bag in an insect-proof container (fine net covered plastic box or pail) in covered area at outdoor ambient temperature and humidity for 6 weeks before roasting.

and disks) were made and used. These could ferment bean batch weights from 60 to 900 g (Gautz, 2009). Unfortunately, the fermenting vessels leaked, it was time consuming to prevent leakage, and they could not hold more than 900 g.

Neither the standard nor nonembedded microfermentation methods were suitable for our needs. The standard method required a minimum fermenting batch weight of 100 kg, which is much greater than what was available. Alternatively, the nonimbedded microfermentation methods were constrained by fermenter size, the need for prepared inoculum, sun drying, and manual temperature control, which added additional labor and material cost.

The objective of the current project was to develop a microfermentation system that did not require embedding samples in a commercial size fermenting batch, was flexible with regards to weight and number of samples, did not require prepared inoculum, did not require sun drying, and was repeatable with regard to the degree of fermentation and cocoa liquor quality. Those efforts evolved into the CTAHR CBS.

Materials and methods

PLANT MATERIALS. The CBS was evaluated using pods harvested from variety trials at four sites on Oahu island, HI; these ranged from full sun, irrigated sites to cloudy, rain-fed sites. Every 3 weeks, ripe pods were cut from trees (up to four grafted trees per variety per site), each pod was

labeled and placed in clean, reused, and woven 50-lb fertilizer bags. Within a site, harvested pods from all varieties were bagged together and kept separate from pods from other sites.

THE CBS. Steps of the CBS are shown in Table 1. Pods from a site were grouped by variety and opened by variety with a heavy cleaver in the laboratory with care not to cut the beans. Beans from the same variety with adhering mucilage were removed, separated from the placenta, and placed in a large bowl. The emptied pods were rolled across their beans to transfer adhering microorganisms from the pod surface to the mucilage-covered beans. This was repeated for each site harvested that day.

Polyethylene bags (two sizes: 10 × 5 × 26 cm and 20 × 10 × 45 cm, both 1.5 mil thick) were used as disposable fermenters. The small bag held 60–1000 g of beans and the large bag 1000–6000 g. Bags were filled with the beans of the same variety from one site and closed by twisting and secured with a 3-inch wood, spring-tensioned clothespin, and placed on stiff, raised netting in trays to collect liquefied mucilage (sweatings) that drain from the bags. Closed bags created anaerobic conditions for the first 48 h. If the sweatings accumulated more than 2 cm in the bottom of a bag at 24 h into the ferment, the bag was pierced with a wooden skewer to allow drainage. Bottom of the small bags were pierced up to six times and the large bags up to 12. After turning

Fig. 1. Photograph of the College of Tropical Agriculture and Human Resources (CTAHR) bag system (CBS) programed cacao fermentery with door open. Wood clothes pins close the opening of fermenters, 1.5-mil-thick (0.04 mm) polyethylene bags. Ambient laboratory air humidified by pumping through a 30-cm (11.8 inches) column of water in 5-L (1.3 gal) container in the fermentery chamber during the aerobic phase, 48–144 h.

the ferment at 48 h, the bags were not closed, a 1 to 2-cm opening remained for aeration (Fig. 1).

A repurposed variable temperature incubator (model 844; Laboratory-Line Instruments, Melrose Park, IL) outfitted with a digital, programmable temperature controller (model 4896; Solo, AutomationDirect, Cummings, GA) served as a fermentery and maintained the set temperature to within

± 1 °C. This fermentery held up to 60 bags with a combined weight of up to 40 kg, with individual bags containing 60–6000 g of beans (Fig. 1). Temperature in the fermentery was programmed to rise gradually (not in 12- or 24-h increments) from 25 to 48 °C over 84 h, held at 48 °C from 84 to 136 h, then gradually decreased to 45 °C by 144 h (Fig. 2). The fermentery was aerated and humidified after 48 h by bubbling ambient air through a 30-cm water column inside the fermentery. Fermentation was stopped at 144 h by removing the bags from the fermentery, emptying the bags, and spreading the beans on racks to dry.

Drying was an adapted protocol of increasing air temperature (Hii et al., 2011). Beans were placed two to three seed diameters deep on food-grade netting (8-mm mesh) on wood frame racks placed side-by-side, three per wire shelf, and stacked with 5 cm of clearance. Drying began at ambient laboratory temperature and humidity (20 to 23 °C and 50% to 55% relative humidity). Air passed over and under the stacked racks at velocities of 0.2–1 m·s⁻¹, generated by a 12-inch-diameter rotary fan (Fig. 3). Fan drying continued for 10- to 14-h periods each day for 2 d. Then, drying racks were stacked in the same formation in a forced air oven (model 645; Precision Scientific/Napco, Winchester, VA) at 35 °C, with air velocities of 0.8–1 m·s⁻¹. Oven drying occurred in 10- to 14-h periods each day for 2 d. Seeds were stirred on the drying racks at least twice in each 24-h period. The oven was turned off and opened when not in use, beans returned to ambient laboratory conditions. If beans were not sufficiently dry after 2 d, the oven temperature was raised to 45 °C for 2-h intervals until moisture content was 6% to 7%, as measured by a moisture meter (model F6 Intec; Sinar Agritec, Camberley, UK). Dried beans were stored for at least 2 months before quality assessment to reduce fermentation aroma. Storage was in paper bags in 20-L plastic pails covered with insect-proof netting in covered, outdoor ambient temperature, and humidity.

QUALITY ASSESSMENT. Quality differences and repeatability of scoring the flavor attributes of dried, fermented beans produced with the CBS were demonstrated using internationally accepted criteria as described below (Sukha and Seguíne, 2015;

Sukha et al., 2008). Beans for sensory analysis were from the two largest harvest dates for each of the 10 varieties from four sites. A cut test was done to examine bean interior for color, appearance, insect, and mold damage. A subsample of 50 beans from each lot was halved with hand pruners through the narrow side of long axis of the bean so that as much interior surface was exposed. International commercial standards indicate that bean color should be purple-brown to brown, not black or slate blue-gray. Cut beans should appear cracked and fissured, not smooth and solid (End and Dand, 2015).

Whole dried beans (one seed deep on perforated metal sheets) were roasted (in the same forced air unit for drying) at 121 °C for 25 min, then cooled and cracked. Seed pieces were winnowed into seedcoat (shell) and cotyledon and embryo pieces (nibs). Nibs were pulverized to less than 20 µm as a paste (cocoa liquor) using a granite stone on granite stone bowl grinder (model ECGC-12 SLTA fitted with a model 05 bowl; Cocoa-Town, Roswell, GA).

The taste panel trained by E. Seguíne included Seguíne, H.C. Bittenbender, J.L. Myers, and two others evaluated cocoa liquor made from the varieties. Flavor attributes (cocoa, acidity, bitter, astringency, sweet, fresh fruit, browned fruit, nutty, floral, woody, spicy, and off flavors) were evaluated based on the intensity (0 = not present, 9 = most intense) (Sukha and Seguíne, 2015). Tasting results were analyzed by the analysis of variance using randomized complete block design (tasters were the blocks) to determine the effects of variety and site on taste using JMP (version 9; SAS Institute, Cary, NC).

Results and discussion

Thermocouples inside fermenter bags tracked fermentery temperatures; even large bags with 6 kg of bean were ± 1 °C of the fermentery air temperature. Cut test evaluation was performed for 50-seed lots from the same four sites and the same 10 varieties over 10 fermentation cycles in the fermentery in 2013 and 2014. All seed lots had over 90% purple-brown or brown color nibs, and none were slate colored. No moldy or insect damaged seeds were found. The CBS produced well-fermented beans

that met or exceeded international standards for cut test evaluation (End and Dand, 2015).

The taste panel found statistically significant effects due to the genetic background of the 10 varieties and environment (four sites) on flavor attributes. Intensity of cocoa, acidity, bitterness, astringency, sweetness, and floral varied significantly between the 10 varieties. Only acidity and brown fruit flavors varied significantly between four sites (Table 2).

Compared with both embedded and not embedded microfermentation systems, the CBS has increased flexibility and convenience (Table 3). The CBS is flexible in terms of the number of fermenters (up to 60) and range of bean weight per

Fig. 2. Graph of the standard programmed temperature curve during fermentation inside the College of Tropical Agriculture and Human Resources (CTAHR) bag system (CBS) cacao fermentery; $(1.8 \times ^\circ\text{C}) + 32 = ^\circ\text{F}$.

Fig. 3. Photograph of fermented cacao drying in mesh bottom, wooden racks with 12-inch-diameter (30.5 cm) oscillating fan 12–14 h·d⁻¹ for 2 d; drying racks are placed in 35 °C (95.0 °F) forced air oven for 12–14 h·d⁻¹ for 2 d or until 6% to 7% moisture content.

fermenter (60–6000 g), in addition to using inexpensive, disposable fermenters. The use of an incubator with programmed temperature profile is more convenient than methods with manual temperature control and requires less labor. The program temperature increase is gradual

like a normal ferment rather than a stepwise incremental increase and can be easily modified for fermentation experiments.

Prepared inoculums from a commercial-size ferment or pure cultured yeasts and bacteria are not required in the CBS. Drying indoors

provides a repeatable drying environment and is unaffected by environmental variables compared with drying in the sun. The programmable temperature controller of the fermentery and separate fermenters simplifies the evaluation of the effects of fermentation variables, such as duration, temperature, inoculums, and pulp composition on flavor attributes.

The CBS also offers benefits over the standard microfermentation method because it does not involve embedding beans of interest into a batch with beans of other varieties or produced elsewhere which can obscure inherent differences between varieties, sites, and treatments. Each ferment of beans from one variety, harvest date, and location is inoculated by the microorganisms from its own pods and fermented in its own mucilage. This increases the likelihood of detecting a *terroir* effect.

Altogether, with the reduction in labor, elimination of the need for a large fermenting batch of beans in

Table 2. Probability that *F* test is significant for 12 flavor attributes of cocoa liquor for the effect of genetic and site differences between cacao varieties fermented using the College of Tropical Agriculture and Human Resources (CTAHR) bag system (CBS) protocol.

Flavor attribute	Level of significance	
	Selection	Site
Cocoa	0.0001	0.3
Acidity	0.0001	0.002
Bitterness	0.002	0.3
Astringency	0.0001	0.8
Sweetness	0.03	0.3
Fresh fruit	0.2	0.5
Browned fruit	0.4	0.02
Nutty	0.7	0.7
Floral	0.0002	0.9
Woody	0.3	0.6
Spicy	0.9	1.0
Off flavors	0.06	0.03

Table 3. College of Tropical Agriculture and Human Resources (CTAHR) bag system (CBS) compared with other methods used to microferment cacao beans.

Criteria	CBS ^z	Other methods ^z
In laboratory fermentation?	Yes	No: Rohan (1963) Yes: Kuman and Hollywood (2010), MacLean (1950), Quesnel and Lopez (1975), Seguire et al. (2013)
In laboratory drying?	Yes	No: Kuman and Hollywood (2010), MacLean (1950), Quesnel and Lopez (1975), Rohan (1963), Seguire et al. (2013)
Prepared inoculum?	No	No: Quesnel and Lopez (1975), Rohan (1963) Yes: Kuman and Hollywood (2010), MacLean (1950), Seguire et al. (2013)
Range of fermenter capacity	50–7,000 g beans	100–1,000 g: Kuman and Hollywood (2010) 100–1,000 g and limited to available funnel sizes: MacLean (1950) 10 kg minimum: Quesnel and Lopez (1975) 200 kg fresh seed in a commercial fermenting batch: Rohan (1963) 50–1,000 g: Seguire et al. (2013)
Number of fermenters or samples	1–100	1–20: Kuman and Hollywood (2010) 1–100: MacLean (1950) 1–50: Quesnel and Lopez (1975) 1–100: Seguire et al. (2013)
Programmed temperature control	Yes	No: Quesnel and Lopez (1975), Rohan (1963) Manual: Kuman and Hollywood (2010), MacLean (1950), Rohan (1963), Seguire et al. (2013)
Seasonality	No, anytime one pod is available.	No: Kuman and Hollywood (2010), MacLean (1950), Seguire et al. (2013) Yes: Quesnel and Lopez (1975), Rohan (1963)
Suitability to study environmental and biological parameters	Yes	Yes: Kuman and Hollywood (2010), MacLean (1950), Seguire et al. (2013) No: Quesnel and Lopez (1975)

^z1 g = 0.0353 oz, 1 kg = 2.2046 lb.

which the “beans of interest ” are embedded, repeatability, and ease of use, the CBS simplifies microfermentation for sensory evaluation. Impacts of environmental variables, such as temperatures, the duration of fermentation, the use of different inoculums, and changes in pulp composition on cacao flavor profile are more readily investigated than with the standard microfermentation method.

Further evaluation and ultimately the release of varieties fermented in the CBS will contribute to the development of the cacao industry in Hawaii.

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