A PRELIMINARY INVESTIGATION INTO THE EFFECT OF VARIETY ON THE CHEMICAL COMPOSITION OF CACAO (*THEOBROMA CACAO* L.) PULP.

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ABSTRACT

The development of flavours during cocoa fermentation is attributed to several components and cacao pulp has been shown to contribute to such developments and expressions of flavours. Selected cultivars were used to investigate whether the type of variety could influence the chemical composition of cacao pulp. Cacao pods from 30 accessions held in the International Cocoa Genebank Trinidad were selected at a uniformly ripened stage. The cacao pulp juice was extracted and analysed for pH, total titratable acidity, vitamin C and reducing sugars content. ANOVA and Tukey post hoc tests showed that there was significant variation (P <5%) for all pulp parameters amongst the accessions. The accessions within the Refractario cluster were highly varied in their chemical compositions and did not form any noticeable subgrouping from the Principal Component Analysis (PCA). The remaining accessions were also moderately scattered from the PCA highlighting the variability of the chemical composition of cacao pulp across varieties. This emphasises the need to understand the composition of cacao pulp for the maximum expression of flavours during processing and production techniques.

INTRODUCTION

The fruit of the tropical tree *Theobroma cacao* L. belonging to the family Malvaceae (Alverson et al 1999; Bayer et al 1999) is used for production of cocoa and cocoa related products. Cacao seeds are enveloped in an aromatic, mucilaginous pulp that is entirely maternal in origin, being developed from the integuments of the ovule (Esau 1977). The pulp undergoes fermentation and drives the chemical changes within the beans that contribute to colour, aroma and flavour precursors of chocolate (Thompson, Miller and Lopez 2001).

Bulk cacao pulp contains, on average, 84.5% water, 2.7% pentosans, 0.7% sucrose, 10% glucose and fructose, 0.6% protein, 0.7% acids and 0.8% inorganic salts (Hardy 1960). This substrate that characterises the microbial succession during native fermentation contributes to the the flavour attributes of fermented cocoa beans. Cacao fermentation studies have shown that there is an influence of genotype on flavour characteristics of the bean (Eskes et al 2007; Sukha et al 2014), and part of the quality of the end product is dependent on the characteristics of the raw material used (Clapperton et al 1994). The alteration of pulp quality through the addition of aromatic fruit pulp has also been shown to have a direct effect on the flavour profile of the bean (Eskes et al n.d) and even appeared to enhance the expression of fruity and floral flavour notes in bulk cocoa (Ali et al 2014). Evaluations on several fine or flavour cacao types have found a strong correlation between sensory traits of the pulp and the flavour notes associated with the cocoa liquor with marked differences in cacao pulp flavour even among accessions within a genetic group (Eskes et al 2007).

In addition to its contribution to the development of flavour, the versatility of cacao pulp is demonstrated through its direst use in the production of various by-products within several

cocoa producing countries. It may be used naturally, added as a flavouring or processed into juices, syrups, nectar, alcoholic beverages, jam, marmalade, yoghurt, milk shakes, ice cream or carbonated drinks (Figueira et al 1993; Freire et al 1999; Ali et al 2008; Escalante 2012).

The International Cocoa Genebank Trinidad (ICGT) is the largest germplasm collection with over 2000 accessions conserved as live trees on approximately 37 hectares of land. The pulp diversity of this collection can be exploited to not only diversify the cocoa flavours but also to increase the range of products from pulp through various processing techniques. Knowledge of chemical properties of the fruit pulp and its genetic variation is essential for improved use. Therefore, this study aimed to conduct chemical analyses on cacao pulp from several accessions within selected cacao genetic groups to determine if cacao genotype influenced the chemical characteristics of cacao pulp.

METHOD

Location

The International Cocoa Genebank Trinidad is located at the University Cocoa Research Station (UCRS) positioned to the southwest of the La Reunion Estate at Centeno, Trinidad. The Caroni River borders the northern end of this site that conserves over 2000 accessions.

Genotypes

Five genetic groups (Guiana, Iquitos, Marañon, Refractario Group I and Refractario Group II) were sampled for this study (Table 1). Within each genetic group, six accessions were selected and two trees per accession were used for selection of fruits.

Extraction of pulp

Three to five mature fruits with an 80 - 90 % colour change indicative of ripeness were harvested per tree.from 30 accessions in the ICGT during the 2013-2014 season (Table 1). All the trees and accessions sampled, were shown from DNA fingerprinting at the Cocoa Research Centre, to be true to type. All fruits were thoroughly washed under tap water and dried before being cracked open. The pulpy seeds and any pulp remnants of a fruit were emptied into a new plastic zipper bag, closed and gently massaged to remove as much pulp and juice as possible. Pulp samples from fruits of an individual tree were bulked and thoroughly mixed with a clean spatula to give a homogenous mixture and then separated equally (by volume) into two sample assays. The pulp juice was poured into clean, sterile labelled 50 mL polypropylene centrifuge tubes and stored at -20°C until analysis.

Table 1. Accessions and genetic groups* sampled for cacao pulp analysis.

Genetic Group	Accession	Genetic Group	Accession
Guiana	GU 114/P	Refractario Group I	B 5/3 [POU]
	GU 265/P		B 13/7 [POU]
	GU 277/G		JA 5/28 [POU]
	GU 307/F		LV 17 [POU]
	GU 310/P		LV 31 [POU]
	GU 353/L		SLC 19
Iquitos	IMC 6	Refractario Group II	AM 2/12 [POU]

	IMC 36	CL 10/3
	IMC 47	CL 15/19-7
	IMC 50	CLM 35
	IMC 55	LP 3/15 [POU]
	IMC 94	LP 3/29 [POU]
Marañon	PA 32 [PER]	
	PA 51 [PER]	
	PA 70 [PER]	
	PA 107 [PER]	
	PA 120 [PER]	
	PA 169 [PER]	

*Groupings according to Motamayor et al (2008); Refractario according to Motilal et al (2013)

The *p*H was measured using the Oakton Multi-Parameter PCSTestr (Eutech Instruments 35 Series, Thermo Fischer Scientific, Singapore) according to manufacturer's instructions. Total Titratable Acidity (TTA) as citric acid/L was assessed using a modification of the colorimetric titration method described by Sadler and Murphy (2010) and Nielsen (2010). The pure pulp juice samples were diluted four – fold and 3 drops of Phenolphthalein indicator were added to each sample. Reducing sugars, measured in g/L, were determined on ×50 dilutions of the pure pulp juice samples using a modification of Miller's (1959) Dinitro-Salicylic Acid Spectrophotometric Method. The ascorbic acid content of the pure pulp juice, measured in mg ascorbic acid/mL, was determined by a modified 2,6-Dichloroindophenol Titrimetric Method (Dhun and Baccus-Taylor 2013; Nielsen 2010).

One-way analysis of variance (ANOVA) was conducted using SPSS 17.0 (IBM Corporation, USA) to examine within group variation using accessions as the factor and the chemical parameters (pH, TTA, ascorbic acid and reducing sugars) as the dependent list. A Tukey post hoc test was then conducted on the data for multiple comparisons of accessions. A principal component analysis (PCA) plot was also constructed in PAST (Hammer et al. 2001) to highlight the relationship amongst the accessions and groups.

RESULTS AND DISCUSSION

There were significant differences ($P < 0.05$) in *p*H and TTA between accessions which varied between 3.27 to 3.94 and 6.1 to 15.9, respectively (Table 2). The TTA showed a 2.5 fold variation among accessions. The acidity of the pulp is a significant parameter in the manufacturing of products from pulp. It also contributes to the flavour of cocoa beans by influencing the microflora associated with fermentation. The total sugar content measured as reducing sugar equivalent showed an almost two-fold variation ($P < 0.05$) among accessions varying from 137 to 228 g/L. This is another parameter that is important for the manufacturing of fruit juices and will also influence the microbial activity during fermentation. The vitamin C concentration of the accessions varied between 10 – 50 mg/L (approximately 7 – 33 mg/100 g) with IMC 55 (Iquitos genetic group), having the highest vitamin C content. Nevertheless, the vitamin C content in cacao pulp was lower than that found in fruits such as oranges (53.2 mg/100 g), sweet red peppers (127.7 mg/100 g) and acerola (1677.6 mg/100 g) (NDL 2011). Our results of cacao pulp as a poor source of vitamin C are congruent with (Anvoh et al 2009). However, there may be accessions within the ICGT collection that may be higher in vitamin C content than IMC 55 and be of significance to the cacao pulp industry so the need for continued evaluation of the germplasm collection is vital.

Table 2. Summary statistics of four chemical pulp parameters in 30 cacao accessions.

Genetic Group	**Accession**	**pH Mean (SE)**	**TTA (citric acid eq/L) Mean (SE)**	**Vitamin C (mg/L) Mean (SE)**	**Reducing Sugars (g/L) Mean (SE)**
Guiana	GU 114	3.43^{a} (0.008)	12.5^{b} (0.22)	13.0^{a} (1.46)	137.4^{a} (4.80)
	GU 265	3.51^{abc} (0.009)	12.1^{b} (0.39)	23.0^{ab} (0.89)	222.2^{b} (17.67)
	GU 277	3.63^{b} (0.006)	8.8^{a} (0.16)	32.9^{b} (3.61)	188.0^{ab} (8.61)
	GU 307	3.53^{abc} (0.035)	10.3^{ab} (0.21)	22.9^{ab} (2.92)	176.9^{ab} (15.42)
	GU 310	3.40^{a} (0.028)	12.5^{b} (0.18)	22.9^{ab} (2.65)	199.0^{ab} (26.76)
	GU 353	3.63^{b} (0.055)	8.5^{a} (1.31)	26.8^{b} (1.93)	202.8^{ab} (9.26)
Iquitos	IMC 6	3.67^{a} (0.025)	8.5^{a} (0.78)	24.7^{b} (3.73)	203.5^{a} (7.32)
	IMC 36	3.62^{a} (0.013)	9.2^{a} (0.12)	24.7^{b} (0.70)	152.9^{a} (3.30)
	IMC 47	3.64^{a} (0.023)	8.6^{a} (0.24)	33.9^{b} (0.00)	160.6^{a} (7.82)
	IMC 50	3.66^{a} (0.005)	8.1^{a} (0.46)	36.0^{ab} (1.76)	197.1^{a} (15.78)
	IMC 55	3.74^{a} (0.022)	9.6^{a} (0.28)	50.1^{a} (2.41)	209.8^{a} (19.36)
	IMC 94	3.64^{a} (0.045)	9.0^{a} (0.33)	24.7^{b} (6.25)	190.9^{a} (14.82)
Marañon	PA 32	3.74^{a} (0.016)	8.2^{bc} (0.49)	15.3^{b} (2.78)	173.8^{ab} (10.01)
	PA 51	3.72^{a} (0.002)	9.7^{c} (0.50)	26.8^{a} (3.15)	228.3^{c} (6.86)
	PA 70	3.60^{b} (0.023)	8.8^{bc} (0.05)	19.1^{ac} (1.46)	136.5^{a} (4.42)
	PA 107	3.68^{ab} (0.005)	8.8^{bc} (0.15)	19.1^{ac} (0.78)	157.1^{ab} (5.96)
	PA 120	3.72^{a} (0.038)	7.8^{ab} (0.58)	12.2^{bc} (1.24)	181.0^{b} (14.80)
	PA 169	3.94^{c} (0.010)	6.5^{a} (0.15)	10.0^{b} (0.75)	160.1^{ab} (2.60)
Refractario Group I	B 5/3	3.51^{b} (0.017)	10.9^{d} (0.56)	34.9^{a} (4.78)	202.7^{a} (21.62)
	B 13/7	3.69^{ad} (0.009)	8.8^{a} (0.31)	12.4^{b} (1.00)	130.9^{b} (2.58)
	JA 5/28	3.27^{c} (0.003)	12.1^{d} (0.76)	20.7^{b} (1.84)	126.2^{b} (11.04)
	LV 17	3.80^{ade} (0.030)	6.3^{c} (0.10)	11.2^{b} (1.59)	155.7^{ab} (3.17)

	LV 31	3.79^{d} (0.036)	6.1^{bc} (0.15)	17.5^{b} (0.65)	133.1^{b} (2.81)
	SLC 19	3.47^{b} (0.033)	8.4^{a} (0.25)	15.3^{b} (2.37)	176.7^{a} (8.80)
Refractario Group II	AM 2/12	3.93^{a} (0.022)	8.3^{b} (0.19)	38.9^{b} (1.98)	125.0^{a} (9.13)
	CL 10/3	3.61^{c} (0.004)	7.8^{b} (0.15)	21.2^{a} (2.71)	128.1^{a} (10.56)
	CL 15/19-7	3.74^{c} (0.060)	9.9^{b} (1.16)	28.5^{ab} (4.60)	168.8^{a} (40.17)
	CLM 35	3.85^{a} (0.050)	9.8^{b} (0.41)	38.2^{b} (0.70)	163.5^{a} (7.44)
	LP 3/15	3.82^{a} (0.009)	9.2^{b} (0.20)	23.3^{ab} (6.15)	139.7^{a} (5.04)
	LP 3/29	3.39^{b} (0.007)	15.9^{a} (1.05)	27.8^{ab} (2.54)	160.9^{a} (13.28)

Values among grouped accessions for each column with different letters are significant at the 5% level
SE – Standard Error

Intra-group variation for the four chemical parameters was evident, at the 5% level, and three of the parameters within three of the five genetic groups being significant at the 0.1% level. Therefore, the chemical profile of cacao pulp is significantly affected by the choice of accessions within a group and this should be used as the deciding factor for processing purposes. The cacao pulp chemical profile of accessions can be used in deciding how best to group accessions for processing purposes. The summary statistics highlighted in Table 2 provide evidence of the accessions at either end of the spectrum, as well as, the range of values within which these samples fall. The *p*H may be considered to be of a small variation, with approximately 14% variation between values, but those samples at the lower end of the scale would be better processed separately to exploit their natural acidity.

When comparing accessions for TTA, reducing sugars and vitamin C, the percentage variation among the parameter values were 45%, 62% and 74% respectively. This emphasises that the variability among accessions is critical to the processes that cacao genotypes would be subjected. For instance, those accessions with high sugar content values, such as PA 51 and GU 265, may be potentially used as complementary accessions for those samples with a low concentration (AM 2/12 and CL 10/3) since this parameter is vital for fermentation. Pulp with high sugar content would be better suited for the production of desserts and alcoholic drinks

In the PCA plot, the first two axes accounted for approximately 77% of the variation of the four parameters assessed (Figure 1). Although the Iquitos and Marañon groups had the closest within group distribution, there was an absence of defined clusters. Several accessions belonging to the same genetic group occupied different quadrants in the PCA plot (Figure 1) based on the chemical parameter measured. The members of the Guiana group were separated across two quadrants based on TTA, reducing sugars and Vitamin C content. This diversity that was demonstrated with the samples showed that the variation of cacao pulp chemical composition was apparent at the accession level and the combination of samples would also influence the degree of clustering. Three accessions (GU 114, JA 5/28, LP 3/29) showed clear distinctions based on their TTA values. This emphasises the effect of variety on the chemical composition of the cacao pulp and the need to identify the specific profiles of these varieties for use in fermentation, as well as, for the commercial use of cacao pulp.

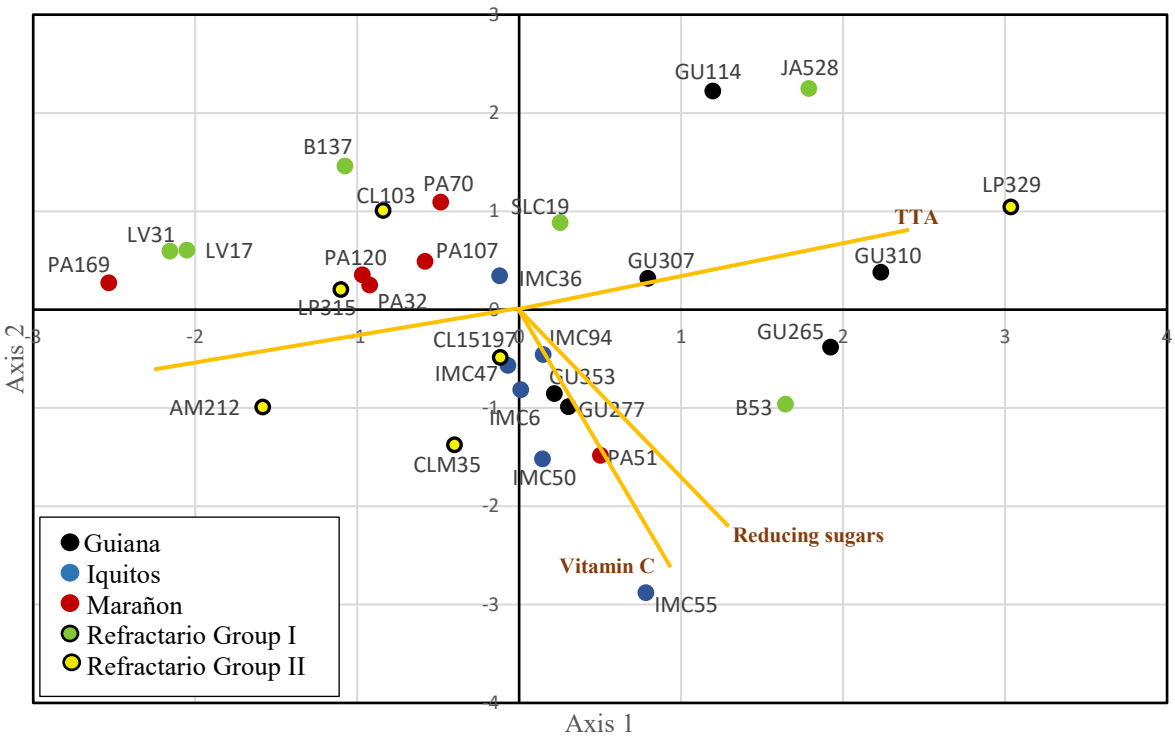

Figure 1. Principal Component Analysis (PCA) on accession variation of the genetic groups across four chemical parameters.

The ICGT, which contains over 2000 cacao accessions, should therefore be assessed in a wider more detailed survey, to evaluate the composition of cacao pulp. The diversification of the cocoa industry would be facilitated by finding accessions that may be readily used for pulp by-products. In addition, the variability encountered for vitamin C, sugar content and TTA may be widened in the larger survey, which would be useful for breeding purposes. If the latter is to be undertaken, the heritability of these traits would need to be assessed. The present study has therefore shown that there is great promise in developing future research projects around cacao pulp.

CONCLUSION

This preliminary investigation was useful in understanding the genetic variation in pulp composition. The study indicated that there was significant variability in cacao pulp composition among accessions which may be unrelated to genetic group membership. The distinctive differences observed at the accession level will be a foundation in understanding the development of the auxiliary flavours during fermentation and product development within the cocoa and the by-product industries.

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ADVANCES ON GENETICAL AND NATURALLY INDUCED VARIATIONS FOR FINE FLAVORS AND AROMAS IN THEOBROMA CACAO

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ABSTRACT

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

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

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

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

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

INTRODUCTION

Pulp vs. fine-flavor chocolate sensory trait relationship

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

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

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

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

express fruity flavors nor aromas. The nutty flavor is not present in the pulp and is known to be formed only during post-harvest processing. These comparisons support the idea of the existence of a pulp *vs*. chocolate fine-flavor relationship, be it with certain modifications such as the grape flavor of SCA6 that is transformed into the related brown fruit/raisin flavor.

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

Selection of cocoa for fine-flavor traits

Objective breeding towards fine-flavor varieties over the last century has probably been restricted to the selection over the last 50 years of the TSH varieties in Trinidad that express high-quality multi-trait pulp and chocolate sensory profiles with strong influence of that of SCA6. The selection for yield and resistance alone has occasionally resulted in lower than average bulk cocoa quality, as is the case with the CCN51 clone selected in Ecuador in the 1980's. Lockwood and Eskes (1995) concluded that selection for cocoa quality was hampered

by differences in objectives among chocolate manufacturers and by lack of efficient individual tree screening methods.
Eskes *et al*. (2012) showed that pulp sensory traits are highly discriminative among nine cocoa clones. Human preference was related to sweetness, flavor and aroma which were correlated to fine-flavor chocolate traits of three well-known clones (EET62, ICS1 and CCN51). Our survey in the native Chuncho variety represents the first systematic study in *T. cacao* demonstrating the feasibility of within-variety single-tree diversity evaluations for pulp and raw bean flavors and aromas.

The native Chuncho cocoa variety

"Chuncho" cocoa is native to the La Convención province in the Cusco region in Peru. Chuncho cocoa was cultivated already in the XVI century, but occurring also spontaneously in association with the indigenous Matsigenkas (Aparicio 1999). This tribe has always had a special interest in consumption of Chuncho fruit pulp (Gade 1975, Missioneros Dominicos 2009) and of slightly fermented and roasted raw beans. Rozas (1861) cited by Aparicio (2000) describes native cocoa in the valley of "high quality, noble and healthy" while Paz Soldan (1852), cited by Gade (1975), labels Chuncho cocoa from the Echerate Estate as "the best cocoa in the world". Chuncho acreage culminated in the 1980s with 14,000 ha and a production of 10.000 tons. The acreage of Chuncho has thereafter declined due to low productivity (150-250 kg/ha) linked to aged trees and competition from CCN51 and from other replacement crops. Chuncho is still mainly used as a low-valued bulk cocoa bean source for butter and powder extraction. However, traditional farmers consume pulp juices from selected Chuncho trees which is a practice not known to occur elsewhere in the world. Our pulp and bean survey was therefore focused on Chuncho trees used by farmers for juice production. Chuncho beans without specific sensory traits express already a certain level of undefined flavor and aroma (Condori Cruz 2015) the intensity and type of which may vary with the collecting site. Even when unfermented or little fermented, Chuncho beans are neither acid nor bitter nor astringent but may occasionally become so with fermentation duration of more than 4.5 days (Condori Cruz 2015). SNP marker studies showed a close relationship of Chuncho with accessions from the Madre de Dios and Beni river basins (Dapeng Zhang Pers. Comm.).

Transformation of bulk cocoas into fine-flavor cocoas

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

MATERIALS AND METHODS

Genetic variation for fine-flavor sensory traits

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

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

Naturally induced variation for fine-flavor sensory traits

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

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

RESULTS AND DISCUSSION

Genetic variation for fine-flavor sensory traits

Pulp flavors and aromas identified outside La Convención, Peru

As described in the introduction, six pulp flavors and aromas have been identified in the traditional four fine-flavor varieties. Between 2007 and 2017 eight hitherto unknown cocoa pulp flavor and aroma traits were discovered, outside La Convención, in very different varieties in Trinidad, Peru, Ecuador and Brazil. The most commonly flavor was soursop identified in six locations. A very interesting mango/rose profile was found in the Ecuadorian clone EET397 that used to be grown in Brazil for its witches' broom resistance. The banana pulp flavor was first discovered in Trinidad in 2008 in an old Trinitario plantation. Chocolates made with beans from this tree by Ed Seguine showed strong and persistent cooked banana/banana jam flavor. Five sensory traits (banana, soursop, jasmine, citrus and annona) were identified by Wilbert Cruz in Amazon genotypes in Satipo, Junin, Peru, in 2012 as verified with molecular markers

(Dapeng Zhang, Pers. Comm). In 2017 it was discovered that the "bulk" Amelonado variety in Brazil harbors the jasmine aroma, which is very surprising, and that the CEPEC 2008 clone expresses the soursop/rose sensory profile.
The discovery of eight hitherto unknown pulp cocoa flavor and aroma traits between 2007 and 2017 showed that different cocoa varieties may harbor unexpected sensory traits that should be of interest for fine-chocolate manufacturing. The surprising discovery in 2017 of three fine-flavor traits in two commercial Brazilian varieties suggests that varieties considered as bulk cocoa may in fact be fine-flavor genotypes with potential for commercial usage as fine-flavor cocoas.

Chuncho pulp and bean sensory traits survey

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

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

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

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

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

Naturally induced variation for fine-flavor traits

Induced sensory trait variation in CCN51

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

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

Transformation of bulk cocoa into fine cocoa

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

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

Naturally induced variation for fine-flavor traits

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

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

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

Treat-ment	N° days	Acidity	Bitter-ness	Astrin-gency	Nutty	Fresh fruit	Mature fruit	Spicy	Floral	Prefe-rence
F	6	0	3	4	0	0	2	0	0	**5**
C	3	0	2	0	4	0	3	2	2	**7**
T1	3	0	2	0	-	0	2	7	3	**9**
T2	3	0	3	0	-	0	8	3	4	**9**
T3	4	2	2	0	-	6	5	2	3	**10**
T4	3	0	3	2	4	4	4	6	6	**10**
T5	5	0	3	2	2	2	5	3	2	**9**
T6	4	0	4	1	5	6	2	6	4	**10**

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

CONCLUSIONS

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

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

The *genetic variation* for 40 sensory traits and 64 sensory profiles disclosed in Chuncho is *enormous* as compared to the four sensory traits and profiles present in the four traditional fine-flavor cocoa varieties. Hence, the potential of commercial valorization of the Chuncho sensory profiles, be it in Chuncho or in other cocoa varieties, is also enormous.

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

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

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

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

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

- The potential for fine-flavor cocoa market diversification is unlimited;
- Fine cocoa may become a mainstream cocoa because of the large potential to increase the offer of fine cocoa worldwide based on existing bulk and fine-flavor varieties;
- Fine-flavor cocoa raw beans or nibs may become an increasing segment in the fine-flavor cocoa market.

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ADAPTATION DU PROCESSUS DE FERMENTATION AUX CONTRAINTES LOCALES. APPLICATION AU CACAO DU SAMBIRANO DE MADAGASCAR

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RESUME

Le cacao de Madagascar est réputé pour donner un chocolat fruité et aromatique. La production (4000-6000 tonnes) est concentrée dans la vallée du Sambirano. Le développement des traits de qualité et de saveur est lié à la variété de cacao, au type de sol, au climat, à la gestion des cultures et aux traitements post-récolte. La fermentation, en particulier, est considérée comme une étape clé dans le développement des arômes. Mais une grande partie de la production du Sambirano provient de petits producteurs avec de faibles quantités de cacao frais et pas d'installation pour les traitements post-récolte. Cette situation conduit à une absence ou à une mauvaise fermentation du cacao, ce qui entraîne une perte de qualité. L'objectif principal de cette étude était d'adapter la fermentation aux contraintes locales: faible volume de cacao frais et pas d'installation adaptée. Cette étude a été réalisée en utilisant le cacao de la plantation biologique Akesson à Ambanja. Au total, 80 échantillons ont été fermentés dans des conditions contrôlées. Trois fermenteurs différents ont été testés: des sacs, des bidons d'huile comestible et les bidons d'huile comestible isolés avec des feuilles de bananier. Chaque fermenteur a été testé pour 3 volumes de cacao: 5, 10 et 20 litres. Des échantillons de contrôle supplémentaires ont été fermentés dans les fermenteurs industriels d'Akesson. La qualité des fermentations a été estimée au travers des profils de températures de fermentation, du test à la coupe, de la couleur et des profils biochimiques et sensoriels. Les paramètres physiques de la fermentation (températures et rendement) et les critères biochimiques (polyphénols et azote ammoniacal) indiquent qu'une fermentation a bien eu lieu dans tous les cas. Les profils sensoriels montrent que la note qualité globale est similaire pour tous les cacaos, sans différence significative avec les contrôles, à l'exception de l'essai "Bidon-isolé - 20 litres" qui a été noté de moindre qualité en raison d'un goût alcoolique plus élevé. Ces résultats permettent le transfert de bonnes pratiques de fermentation aux agriculteurs, avec du matériel disponible partout à un prix minimum. Cette approche se traduira par une mise à niveau de la qualité, similaire aux plantations industrielles, et nous l'espérons, une croissance des revenus des petits agriculteurs.

INTRODUCTION

A Madagascar, les premiers cacaoyers Criollo ont été introduits vers les années 1900. Le faible rendement, la sensibilité aux parasites et aux cyclones ont suscité la création des hybrides Trinitario, issus de croisement entre les variétés Criollo et Forastero, par l'IFCC dans les années 60. La sélection et la vulgarisation d'hybrides Trinitario a été poursuivie par le FOFIFA (Centre National de Recherche Appliquée au Développement Rural Malgache) dans les années 70-80. Durant cette période, les plantations coloniales cédèrent la place aux grandes sociétés privées et les petits exploitants malgaches commençaient à planter le cacaoyer. La disjonction des caractères dans ces descendances est telle que les vieilles cacaoyères malgaches sont encore actuellement occupées aussi bien par les 3 variétés classiques, que par une multitude de génotypes intermédiaires. Du fait de l'effet terroir, le cacao de Madagascar est encore considéré comme étant parmi les meilleurs du monde. Cependant la production malgache reste faible (6000 tonnes, soit seulement 0,12% de la production mondiale). Aujourd'hui la culture du cacao est concentrée dans la vallée du Sambirano (95 % de la production malgache) qui se situe dans le Nord-Ouest de Madagascar, dans la région de Diana[1]. Avec 23 796 hectares, le verger

cacaoyer du Sambirano, installé sous un couvert forestier, est réparti entre quelques dizaines de milliers de familles rurales et des plantations industrielles. Les paysans, sans encadrement depuis de nombreuses années, méconnaissent la culture cacaoyère et les procédés de transformation primaire. De plus, le manque d'organisation et d'infrastructure (route, unités de transformation) ne permettent pas une collecte efficace du cacao et donc un traitement collectif du cacao. Du fait de la faible production des exploitations familiales, le cacao est souvent vendu en frais ou mal fermenté et mal séché. Ce qui entraine une baisse de la qualité générale des lots à l'exportation en dehors des lots des plantations industrielles. L'objectif principal de cette étude était d'adapter la fermentation aux contraintes locales: faible volume de cacao frais et pas d'installation adaptée pour le traitement post récolte.

MATERIEL ET METHODE

Matériel végétal

Pour cette étude 80 échantillons de cacaos de la plantation Akesson Organic, située à Ambaja dans le Sambirano à Madagascar, ont été fermentés dans des conditions contrôlées. Deux types d'échantillons ont été sélectionnés en fonction de la couleur des fèves fraîches à la coupe : Casse claire et Casse foncée en fonction du % de fèves par couleur. Par classe de couleur de fèves, 4 récoltes ont été faites, soit un total de 8 récoltes entre le 20 aout et le 20 septembre 2014. Pour chaque récolte Les fèves fraîches (environ 100 kg récoltés) ont été mises à fermentées selon le plan d'expérience suivant :

-En sac avec 5, 10 et 20 litres de cacao (S5, S10 et S20)
-En bidon avec 5, 10 et 20 litres de cacao (B5, B10 et B20)
-En bidon isolé avec des feuilles de bananier avec 5, 10 et 20 litres de cacao (Bi5, Bi10 et Bi20)

En plus de ces échantillons, à chaque récolte un échantillon témoin, correspondant à la couleur de la récolte, a été récolté puis mis à fermenter dans un filet dans les conditions industrielles (caisses en bois en cascade, de 1 m^3) au sein de la masse de cacao issue de la même parcelle.
En fin de fermentation les échantillons ont été séchés au soleil en couche de 1 cm d'épaisseur afin de ramener la teneur en eau à moins de 8%. Puis chaque échantillon a été trié manuellement pour retirer les corps étrangers les fèves abimées et les fèves plates. Les échantillons d'un même essai (couleur/fermenteur/volume) ont été ensuite regroupés en lot. En opérant ainsi, 18 lots ont été réalisés + 2 lots témoin, soit 20 échantillons d'environ 5 kg de fèves de cacao fermentées séchées.

Fermentation

Le protocole industriel a été appliqué à tous les essais, à savoir : durée de fermentation 144 heures (6 jours) avec les rythmes de brassage suivants : 48 heures, 96 heures et 120 heures. Pour chaque essai le poids de cacao frais, mis à fermenter, a été mesuré ainsi que le poids de cacao sec final. Ce qui a permis de calculer le rendement de chaque fermentation.
La température du cacao pendant la fermentation a été suivie à l'aide de capteurs (Thermo boutons IP65 -40/+85 +/-0.5, Laboratoires Humeau, France) avec un enregistrement toutes les deux heures. La température ambiante extérieure a également été suivie, pour chaque essai, avec le même rythme d'enregistrement, pendant toute la durée de fermentation. Toutes les fermentations ont été réalisées sur site de production, dans le même local, à l'abri des intempéries et des courants d'air. Les fermentations en sacs ont été réalisées dans des sacs en polypropylène, les fermentations en bidon ont été réalisées dans des bidons d'huile alimentaire (préalablement nettoyés de toutes traces d'huile) de 20 litres dont le fond a été percé de trous de 1 cm de diamètre espacés de 5 cm. Les fermentations en bidon isolés ont été conduites dans

les mêmes types de bidons, placés dans un sac avec des feuilles de bananier entourant le bidon (fig.1). Dans tous les cas les cacaos mis à fermenter ont été couverts de feuilles de bananier pour minimiser les échanges de température. Les brassages ont été réalisés en vidant complément le fermenteur et en le remplissant à nouveau dans un temps minimum afin d'éviter les pertes de température.
En fin de fermentation les lots de cacaos ont été séchés au soleil jusqu'à une teneur en eau < 8%. Le cacao sec a ensuite été stocké dans des sacs en tissu dans un local sec et ventilé, avant triage manuel et assemblage des lots.

Figure 1 : Fermentations en bidons, sacs et bidons isolés

Analyses physico-chimique et sensorielle

La qualité de la fermentation a été évaluée par une épreuve à la coupe (cut test) à l'aide d'une guillotine (Dried Fruit Cutting unit, TESERBA, RÜTI, Suisse). Pour cela, 300 fèves sont analysées en 3 fois, soit 100 fèves par coupe, et le nombre (en %) de fèves violettes, 1/2 violettes, brunes, à casse claire, moisies, germées et attaquées par des insectes a été comptabilisé. Une mesure du grainage a été réalisée : les 300 fèves prélevées ont été pesées (± 0.01g) et le poids (g) d'une fève a été calculé. Pour les analyses de couleur et en spectrométrie proche infrarouge (SPIR), 100 g de fèves par lot ont été décortiqué manuellement à l'aide d'un scalpel. Puis les fèves décortiquées ont été refroidies à l'azote liquide et broyées à l'aide d'un broyeur à lame (IKA A11, IKA®, Staufen, Allemagne). Les coordonnées trichromatiques (L, a, b) des poudres ainsi obtenues ont été mesurées à l'aide d'un Chromamètre CR-410C (Konica, Minolta). Les acquisitions SPIR ont été réalisées avec un spectromètre FOSS 6500 (Foss, Silver Spring, MD) en utilisant un module de mesure tournant et des cellules en quartz de 50 mm de diamètre. Environ 3 g de poudre de cacao a été analysé par réflexion diffuse pour la gamme de longueurs d'onde de 400 nm à 2500 nm avec un pas d'acquisition de 2 nm. Les spectres ont été enregistrés en tant que la moyenne de 32 balayages et stockés sous forme de log (1 / R) où R est la réflectance à chaque longueur d'onde. Pour chaque échantillon trois spectres ont été réalisés, la moyenne des trois spectres a été conservée pour la suite de l'étude.
Les teneurs en azote ammoniacal et en polyphénols ont été prédites sur la base des spectres SPIR en utilisant les équations de calibrages développées au CIRAD[2, 3].
Pour l'analyse sensorielle, les fèves de cacaos ont été torréfiés 25 min à 125°C. Puis, après décorticage les cotylédons ont été malaxés dans un pétrin (Capco) à 45 °C pendant 30 min afin d'obtenir une liqueur homogène. Les liqueurs ont ensuite été raffinées (raffineur Exakt, <25µm) et conservées dans des flacons en verre foncé à 16°c avant dégustation. Les 20 échantillons de liqueurs ont été analysés 3 fois (répétitions) pour leur profil sensoriel (22 descripteurs dont 5 olfactifs et 17 saveurs) par 9 juges experts. Les notes moyennes par produit et juge ont été retenues pour la suite de l'étude.
Les analyses statistiques ont été réalisées avec le logiciel Win-ISI IV (Infrasoft International, Port Matilda, PA) pour le traitement des spectres et le logiciel XLStat (Addinsoft, Paris, France) pour les analyses descriptives, les analyses de variance, les analyses en composantes principales, les classifications non supervisées et l'analyse des données sensorielles.

RESULTATS

Les profils de températures (fig.1), quel que soit le fermenteur, sont conformes à ce qui est classiquement observée pour le cacao, avec une légère augmentation de la température en début de fermentation jusqu'au premier brassage (48h), correspondant à la fermentation (anaérobie). Suivie d'une brusque élévation de température due à la fermentation alcoolique (aérobie), la température maximale moyenne est de 49,2°c (sac 10L) et la température moyenne est de 37,3°c. Les profils sont similaires pour tous les types de fermenteurs en début de fermentation, donc pendant les phases les plus importantes.

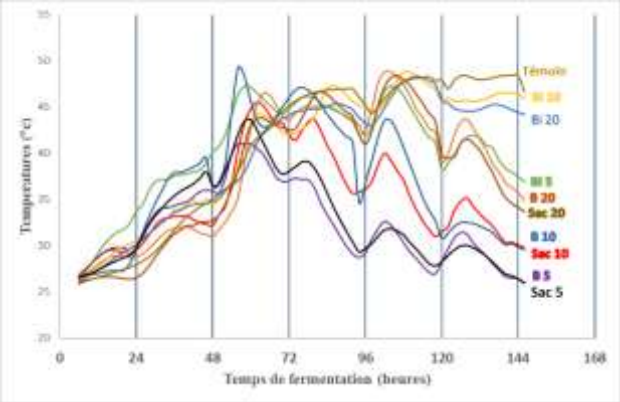

Figure 2 : Profils moyens des températures en cours de fermentation

Ces profils sont différents en fin de fermentation du fait de la quantité de cacao qui ne permet pas de maintenir le même volant thermique, surtout après brassage. Mais, même pour de faibles volumes (sac et bidon 5 litres), les reprises de températures après brassage sont nettes, témoignant d'une activité fermentaire. Les fermenteurs petits volumes (Bidon 5 litres) isolés se comportent comme les fermenteurs de 20 litres. L'épreuve à la coupe n'a pas relevé de fèves ardoisées, mitées ou moisies dans les 20 lots analysés. Les % de fèves violettes, claires ou brunes ne sont pas significativement différents entre les lots et les témoins (ANOVA à un facteur (type de fermenteur) et test de Newman-Keuls (SNK) au seuil α de 5%). La même observation est faite pour les coordonnées trichromatiques des poudres. Le % moyen de fèves violettes est de 20%, celui de fèves claires est de 11% et celui de fèves brunes est de 69%.

La teneur moyenne en azote ammoniacal (NH_3-N) observée en fin de fermentation est de 260 ppm. La teneur minimum est de 158 ppm (Bidon isolé, 20 litres) et la teneur maximum est de 299 ppm. Ces résultats confirment le bon déroulement de la fermentation quel que soit le type de fermenteur et le volume mis à fermenter. En effet, il est connu que les cacaos du Sambirano, fermentés 6 jours, présentent des teneurs en NH_3-N de l'ordre de 300 ppm[3]. Ce paramètre, classiquement utilisé par les industriels pour estimer la qualité de la fermentation, correspond au stade ultime de la dégradation des protéines en cours de fermentation[4]. Les teneurs en NH_3-N observées sont très peu dispersées avec un écart type de 30 ppm, ce qui correspond à des processus biochimiques similaires pour tous les essais réalisés. Cette observation est confirmée par une ANOVA à un facteur (type de fermenteur) associée à un test de Dunnett (bilatéral) avec les lots témoins comme référence, au seuil $\alpha = 5\%$. Il n'y a pas d'effet significatif du type de fermenteur sur la teneur finale en NH_3-N et aucun lot de cacao ne présente de teneur significativement différente des lots témoins.
La teneur moyenne en polyphénols observée en fin de fermentation est de 12 mg/g. La teneur minimum est de 9,4 mg/g (Bidon isolé, 20 litres) et la teneur maximum est de 14 mg/g (bidon, 5 L). Pendant la fermentation la couleur du cotylédon passe du violet au brun en raison de l'oxydation et de la polymérisation des polyphénols dans les structures à haute molécules. Les

polyphénols sont parmi les principaux métabolites secondaires[5], ils représentent jusqu'à 18% du poids sec sans graisse dans les graines non transformées. L'oxydation des flavan-3-ol (catechine, épicatéchine et leurs dérivés[6]) sont responsables du changement de couleur des cotylédons. Les teneurs en polyphénols observées sont très peu dispersées avec un écart type de 1,5 mg/g, les fermentations se sont déroulées de façon identiques pour tous les types de fermenteurs. Une ANOVA à un facteur (type de fermenteur) associée à un test de Dunnett (bilatéral) avec les lots témoins comme référence, au seuil $\alpha = 5\%$ confirme cette observation. Il n'y a pas d'effet significatif du type de fermenteur sur la teneur finale en polyphénols, aucun lot de cacao ne présente de teneur significativement différente des lots témoins.

Sur la base des notes moyennes des 9 juges pour les 22 descripteurs sensoriels, une analyse en composantes principales est réalisée, les 3 premières composantes principales expliquent 75.2% de variance totale des 20 lots. La représentation des échantillons selon le premier plan principal (66,2% de la variance totale) met en évidence (fig.3) une répartition des échantillons selon le sens des aiguilles d'une montre, allant des notes défavorables (végétal, astringent, boisé, amertume) pour les fermentation Bi5, S10, B10, aux notes fruits frais, acide floral et alcoolique pour les fermentations témoins et Bi20. Il n'y a pas de différences sensorielles (clusters) en fonction de la couleur de la casse des fèves.

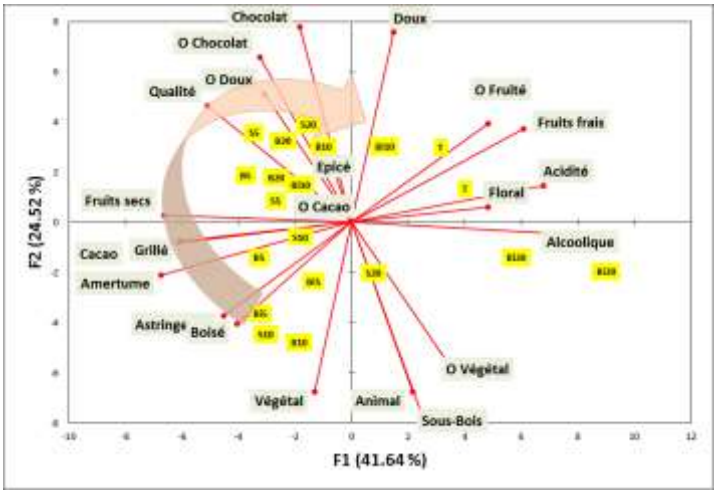

Figure 3 : Répartitions des différents lots selon le premier plan principal de l'ACP sur données sensorielles. (o, pour

Les notes de qualité sont très resserrées (écart type de 0,5) avec une note moyenne de 6. Les notes des témoins (fermentation industrielle) sont respectivement 5,9 et 6,2 pour les fèves casse foncée et casse claire.
Le lot Bi20 (bidon isolé 20L, casse foncée) a obtenu la note de qualité la plus basse (4,2) et la note maximale (6,6) a été attribuée au lot B20 (bidon 20L, casse claire). Une ANOVA a un facteur (fermenteur) au seuil 5% couplée à un test de Dunnett (bilatéral) confirme ces observations, seul le lot Bi20 est significativement différent du témoin (fig.4).

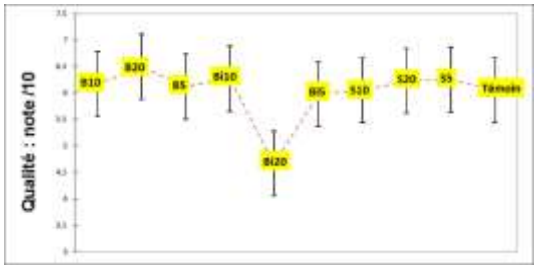

Figure 4 : Moyennes des notes sensorielles : qualité globale

Ces différents lots de cacaos présentent des profils sensoriels très proches (fig.5) caractérisés par une note olfactive fruitée (5,4), une note gustatives cacao (5,1), un ratio acidité/amertume équilibré (1,04) et une absence de notes défavorables (notes < 1,5 pour : animal, boisé, sous-bois et alcoolique)

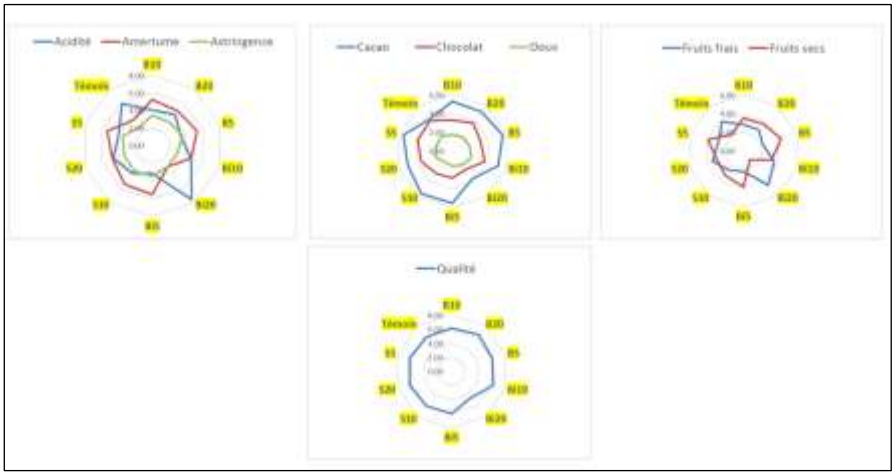

Figure 5 : profils sensoriels des 20 lots de cacao

CONCLUSION

Cette étude montre que d'un point de vue physique (profils de températures de fermentation, couleurs et cut-test) les fermentations en petit volumes (5 litres) sont possibles et identiques aux fermentations industrielles. Il est, toutefois, préférable d'utiliser des bidons isolés pour des volumes inférieurs à 10 litres, afin de conserver un volant thermique suffisant. Ces observations sont confirmées par les paramètres chimiques, marqueurs du niveau de fermentation (NH_3-N et polyphénols). Les mécanismes biochimiques de la fermentation ont bien eu lieu.
Les profils sensoriels des chocolats obtenus à partir de ces différentes fermentations sont proches de ceux des lots fermentés en conditions industrielles (témoins). Aucune note de défaut de fermentation n'est ressortie. La note moyenne de qualité obtenue est satisfaisante (6.1) et surtout identique à la note du témoin.

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COMPARISON OF THE DRYING BEHAVIOR OF FERMENTED COCOA (THEOBROMA CACAO L.) BEANS DRIED IN A COCOA HOUSE, GREENHOUSE AND MECHANICAL OVEN.

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ABSTRACT

The objective of this study was to compare the drying behavior of fermented cocoa (Theobroma cacao L.) beans dried in a traditional cocoa house replica and greenhouse-type dryer with that of beans dried in a mechanical cabinet oven at 40°C (drying for 8h with a rest period of 16h). Weight measurements were taken at the start of drying and at regular intervals during the drying process, and drying continued until there was no change in bean weight. Moisture content, water activity, pH and color attributes were measured at the start and at the end of drying. Sensory evaluation of the cocoa liquor was done on the dried samples. Initial moisture content of beans averaged 1.04 g H2O/g DM (50.9% wb). Final (equilibrium) moisture values attained after 11.4 days of drying in the cocoa house and the greenhouse and after 9.3 days of drying in the oven averaged 0.60, 0.70 and 0.50 g H2O/g DM (2.3-4.6 % wb), respectively. During the first 3 days of drying, the decline in moisture content was similar in oven-dried beans and beans dried in the cocoa house, while the decline in moisture in beans dried in the greenhouse was not as pronounced. The industry-accepted moisture of 6-8% (wb) was attained after 4.8, 6.3 and 4.3 days of drying for beans dried in the cocoa house, greenhouse and oven, respectively. Drying rate constants for the first day of drying in the cocoa house, greenhouse and oven averaged 0.1194, 0.0840 and 0.1124 1/h, respectively, and diffusivity values averaged 3.36, 2.37 and 3.17 x 10-10 m2/s. Moisture ratio curves were successfully modelled using the Hii et al. model. With respect to quality attributes, the pH of the cotyledon and testa of fresh (undried) beans averaged 4.98 and 4.86, respectively. Cotyledon pH did not change significantly with drying method, but was highest at 5.22 in oven dried beans. The pH of the testa of beans dried in the greenhouse was significantly higher than for the other drying methods. Hue angle (°) was higher in dried beans compared with fresh beans, with no effect of drying method seen.

The results of the sensory evaluation exercise revealed very little differences in bean acidity and astringency, with beans dried in the cocoa house obtaining a moderately higher score for cocoa flavor compared with beans dried in the greenhouse and in the oven.

INTRODUCTION

In the primary processing of cocoa beans, drying is a critical step that follows fermentation.

After fermentation, beans are dried to reduce the moisture content from about 60% to 6-8% (wb) for safe storage and transportation, as well as to reduce the astringency and bitterness of

the beans. Sun-drying of fermented cocoa beans is the most widely practiced method of drying. Beans are spread on raised mats or on concrete floors during periods of sunshine and allowed to dry (Lasisi, 2014). In the West Indies, drying takes place on wooden or concrete drying floors with moveable roofs (called cocoa houses) and beans are mixed regularly by walking through the layers or raked by using a wooden palette. Depending on weather conditions, beans are sun-dried anywhere from 7 to 22 days. Slow drying in thick beds can sometimes result in over-fermentation of the beans and in mold growth while too rapid drying can result in in acidic beans with shriveling and/or case hardening of the shells (Jinap and Thien, 1994; Bonaparte et al., 1997, Sukha, 2003; Lasisi, 2014; Fagunwa et al., 2009; Zahouli et al., 2010).

To offset the drawbacks associated with sun drying of cocoa beans, several researchers have investigated the use of solar dryers. From these works, it is evident that compared with open sun-drying of beans, use of solar drying can reduce the drying time during periods of poor weather conditions, but increase the drying times under good weather conditions. Bonaparte et al. (1997) found that under adverse weather conditions, beans dried in an indirect solar dryer showed the highest cut test score, and that beans dried in the solar dryer attained the desired moisture content of <8% (wb) in 6.5 days compared with sun-dried beans, which took 12.5 days. Sankat and Ramlochan (1997) reported a reduction in drying time in beans dried in a solar cabinet fermenter/dryer, with beans attaining the desired moisture value of 6-7% moisture content within 6 days. The study also revealed that an increase in drying load affects the drying behavior. Hii et al. (2006) also reported that loading density has an impact on the quality of beans dried in a direct solar. With respect to bean quality, no significant differences were reported for cocoa dried in a solar type dryer when compared to sun drying (McDonald 1981).

Artificial drying can increase moisture removal rate and save time, and so has been the focus of many drying studies on cocoa beans. Amongst these studies are wide variations in experimental variables, for example, drying temperatures, pre-treatment of beans, depth and loading density, rest intervals, bean mixing and the end-point of the drying process. Several researchers have reported that while drying is enhanced at higher temperatures, artificially dried beans, especially beans dried at higher temperatures of 55-60°C, are more acidic than sun-dried beans. Jinap and Thien (1994) found that sun- dried beans produced the lowest concentration of volatile fatty acids and were higher in pH (5.12) compared with the oven-dried (60°C) beans which had the lowest pH of 5.02 and highest titratable acidity. Irie et al. (2010) also showed that sun-dried samples were less acidic with a pH of 4.0 than oven drying at 60°C which produced a pH of 3.7. Oke and Omotayo (2011) showed that intermittent drying in an oven at 55°C produced highly acidic beans with a pH of 4.70 while the pH of sun-dried samples averaged 5.03.

The objective of this study was to compare the drying behavior of fermented cocoa (Theobroma cacao L.) beans dried using three methods, namely; a traditional cocoa house replica, a greenhouse-type dryer and a mechanical cabinet oven at 40°C. Drying rate constants and moisture diffusivities were determined and an assessment of the impact of drying method on selected quality attributes of the dried beans and cocoa liquors carried out.

MATERIALS AND METHODS

Fermented cocoa beans of mixed Trinitario varieties, were obtained from the Cocoa Research Centre of the University of the West Indies, St. Augustine Campus. Drying of fermented beans was carried out in a small cocoa house replica, a greenhouse-type dryer and a mechanical oven. The cocoa house was made of metal beams and a moveable roof made of galvanized sheets (Figure 1). The drying tray was made of wood with dimensions of L 2.13 x W 1.22 x D 0.10 m and elevated 0.09m above the ground. Each day the roof of the cocoa house was opened at 9am and closed at 5pm.

Figure 1. Miniature Cocoa House used for drying beans

a)Opened roof, exposing the cocoa beans to the sun b) Cocoa house with roof closed

Beans were dried in a greenhouse (Figure 2) approximately L 5.5 x W 2.7 m. The beans were placed onto wooden tables with built-in trays L 0.81 x W 0.61 x D 0.05 m, elevated 0.84m off the ground.

Figure 2. Greenhouse used for drying beans

a) Exterior view b) Interior view showing the drying tables on both sides

For oven drying, cocoa beans were dried at 40°C in a natural convection Unitemp Drying Cabinet (LTE Scientific Ltd., Greenfield, Oldham) with a glass door and metal shelves (Figure 3). Internal dimensions of the dryer were H 1.12 x W 0.79 x D 0.61 m.

Figure 3. Oven drying of fermented cocoa beans

a) Cabinet oven b) Arrangement of cocoa beans on wire tray

For drying in the cocoa house and greenhouse, weight (g) and temperature measurements (°C) were taken three times a day, at 9am, 12pm and at 5pm, and drying continued until there was no change in weight. For oven drying, beans were spread in a single layer (500g) onto wire mesh trays (L 0.33 x W

0.22 x H 0.06 m). The loaded trays were placed into the oven and at regular intervals the trays were taken out, quickly weighed (0.01±0.005g) using an Ohaus Explorer Pro Balance, Model EP2102C (Ohaus Corporation, NJ, USA), and returned to the oven until there was no change in weight. To mimic the traditional sun-drying regime, beans were dried for 8 hours and at the end of each drying day, the beans were placed in re-sealable plastic bags and stored in a cool room (24°C) overnight, for 16 hours. At the start of the next day, the beans were re-loaded onto the trays and drying resumed.

The moisture content of fermented and dried beans was measured in triplicate using a Halogen Moisture Analyzer HB43-S (Mettler Toledo-AG, Zurich, Switzerland). Water activity (aw) was measured using an Aqua Lab CX-2 1021 water activity meter (Aqualab, Pullman, Washington, USA). The pH of the bean testa and cotyledon (nib) was determined using a Model HI991002 pH Meter (Model HI 991002, Woonsocket RI, USA). The color of the cotyledon or cocoa nib (Hii et al, 2009a) was measured using a Konica Minolta CR-410 Choma Meter (Konica Minolta Sensing Americas, Inc., NJ, USA). Triplicate L*, a* and b* readings were recorded for each sample. L* represents lightness (0-black to 100-white), a* represents red (+ve value) and green (-ve value) while b* represents yellow (+ve value) and blue (-ve value). Hue angle (°) was calculated as given in Equation 1:

$$Hue = Arc \tan \left(\frac{b^*}{a^*}\right) \quad (1)$$

The sensory evaluation of the cocoa liquor from dried beans was carried out (in triplicate) according to methods outlined by CAOBISCO/ECA/FCC (2015) and Sukha et al. (2008). Major attributes assessed included Cocoa flavour, Total Acidity, Bitterness, Astringency, Total Fruity and Total Floral notes, rated in intensity from 0 (none) to 10 (maximum).

Drying curves, drying rates, moisture ratio (MR) values, drying rate constants (k) and effective diffusivity (Deff) values were determined as outlined by Mujaffar and Sankat (2014). Using the approach reported by Mujaffar and Lee Loy (2016), the MR data was modelled using a new thin layer model proposed for cocoa bean drying by Hii et al. (2009b), as given in Equation 2.

$$MR = a\ exp(-kt^n) + c\ exp(-gt^n) \quad (2)$$

Drying and quality data was analysed using one-way ANOVA (Assaad, 2014; Rapid publication-ready MS-Word tables for one-way ANOVA) and drying data modelled Curve Expert Professional software, Version 2.3.0 (Hyams, 2016).

RESULTS AND DISCUSSION

Drying data analysis

The average air temperatures recorded in the cocoa house and greenhouse for the drying period are given in Table 1.

Table 1. Average air temperatures

	Air Temperature °C	
Time of day	**Cocoa house**	**Greenhouse**
9 am	33.0 ± 3.4	31.8 ± 3.0
12 pm	35.1 ± 3.8	34.9 ± 2.1

5 pm	30.9 ± 3.5	31.4 ± 2.1

The initial moisture content of fermented beans averaged 1.04 g H2O/g DM (50.9% wb) with an initial water activity value of 0.958. The decline in moisture content is shown in Figure 3. As expected, the decline was more pronounced for beans dried in the cocoa house and greenhouse during the daytime. It was also noted that the beans did continue to lose weight during the evening time due to exposure to the air in the evening as well as in the early morning. The decline in moisture content, although stepwise for beans dried in the oven, was similar to that seen in beans dried in the cocoa house.

During the first three days (72h) of drying, the decline in moisture content was similar in oven-dried beans and beans dried in the cocoa house, while the decline in moisture in beans dried in the greenhouse was not as pronounced. This could have been due to the windy conditions and higher temperatures experienced at the cocoa house. Final (equilibrium) moisture values attained after 11.4 days (274h) of drying in the cocoa house and the greenhouse and after 9.4 days (224h) of drying in the oven averaged 0.0617, 0.0670 and 0.0464 g H2O/g DM (2.3-4.6 % wb), respectively. Water activity values of the beans at the end of drying averaged 0.565, 0.600 and 0.518 for beans dried in the cocoa house, greenhouse and oven, respectively. The industry-accepted moisture of 6-8% (wb) was attained after 6.3 days (151h) for beans dried in the greenhouse, which was significantly ($p \leq 0.05$) longer than the 4.8 days (116h) taken by beans dried in the cocoa house. Oven-dried beans attained this moisture value in a shortest time of 4.25 days (102h).

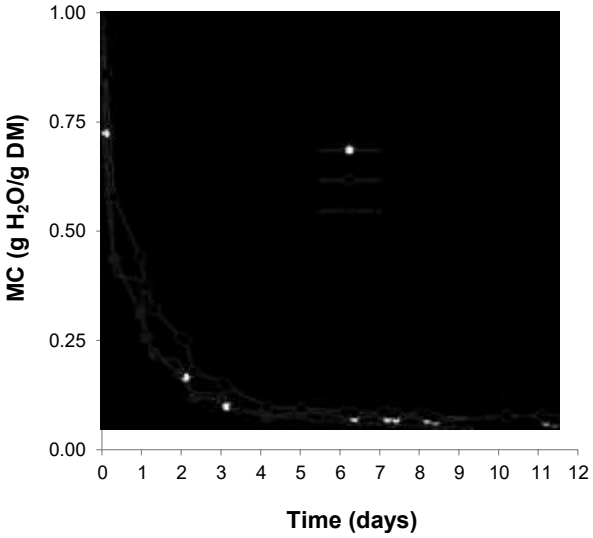

Figure 4. Drying curves for cocoa beans as a function of total drying time.

Drying rates calculated as the change in moisture content with respect to drying time revealed that initial drying rates were highest in oven dried beans (0.121 g H2O/g DM/h) and lowest in beans dried in the greenhouse (0.068 g H2O/g DM/h). For the duration of the drying period, rates were generally highest in beans dried in the oven at 40°C, followed by beans dried in the cocoa house. Drying rates were generally lowest in beans dried in the greenhouse.

Drying rate constants and diffusivity values were similar for beans dried in the cocoa house and in the oven, while these values were significantly ($p \leq 0.05$) lower in beans dried in the greenhouse. For the first day of drying in the cocoa house, greenhouse and oven, rate constants averaged 0.1194, 0.0840 and 0.1124 1/h, while diffusivity values averaged 3.36, 2.37 and 3.17 x 10-10 m2/s. Drying rate constants of 0.009583 to 0.12666 1/h were reported by Chineye (2009) for the drying of beans three kernels deep in a heated batch cocoa bean dryer at three temperatures ranging from 55-81°C and three air velocity values ranging from 1.3 -3.7 m/s.

Moisture ratio (MR) data was successfully modelled using the thin layer model proposed by Hii et al. (2009b) given in Equation 2. As shown in Figure 5, there was good agreement between the Predicted and Experimental MR values (R2 values of 0.9972 and 0.9923 for beans dried in the cocoa house and greenhouse, respectively). The MR data for oven dried beans was adequately modelled but with some deviation (R2 value of 0.9811) due to the stepwise reduction on MR at the start of the drying process.

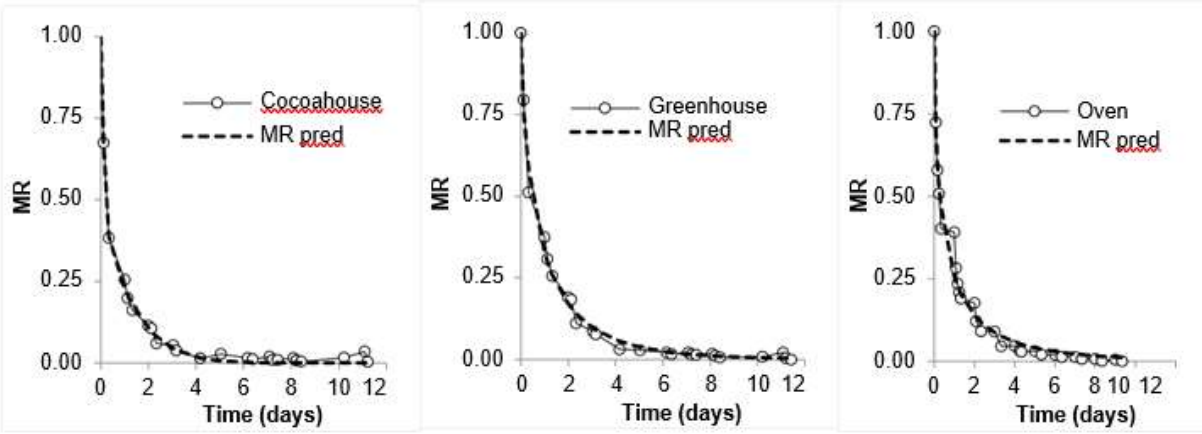

Figure 5. Predicted versus experimental Moisture Ratio (MR) values for beans.

Bean quality and liquor assessment

As shown in Figure 6, the pH of the testa of all dried beans was higher than for fresh beans (4.86), indicative of a decrease in acidity of beans as they dried. The pH of the testa of beans dried in the greenhouse was significantly higher than for the other drying methods, averaging 5.46. The pH of the testa of beans dried in the oven was the lowest at 4.97. Cotyledon pH averaged 4.98 in fresh beans and did not change significantly with drying method, but was highest at 5.22 in oven dried beans compared with 5.02 for beans dried in the cocoa house. Hii et al. (2006) reported pH values of 4.64 in fermented beans, which increased to 4.91 to 5.39 in beans dried in a solar dryer at different loading densities.

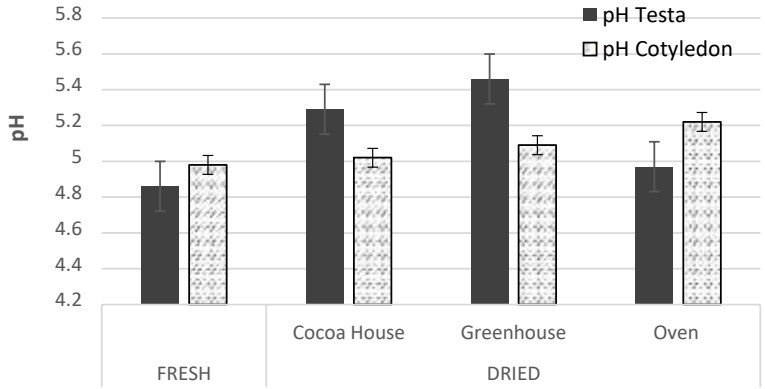

Figure 6. The pH values of fresh and dried beans

The Hunter L*, a* and b* values for fresh and dried beans are given in Table 2. Drying method did not affect bean lightness (L* values), but oven dried beans were found to be more brown/red (higher a* value) and more yellow (higher b* value) than beans dried using the sun's energy. Hue angle (°) was higher in dried beans compared with fresh beans, but no effect of drying method was seen.

Table 2. Colour attributes of fresh and dried cocoa beans.

	Fresh Beans	Dried Beans		
		Cocoa house	Greenhouse	Oven
*L**	27.0 ± 0.28	26.0 ± 1.34	25.9 ± 0.14	28.2 ± 0.14
*a**	11.6 ± 0.32^{a}	9.02 ± 0.12^{c}	8.48 ± 0.36^{c}	10.4 ± 0.06^{b}
*b**	6.59 ± 0.37^{ab}	5.8 ± 0.34^{b}	5.85 ± 0.41^{b}	7.74 ± 0.35^{a}
Hue (°)	29.5 ± 0.72^{b}	32.7 ± 1.70^{ab}	34.5 ± 1.10^{a}	36.6 ± 1.40^{a}

Values are means ± SEM, n = 3 per treatment group.
Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the LSD test.

Bonaparte et al. (1997) found that the L* values of cocoa nibs from beans dried in the sun and in an indirect solar dryer were slightly higher at 32.15 compared with the L* values of 33.93 for beans dried in a hot plate assisted direct type solar dryer. Hii (2008a) studied the effect of drying on colour change of cocoa beans that were freeze dried, sun-dried and dried in an artificial hot air dryer 60-80°C. Hii et al (2009a) reported high a* values for nibs of oven dried beans (7.96) at 60°C, indicative of more browning when compared with beans dried in the sun (7.06). While not statistically significant, beans dried in the oven were more yellow in colour (b* value of 12.51) compared with sun-dried beans (b* value of 10.40). In that study, Hue angle values were found to be higher in sun-dried beans compared with oven-dried beans.

While it is noted that the beans were dried to equilibrium moisture well below the 7% (wb) minimum moisture value that is the industry standard for storage, the results of the sensory evaluation exercise revealed very little differences in Bean Acidity, Astringency and Bitterness. Cocoa flavor was significantly ($p \leq 0.05$) higher in beans dried in the cocoa house (score of 5.0), compared with that of beans dried in the greenhouse (4.3) and oven (4.0), respectively. The acid-fruit score was lowest (0.9) in oven- dried beans and significantly higher in beans dried in the cocoa house (2.0) greenhouse (2.4). Total floral notes were present in oven-dried beans (score of 2.0) compared with significantly ($p \leq 0.05$) lower values for beans dried in the cocoa house and greenhouse (0.3-0.5).

CONCLUSIONS

The results show that fermented cocoa beans can be successfully dried in a greenhouse as well as a mechanical oven as an alternative to the traditional cocoa-house drying method. With respect to drying rates and drying time, drying can take up to 30% longer in a greenhouse-type dryer, while drying beans intermittently a mechanical oven set at 40°C can reduce drying time by approximately 10% compared with drying in a traditional cocoa-house. For both fresh and dried beans, pH was found to differ between the testa and the cotyledon (nib). Based on the pH values, the testa of the beans dried in the greenhouse, which was the slowest method of drying, was found to be the least acidic while the pH of the cotyledon of beans dried in the oven was the least acidic of the three treatments. The nibs of beans dried in the oven were found to be more red/brown and yellow than for beans dried in the cocoa house and greenhouse.

There were detectable differences in the ratings for some sensory attributes of the liquors with regard to cocoa flavour, total floral and acid-fruit, however it is noted that these beans were dried to equilibrium moisture values of 4.6 to 6.3% (wb), well below the industry standard for safe storage of beans.

NOMENCLATURE

A	Drying constant
a_w	Water activity
D_{eff}	Effective diffusivity $D = k\ (4L2/\square 2)$ where L = 0.50 x 10-2 m

DM	Dry matter (g)
FW	Fresh weight (g)
k	Drying rate constant (1/h)
L	Half-thickness of sample (m)
L, a*, b**	Colour attributes of dried beans
M	Moisture content (g H_2O/g DM) at time = t
M_o	Moisture Content (g H_2O/g DM) at time = 0
M_e	Equilibrium Moisture Content (g H_2O/g DM)
MR	Moisture Ratio $MR = (M - M_e / M_o - M_e) = A\ e^{-kt}$
R2	Coefficient of determination
t	Time (h)
wb	Wet basis (g H_2O/100g FW)

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SENSING COCOA (THEOBROMA CACAO L.) BEANS FERMENTATION BY ELECTRONIC NOSE SYSTEM

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ABSTRACT

Fermentation is a very important postharvest process where many processing properties and sensory attributes are developed. However, cocoa fermentation still remains empirical due to its complex mechanisms that evolved many microbiological changes. Some equipment such as HPLC, GC-MS, and near infrared spectroscopy may be useful to study cocoa fermentation, however they are relatively expensive, timing consuming and inaccessible to cocoa farmers. In this study, a machine learning based electronic nose system was developed to determine the fermentation time of cocoa beans. The system achieved a misclassification rate as low as 14.2 % with relatively show time and low cost.

Keyword: cocoa; fermentation; electronic nose; machine learning

INTRODUCTION

Chocolate is one of the most profitable merchandise of the global confectionary industry. The chocolate market worth 98.3 billion dollars in 2016 and the retail sale of chocolate in US alone is estimated to be 22.4 billion for 2017 (Duncan 2017). Cocoa bean (Theobroma cacao L.) is the major raw material in chocolate products. Globally, the production of cocoa bean was 4.031 million tons in 2016. Consumers are willing to pay more money for better quality chocolate, which creates price gap between mediocre chocolate and fine making chocolate. In most cases, the quality of cocoa bean is pivotal to the value of the final the chocolate products (Afoakwa et al., 2008).

The quality of cocoa bean is influenced by its variety, soil, climate, crop management and mainly by post-harvest processing (De Brito et al., 2001). Fermentation is a prerequisite for the development of cocoa flavor precursors and better processing properties (Hue et al., 2016). During there are many microbial, physiochemical and enzymatic effects that greatly change the properties of cocoa. Some researchers (Biehl et al., 1982; Biehl et al., 1985) have reported that the PH of cocoa beans can influence the formation of flavor precursors by either inhibiting or stimulating the activities of proteolytic enzymes such as endoprotease (Biehl et al., 1982; Biehl et al., 1985). Those proteolytic enzymes transform seed proteins into precursors for Maillard reaction triggered at roasting process (Biehl et al., 1993).

Cocoa fermentation still remains empirical even it has been studied for more than one hundred years. Fermentation conditions and fresh bean qualities are very difficult to control which give rise to beans of inconsistent fermentation quality, which obliges processors continuously to make changes of their formulations (Zhao et al., 2015). The formation of flavor compounds during fermentation involves a successional growth of various species of yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and, possibly, species of Bacillus, other bacteria and filamentous fungi (De Vuyst et al., 2010). In the beginning of fermentation, yeasts transform carbohydrates in cocoa pulp into ethanol and carbon dioxide. In the meantime, LAB

converts citric acid and other remaining carbohydrates in the pulp to lactic acid, slightly increasing the pH of cocoa beans (Lefeber et al., 2012). In the following stage, AAB oxidizes the produced ethanol into acetic acid (Camu et al., 2007; Sandhya et al., 2016). The microbial oxidation of ethanol into acetic acid increases the temperature, which kill the seed embryo and diffusing acetic acid inside the beans. The diffused acetic acid disintegrates the cellular membranes inside cocoa beans and triggers enzymatic conversions of substrates in the cotyledon to develop characteristic flavor precursors and color of fully fermented cocoa beans (Thompson et al., 2013). In the last stage, various species of Bacillus grow when the pH of the cocoa bean becomes less acidic and the temperature increases to 40– 50 °C due to the oxidative metabolism of ethanol.

Currently, the standard methods for determining the fermentation degree of cocoa bean is cut test. This method consists in longitudinally cutting and counting the proportion of purple and brown beans on a representative dried sample of 300 beans (Wood and Lass 2008). However, cut test is relatively time consuming and the determination is based on human observations which are inevitable inconsistent and bias. Sensory tests are alternative methods for cut test, however, it is also time consuming and required a well-trained sensory panel. Some chocolate manufacturers and researchers have applied techniques such as gas chromatography-Mass spectrometry (GC-MS) (Grün et al., 2008; Caligiani et al., 2007), High-performance liquid chromatography (HPLC) (Pätzold et al., 2006; Tomlins et al., 1990; Sandhya et al., 2016) and near infrared spectroscopy (Hue et al., 2014) to determining cocoa fermentation degree by mapping the profiles of compounds such as ammonia nitrogen, free amino acids, and volatile compounds. Those methods were reported to be useful, however, those technologies are expensive and difficult to conduct.

Electronic nose is an array of many gas sensor, mimicking the discrimination of the mammalian olfactory system for smells (Persaud and Dodd 1982). Each gas sensor gives a fingerprint response to given odors, and the response pattern of gas sensor can be recognized by certain algorithms and then performs odor identification and discrimination (Arshak et al., 2004). E-nose has been applied to access the qualities of some food materials include sausages (Eklöv et al., 1998), vegetable oils (Hai and Wang 2006), milk (Capone et al., 2001), meats (Rajamäki et al., 2006) and fruits (Saevels et al., 2004). In addition, the applications of e-nose in food quality evaluation, discrimination, and control are also very broad. However, the applications of e-nose in cocoa quality and processing controls were barely reported. Therefore, it is potentially useful to develop a universal, affordable, and fast measuring methods for cocoa bean quality determination.

Artificial neural network (ANN) is computational model used in machine learning, mimicking the cognitive processes of human. Like the human cerebral cortex, a ANN consist of layers of artificial nodes. In the basic model of the ANN, nodes are separated into different layers and connections are built between nodes that are in adjacent layers. The weight is assigned to connection between two nodes. each node calculates all the weighted inputs from connected nodes in the previous layers and processed them by transfer function. The results from the function are transferred to the connected nodes in the next layer. The effects of the synapses are represented by connection weights that modulate the effect of the associated input signals, and the nonlinear characteristic exhibited by neurons is represented by a transfer function. The learning capability of an artificial neuron is achieved by adjusting the weights in accordance to the chosen learning algorithm (Abraham 2005).

In this study, the fermentation of cocoa (Theobroma cacao L.) beans was monitor by self-built electronic nose system. The responses of the e-nose were processed by artificial neural network. The temperature and PH of cocoa beans during fermentation were recorded and cut tests were conducted as reference.

MATERIALS & METHODS

Cocoa fermentation

75 kg fresh cocoa beans (Theobroma cacao L.) were evenly distributed to 3 Styrofoam coolers (60 × 30 × 30 cm). The three coolers were placed adjacent to each other in a fermentation room with ambient temperatures varied from 20-30 °C. The cocoa beans were turned and mixed every two days.

PH, temperature measurements

Temperature, PH measurements and cut tests were taken every day (Days 0-7) after the first electronic nose reading was obtained. A thermometer (model EW-94469-40, Cole-Parmer, Vernon Hills, IL) was inserted at three different depths (top, middle and bottom) in each of the three Styrofoam coolers in order to obtain three replicates of readings for each treatment. PH measurements were carried out using an Oakton Acorn series PH meter (model WD-35613-70, Oakton, IL). the testa was separated from the cotyledons and placed in separate ceramic mortars. 10mL of distilled water was added to each and then the mixture was grounded using a ceramic pestle.

E-nose system

The design of the e-nose is based on Tan and Kerr (2018)'s work with some upgrade. The diagram of the e-nose system is shown in Fig. 1. The system consisted of five major components, including a micro pump (NMP830, KNF, Trenton, NJ), a 3-way solenoid valve (225T031, NR, Caldwell, NJ), an Arduino board microcontroller (Uno, Arduino), e-nose (gas sensors and chamber), and data acquisition system. The e-nose chamber was built from a 10cm χ 10cm χ 5cm nylon box with a 1.5cm thick Teflon top. Sensors alone with their socket were inserted into the top with sensor head inside the chamber. The e-nose had nine gas sensors from Figaro USA, INC (Arlington Heights, IL). The specification of each sensor was summarized in Table 1. The pump is always open during sampling (30s) and cleaning (100s) and closed when e-nose is reacting with gas. The valve alternated its direction to switch the e-nose from sampling model to cleaning model.

The signals (output voltage as a function of time) were collected by three data acquisition boards (Model NI9219, National Instruments, Austin, TX). A program was developed using LabView software (Version 2015, National Instruments, Austin, TX) to collect data from the DAQ. Three characters (relative peak, relaxation time, and rising time) of the responses of each gas sensor were extracted. The 'relative peak' was defined as the output peak value minus the baseline values of each sensor. The 'relaxation time' was defined as the time that the output voltage decreased from the peak value to 80% of its relative peak value. The 'rising time' was defined as the time needed before the responses of each sensor reached its relative peak.

Artificial Neural Network (ANN) setup

The three characters of each sensor were scaled to 0-1 before serving as training data. ANN training was conducted by neural Matlab network toolbox (R2017a, MathWorks, Natick, MA). There were 60 repetitions at each day of fermentation, of which 50 % repetitions were used for training the ANNs while the rest were used for validation. The scaled target data were 0, 0.13, 0.28, 0.42, 0.57, 0.71, 0.85 and 1, representing fermentation times of 0, 1,2,3,4,5,6 7 days respectively. At the beginning of training, initial weights between 0 to 1 were randomly assigned. Training was done using a backpropagation function, which updates weight and bias values according to the Levenberg-Marquardt optimization. Settings for the routine are shown in Table 2. Hyperbolic tangent sigmoid ("tansig") functions were used for hidden layers and output layers

Statistical methods

All results presented as the mean and superscript letters which indicated significant differences amongst treatments at the 95% level of confidence by Tukey's HSD. The results were compared by one-way ANOVA using JMP (Pro 13, SAS Institute Inc., Cary NC).

RESULTS AND DISCUSSION

Temperature and PH variation during cocoa fermentation

The trendlines in Fig. 1 and Fig. 2 shown the change of temperature and PH respectively during fermentation. Generally, in the fermentation process, the temperature varied between 28 to 50 °C, and the peak temperature was observed in the fourth day of fermentation when microbial action on producing ethanol and acids was about to over. The temperature of cocoa beans changes in the fermentation process was due to heat generated activities of microorganisms which transformed the substances in pulp into alcohol, carbon dioxide, organic acid and other volatiles.

The PH in testa increased from 3.6-4.5 during fermentation, however, the PH in cotyledon during drastically from 6.3 to 4.5. The observations were due to the organic acids including acetic, oxalic, phosphoric, succinic, and malic acids produced by several yeasts, penetrating the testa and gradually absorbed by the cotyledon.

Fermentation time determination by ANN

Table 3 shown the performance of the trained ANN. 14.2% overall misclassification rate was achieved. The ANN misclassified 33.3% of the verification samples from the first fermentation day. This was because cocoa fermentation didn't produce enough volatiles to reach the thresholding sensitivity of some gas sensor in the first day. In addition, we cocoa bean generated high content of water vapor in the headspace, camouflaging the volatiles. In addition, ANN may scarify the accuracy for samples from the first day in order to achieve high overall performance.

CONCLUSION

The ANN based e-nose system was proved to be successful in determining the fermentation degree of cocoa bean. Compared to traditional methods, the proposed method is much cheaper and fast. However, to make more powerful system that works for other cocoa beans, a massive data library need to be established to provide enough number of training data.

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ANNEX

Figures

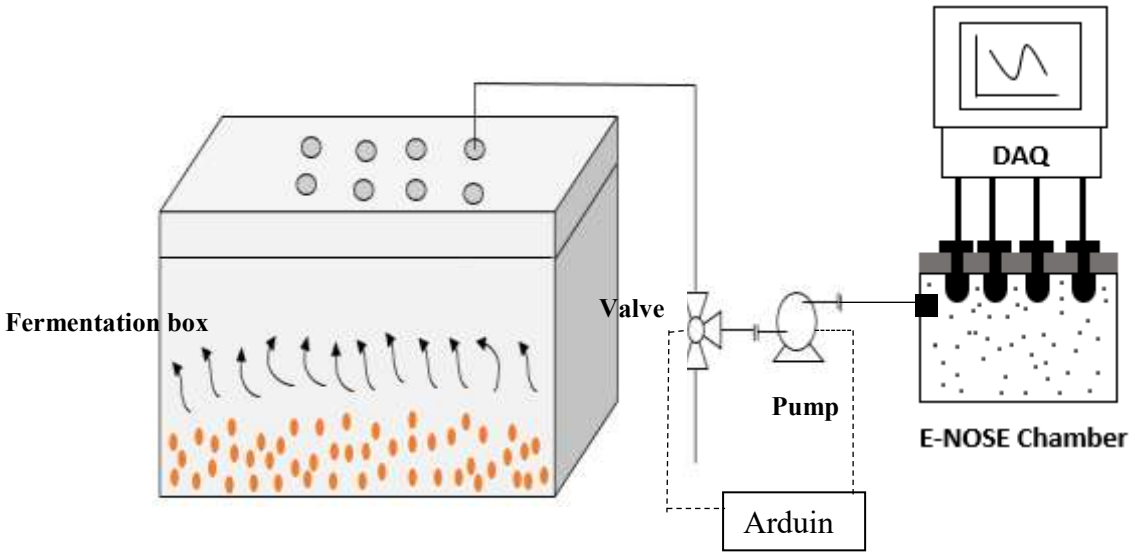

Fig. 1: The diagram of the e-nose system for cocoa fermentation

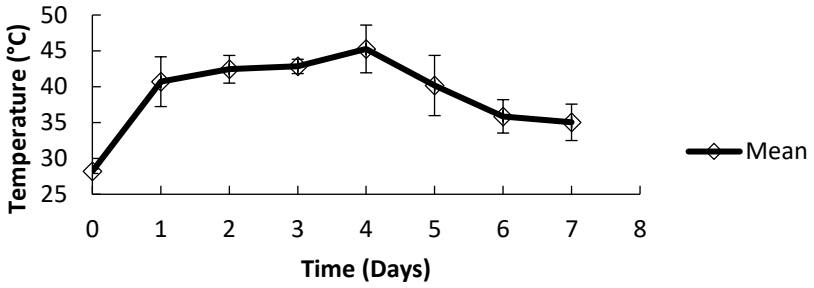

Fig. 2: The mean Temperature for the cocoa beans in the process of fermentation

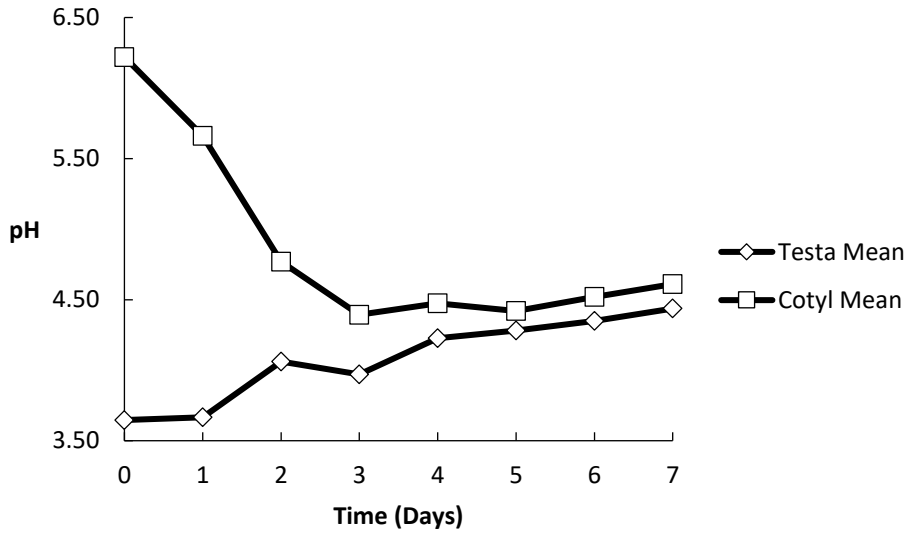

Fig. 3: The mean pH for the cocoa testa and cotyledon as a function of time in the fermentation

Table

Table 1: features and specification of the gas sensors being used for e-nose system

Sensors	Features & specification
TGS821	Hydrogen
TGS 826	High sensitivity to ammonia and ethanol
TGS813	High sensitivity to methane, propane, and butane
TGS2602	High sensitivity to VOCs and odorous gases
TGS822	High sensitivity organic solvent vapors such as ethanol

TGS2610	High sensitivity to LP and its component gases (e.g. propane and butane)
TGS2620	High sensitivity to alcohol and organic solvent vapors
TGS830	R11, R113, other halocarbons
TGS823	High sensitivity to organic solvent vapors such as ethanol

Table 2: Initial settings for training artificial neural network (ANN)

Mu	Mu-dec	Mu-inc	Iterations	Validation check
0.001	0.1	0.1	1000	5000

Table 3: Performance of ANN for classify the fermentation time of cocoa

Fermentation time (day)	Misclassification rate (%)
0	33.3
1	16.7
2	6.7
3	13.3
4	13.3
5	16.7
6	6.7
7	6.7
Overall	14.2

THE IMPACT OF POLLEN DONOR ON FLAVOR IN COCOA

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ABSTRACT

Past studies have shown evidence of pollen parent effects on yield, bean size and pod characteristics but its effect on flavor attributes is not clearly understood. An incomplete diallel mating design involving five cacao cultivars (West African Amelonado (WAA), Imperial College Selection (ICS) 1, Iquitos Mixed Calabacillo (IMC) 67 and two Trinidad Selected Hybrids (TSH) coded as CCL 200 and CCL 201) with widely differing flavor attributes were used to investigate the magnitude of female and male parent effects on key intrinsic flavor attributes. The seeds derived from pods arising from these pollinations were fermented, dried and made into cocoa liquor according to standardized methods. Flavor evaluations were carried out by a trained sensory panel for nine flavor attributes with five repetitions and hidden flavor reference controls. The study was conducted over two cocoa crop years. The results failed to detect dominant xenia effects for important ancillary flavor attributes i.e., cocoa flavor, acidity, fruitiness and floral flavors but showed significant female parent effects for cocoa and floral flavors. Small but inconsistent male parent effects were seen for astringency. Lack of xenia effect for the major flavor attributes implies that the flavor quality of cocoa beans is determined principally by the genotype of the female parent.

INTRODUCTION

Cacao (*Theobroma cacao* **L**.) possesses an unusual incompatibility mechanism, first discovered by Pound (1932), which exhibits features of both sporophytic and gametophythic systems. Many studies have investigated this incompatibility system (Cope, 1939; Posnette, 1944, 1945; Voelcker, 1936) in cacao and its effect on planting systems and yield (Lockwood, 1977; Warren et al., 1995). Over the years, extensive work has been done on pollination biology of cacao (Dias and Kageyama, 1995; Dos Santos Dias et al., 2003; Lanaud, 1987, 1988; Young, 1986, 2007; Young et al., 1987; Yong Tan, 1990). Past studies have highlighted the many differences in fruit set, bean size, shape, color and quality of the beans both within and between, particular cacao cultivars under natural and artificial pollination (Enriquez and Soria, 1968; Falque et al., 1995, 1996; Glendinning, 1963; Iwaro et al., 2003; Lachenaud, 1994, 1995).

A number of studies have described the specific effect of pollen parent on yield and some pod characteristics (Iwaro et al., 2003; Jacob and Toxopeus, 1969; Lockwood and Edwards, 1980). Although, Smulders et al. (2008) were able to differentiate the pollen donor contribution to particular pods from a known location using 15 microsatellite markers and were able to trace it to the chocolate made from these pods, they acknowledged that a link to flavor and pollen donor was needed but was outside the scope of their study. The only previous works attempting to link flavor to pollen donor effects in cocoa were Clapperton et al. (1994a, 1994b) and Lockwood and Eskes (1996). These studies reported lack of pollen donor effect on astringency involving cacao cultivars with markedly different levels of astringency. Lockwood and Eskes (1996) expressed surprise at the lack of xenia effect on flavor since xenia effects have been reported on cotyledon color. They concluded that more work needed to be done in this specific

area to arrive at a clear answer, but also recognized that experiments to investigate this phenomenon posed *"formidable technical difficulties"*. The objective of the present study was therefore to investigate if xenia effects exist for the various flavor attributes of cocoa over a range of crosses as part of a broader investigation examining factors possibly contributing to *"terroir"* in cocoa (Guittard, 2005; Nesto, 2010; Sukha et al., 2014).

MATERIALS AND METHODS

Tree selection and experimental design

The cacao cultivars chosen for this study were West African Amelonado (WAA), Imperial College Selection (ICS) 1, Iquitos Mixed Calabacillo (IMC) 67 and two Trinidad Selected Hybrids (TSH) coded as CCL 200 and CCL 201. Selection was based on the availability of sufficient numbers of verified true-to-type trees (determined by morphological characterization and simple sequence repeat (SSR) molecular marker analysis) and their widely differing flavor attributes (Sukha et al., 2008). Each of the five parents was selfed where possible and mated with the other four parents in all possible combinations analogous to a 5 × 5 diallel mating design. Selfing was possible only with WAA and ICS 1 as the other cultivars were self-incompatible. The study was conducted over two cocoa crop years, as two trials. Selected crosses were done over both crop years to validate the repeatability of results (Table 1).

Table 1. The five cacao cultivars selected for the study arranged in a 5 × 5 diallel mating design for pollinations.[z]

		Male parent				
		WAA	ICS 1	IMC 67	CCL 200	CCL 201
Female parent	WAA	1,2[y]	1,2	1,2	1,2	1,2
	ICS 1	1,2	1,2	1,2	1	1,2
	IMC 67	1	1	SI	1	1
	CCL 200	1,2	1	1	SI	1
	CCL 201	1,2	1,2	1,2	1,2	SI

[z]The cultivars West African Amelonado (WAA), Imperial College Selection (ICS) 1, Iquitos Mixed Calabacillo (IMC) 67 and two Trinidad Selected Hybrids (TSH) coded as CCL 200 and CCL 201) were selfed where possible and mated with the other four parents in all possible combinations analogous to a 5 × 5 diallel mating design carried out over two crop years, trial 1 and 2, to study the effect of male and female parent effects of flavor attributes in cocoa.

[y]1,2 = Crosses done in both trial 1 and 2; 1 = Crosses done in trial 1 alone; SI = self-incompatible types.

Pollinations, sample preparation and sensory evaluation

Trees selected for this study were pruned and fertilized 3 months before hand pollination to increase flowering and tree yield. These trees were sprayed with a broad-spectrum pyrethroid insecticide and copper fungicide 1 week before hand pollination to remove ants (family Formicidae) and other crawling insects that could cause unwanted open pollinations and to eliminate any moss covering flower cushions. Flower buds on trees selected as pollen donors and pollen recipients that would open within 24 h were identified and covered with pollination hoods and secured to the tree with 25 mm long stainless steel pins.

Hand pollinations were done between the hours of 0700 and 1200 HR daily using the method described by Doodnath (1996). Pollinated flowers were monitored daily and a successful pollination was considered to have occurred when after 3 d the flower was in a "swollen ovary" state. This was the first visible sign that the ovules had been fertilized. Flowers usually abscised and fell off the tree within 24 to 48 h with unsuccessful pollinations.

Trees in this study were kept in an optimal physiological state by irrigation and application of a foliar fertilizer (7N−9.6P−5.8K) to minimize the incidence of cherelle wilt. After 50 to 70 d when the susceptible period for cherelle wilt had passed, the immature pods were covered, where possible, with wire cages to prevent attacks by neotropical red squirrels (*Sciurus granatensis*) and orange-winged parrots (*Amazona amazonica*). Immature pods were checked weekly and sprayed with copper fungicide in two heavy applications (30 $g \cdot m^{-2}$) mixed with a sticker solution (0.5 $mL \cdot m^{-2}$) during the 6-month growing period to protect against black pod disease (*Phytophthora* spp.)

Fully mature disease-free pods from successful pollinations were harvested and both primary (fermentation and drying) and secondary (bean roasting and liquor preparation) processing were conducted using the methods described in Sukha et al. (2008). Sensory panel training as well as flavor evaluations were also carried out according to the methods described in Sukha et al. (2008).

Data analysis

Individual flavor attribute scores from the two trials were entered into Microsoft Excel (Microsoft, Redmond, WA) for a pooled analysis. Analysis of variance (ANOVA), paired *t* tests, Tukey-Kramer test and linear regression analysis were carried out on the sensory data, as well as, female parent × pollen donor interactions determined from ANOVA data.

It was not possible to use the entire dataset at the same time for ANOVA, due to the presence of self-incompatible crosses in the diallel mating design and because the study was conducted as two trials over two crop years. To address this, a number of smaller diallels on combined trial 1 and trial 2 data were created from the crosses in the main 5 × 5 diallel design (Data not presented).

General linear model (GLM) ANOVA using MINITAB (release 14; Minitab, State College, PA) was carried out on data from each smaller diallel for trial 1 and trial 2 to determine the significance of female parent, pollen donor and female parent × pollen donor interaction effects over the five repetitions of flavor evaluations per sample. The F test values from the GLM ANOVA were used to gauge the relative magnitude of the effects.

Paired *t* tests gave better resolution to the ANOVA results by focusing on the difference between paired sets of reciprocal crosses and presenting the probability that the actual mean difference was consistent with zero. Comparisons of reciprocal crosses allowed examination of significant effects on flavor with a specific combination of crosses and involved the same pair of accessions where each accession has the opportunity to be the female parent and pollen donor in turn.

RESULTS

The ANOVA results from the pooled trial 1 and trial 2 data (Table 2) showed a general trend indicating that the female parent effects were significant ($P \leq 0.001$ to $P \leq 0.05$) in majority of the diallels for cocoa, acid, floral, nutty, raw/beany/green and other flavor attributes, whereas the female parent effects for astringency and fruity flavors were significant only in one diallel, each.

In contrast, the male parent effects were not significant for all flavor attributes in vast majority of the diallels, except nutty and other which showed significant male parent effects in some diallel designs. Significant interaction effects ($P \leq 0.001$ to $P \leq 0.05$) were evident for nutty and other flavor attributes in several diallels. Even when the interaction was significant in two diallels for cocoa flavor and one diallel for floral, the magnitude of the F value was smaller than the female parent effects. Bitterness was the only flavor attribute that did not show significance ($P \leq 0.001$ to $P \leq 0.05$) for male parent, female parent, or male parent × female parent interaction effects.

Paired *t* test between reciprocal crosses revealed that the greatest significant ($P < 0.001$) effect was observed for floral flavor. This effect was strongest (greatest difference in average scores) when CCL 200 was the female parent in the cross. The largest difference in floral flavor occurred when crosses ICS 1 × CCL 200 and CCL 200 × ICS 1 were compared to each other. Their average scores for floral were 0.55 and 5.78, respectively on the 10-point attribute scale. Other reciprocal crosses with big differences in floral flavor occurred with CCL 201 × CCL 200 and CCL 200 × CCL 201 as well as WAA × CCL 200 and CCL 200 × WAA (0.98 and 6.15; 0.35 and 5.14, respectively).

Significant ($P \leq 0.001$ to $P \leq 0.05$) reciprocal effects, albeit with smaller differences between reciprocals, were observed for cocoa flavor, astringency and other flavors, whilst no significant reciprocal effects ($P > 0.05$) were observed for acid and fruity flavors. Whilst reciprocal crosses do not specifically identify female parent or pollen donor effects they do allow examination of significant effects on flavor, with a specific combination of crosses. These effects occurred in reciprocal crosses involving CCL 200, CCL 201 (cocoa flavor and astringency) and WAA, CCL 200 (other flavors). Similarly, significant (P □ 0.01) reciprocal effects were observed for nutty flavor when WAA, ICS 1; WAA, CCL 200 and CCL 200, CCL 201 were the parents in reciprocal crosses.

Table 2. Summary of significant effects across nine flavor attributes for cacao female parent, pollen donor and female parent × pollen donor interactions from general linear model (GLM) analysis of variance (ANOVA).[z]

Dialle l	Cocoa		Acidity		Astringency		Bitterness		Fruity		Floral		Nutty		R/B/G[y]		Other	
	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value
Female parent effects																		
1	NS	2.72	*	4.90	NS	0.24	NS	2.28	NS	2.81	**	7.78	NS	1.71	**	8.02	**	13.35
2	**	13.14	NS	1.39	NS	1.09	NS	0.33	NS	1.81	NS	1.11	**	6.28	*	3.12	**	5.29
3	**	51.06	**	13.96	*	5.14	NS	1.37	NS	3.30	**	111.88	**	14.7	---	---	NS	0.48
4	NS	1.95	NS	1.98	NS	0.57	NS	2.89	NS	0.59	NS	0.18	NS	2.17	**	9.89	NS	3.09
5	**	15.47	*	2.97	NS	0.42	NS	1.73	NS	2.60	**	103.09	**	7.51	*	2.82	**	6.76
6	**	9.47	NS	0.34	NS	0.37	NS	1.25	*	4.55	**	10.32	**	6.84	**	8.88	**	17.68
Pollen donor effects																		
1	NS	1.95	NS	1.29	NS	1.81	NS	0.8	NS	1	NS	1.91	**	5.58	NS	1.08	*	2.96
2	NS	0.51	NS	1.29	NS	0.56	NS	0.99	NS	0.61	NS	0.59	**	7.06	NS	0.74	NS	0.75
3	NS	0.07	NS	2.57	NS	0.62	NS	0.97	NS	0.42	NS	0.72	NS	0.23	---	---	NS	1.29
4	NS	2.39	NS	0.02	NS	1.0	NS	0.21	NS	1.57	NS	1.51	NS	3.03	NS	2.01	NS	0.01
5	NS	0.39	NS	1.35	**	5.1	NS	0.27	NS	1.95	NS	0.25	**	5.35	NS	1.16	NS	1.65
6	NS	2.33	NS	0.46	NS	1.23	NS	1.2	NS	0.18	NS	1.13	**	5.23	NS	1.42	*	3.78
Female parent × Pollen donor interactions																		
1	NS	1.28	NS	2.32	NS	0.71	NS	1.93	NS	1.35	NS	2.41	**	5.73	NS	1.85	NS	2.14
2	**	4.09	NS	1.31	NS	1.48	NS	1.90	NS	1.32	**	50.48	**	7.22	NS	0.70	**	7.06
3	NS	0.66	NS	0.01	NS	0.77	NS	0.19	NS	0.22	NS	0.33	NS	0.71	---	---	*	3.59
4	NS	0.33	NS	2.56	NS	1.54	NS	0.64	NS	0.18	NS	0.21	*	4.59	*	3.18	NS	0.24
5	**	3.60	NS	1.72	NS	1.97	NS	1.69	NS	1.06	NS	1.43	**	3.99	NS	0.70	**	3.24
6	NS	1.99	NS	1.53	NS	0.91	NS	1.89	NS	1.19	NS	1.40	**	5.67	NS	1.79	**	3.32

[z] Analysis of variance carried out on data from each smaller diallel for trial 1 and trial 2 (listed in Table 2) over five repetitions of flavor evaluations per sample. The F test values were used to gauge the relative magnitude of the effects.
[y]R/B/G = raw/beany/green flavors.
*, **, *** significant differences by GLM ANOVA at P □ 0.05; 0.01 and 0.001 respectively; NS = nonsignificant; --- = Analysis of variance not possible due to zero scores.

Linear regression analyses from pooled trial 1 and trial 2 sensory data was carried out to determine whether the average score for each cultivar over all crosses as male or female parent was related to the score of the actual cross. The independent variable in the linear regression was obtained from the average scores for all possible combinations of crosses, repetitions and trials in the overall diallel mating design (Table 1) for each female parent in turn. Averages for similar combinations were also calculated for each pollen donor in turn. The dependent variable was derived from the average score for a particular flavor attribute, for each individual cross.

A summary of significance of linear relationships between ($P \leq 0.001$ to $P \leq 0.05$) female parent and progeny and pollen donor and progeny are presented in Table 4 using the values of slope and the significance of these values, as well as, magnitude of these effects (given by the t statistic values) from linear regression analyses. There were significant ($P \leq 0.001$ to $P \leq 0.05$) female parent effects for all flavor attributes except astringency, indicated by slopes significantly ($P \leq 0.001$ to $P \leq 0.05$) larger than 0. The slopes were particularly large for floral and cocoa flavors (0.72 and 0.96, respectively) and significantly different from zero indicating a strong dependency of the female parent for floral and cocoa flavors (Table 4). The coefficient of determination for the regressions were 0.72 and 0.97 for cocoa and floral flavors respectively. In contrast the male parent effect on bean quality were largely not significant except for astringency which was significant at $P < 0.05$.

DISCUSSION AND CONCLUSIONS

The objective of this study was to examine the effect of pollen donor on the flavor attributes of selected cacao cultivars to determine if there was a xenia effect on flavor and the diallel mating design provided an effective framework to investigate this premise. Pollen donor effects have been studied in other crops, such as apple and cherry, for other quality attributes such as firmness and acid content (Davarynejad et al., 1994) and fruit size and total soluble solids (Ansari and Davarynejad, 2008) but rarely on flavor.

The optimized organoleptic evaluation methods (Sukha et al., 2008) provided robust data which were able to provide statistically valid results. This highlights the effectiveness of the sensory design in being able to provide data from which we are able to resolve significant effects from small differences in scores. The results showed that flavor of cocoa beans was determined largely by the genotype of the female parent and with a possible marginal effect of pollen donor on flavor, confined mainly to astringency, nutty and other flavors. Due to the inconsistency of significance of male parent effects over crosses, and the relatively small effects one cannot preclude type II error. However, F test values and t statistic values were presented where possible for each flavor attribute to gauge the relative magnitude of the male and female parent effects observed.

The possibility of the pollen donor contributing to bean flavor originates from the fact that the endosperm of the seed is determined by male and female gametes. However, this appears to be superseded by the strong influence of the seed coat and mucilage (pulp), that is entirely maternally derived since the integument of the matured ovule develops into the seed coat which shares the same diploid genotype of the mother tree (Fang et al., 2014). Fresh cocoa seeds are characterized by an astringent and bitter taste due to the high content of phenolics, especially anthocyanins

(Jinap et al., 2005) and because they do not contain the necessary aroma precursors (Ziegleder and Biehl, 1988).

Many authors including Andersson et al. (2006) have highlighted the kinetics of bean acidification during fermentation, on flavor development. The maternally derived seed coat and pulp is degraded by yeasts, lactic- and acetic acid bacteria resulting in lactic and acetic acid formation as a result of exothermic reactions. The acids permeate though the differentially permeable seed coat into the seed tissue and, together with the heat evolved during fermentation, cause seed death. The storage proteins and carbohydrates are subsequently degraded by seed enzymes yielding aroma precursors which are peptides, free amino acids and reducing sugars (Afoakwa et al., 2008; Andersson et al., 2006; Kadow et al., 2013; Schwan and Wheals, 2004; Ziegleder and Biehl, 1988).

In addition to the endogenous formation of flavor and aroma precursors from fermentation and drying, there is also mounting evidence for the exogenous influence of the pulp directly on aroma and flavor development. Eskes et al. (2007, 2012) discuss the direct permeation of aroma components from the pulp into the seed tissue during fermentation that may be retained during the drying process.

The study therefore failed to detect xenia effects for most of the important flavor attributes i.e., cocoa flavor, acidity, fruitiness and floral flavor which agrees with the preliminary work of Clapperton et al. (1994a, 1994b) and Lockwood and Eskes (1996) suggesting a general lack of xenia effect on flavor in cacao and implies that the flavor quality of cocoa beans is determined principally by the genotype of the female parent.

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EVIDENCE FOR APPLYING THE CONCEPT OF "TERROIR" IN COCOA (*THEOBROMA CACAO* L.) FLAVOUR AND QUALITY ATTRIBUTES

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ABSTRACT

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

INTRODUCTION

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

role of the main operations during chocolate manufacturing in expressing the genetic flavour potential of the cocoa beans as a chocolate.

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

MATERIALS AND METHODS

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

Processing Location study

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

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

Growing Environment study

In the GE study, all the major factors affecting flavour were kept constant except for the growing environment. Samples from the same clones were taken from four growing locations with different soils and rainfall patterns in the north and east of Trinidad: LRE, Centeno (North Trinidad), UWI, St Augustine (North Trinidad), the University Cocoa Research Station (UCRS), Centeno (East of LRE) and Marper Farm, Manzanilla (East Trinidad). Micro-fermentations were all conducted in a fermentation box at one processing location and the samples were sun-dried in wooden trays until reaching 6 - 7% moisture content. The number

of clones used in each location depended on the availability of verified true-to-type trees. Samples from two clones (IMC 67 and ICS 1) were taken from UCRS, UWI and LRE, those from four clones (West Africa Amelonado (WAA), GU 243/H, ICS 84 and SCA 6) were taken from UCRS and UWI and those from two clones (JA 5/5 [POU] and PA 165 [PER]) were taken from UCRS and Marper Farm.

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

Liquor preparation and sensory evaluation

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

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

Near infrared reflectance spectroscopy (NIRS)

Representative fermented and dried bean samples from clones in the processing location and growing environment experiments were sent to the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France. NIRS acquisitions were performed on a Foss-Perstorp 6500 using a spin cell. Three grams taken from 100 g of hulled, ground and sieved (<0.5 mm) cocoa samples were analysed in diffuse reflectance from 400 nm to 2,500 nm in 2 nm steps.

Data analysis

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

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

RESULTS

Sensory data – PL effects

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

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

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

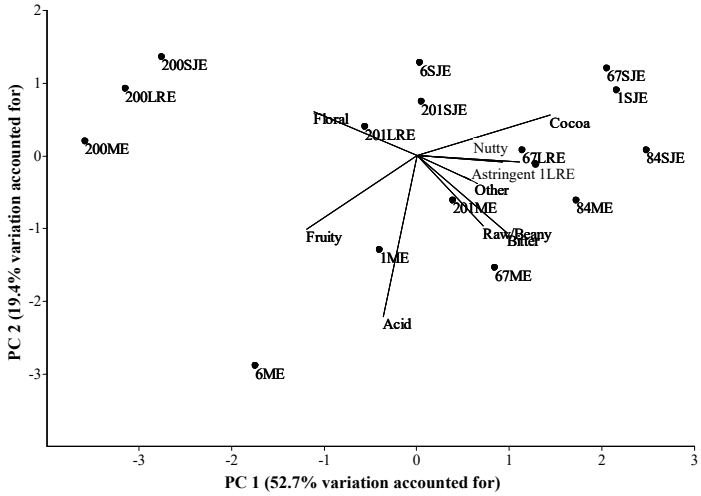


Figure 1. PCA plot of different flavour attribute scores for cocoa samples processed at different locations in Trinidad.

Sensory data – GE effects

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

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

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

Acid and fruity flavours were significantly higher ($p \leq 0.05$ and ($p \leq 0.01$ respectively) for clone WAA grown at UWI compared to UCRS (Figure 3). Scores for 'other' flavours were generally

less than one and the only significant effect was a higher score at the UCRS for WAA ($p \leq 0.01$) (Figure 3). The descriptors used for 'other' flavours by panellists were for an off flavour described as "over-roasted" or "putrid".

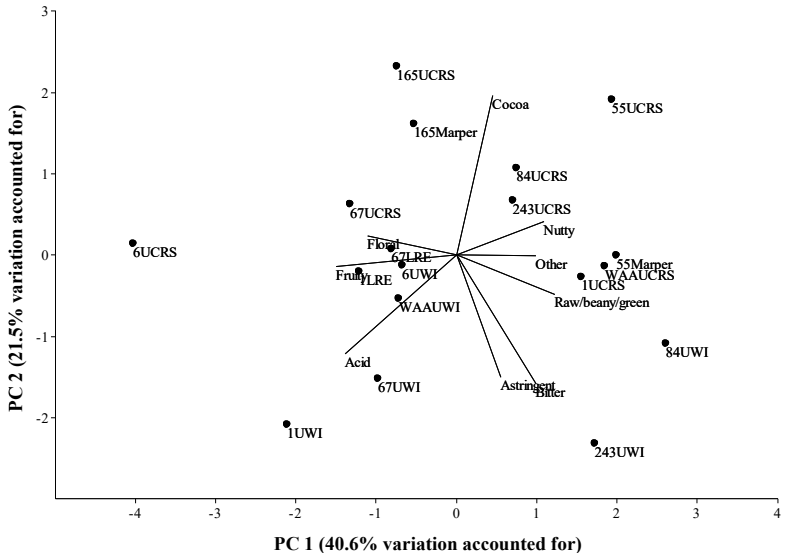
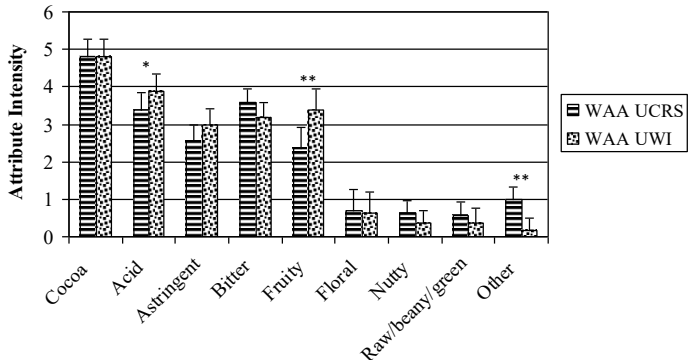


Figure 2. PCA plot of different flavour attribute scores for cocoa samples grown at different locations in Trinidad.



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

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

Near infrared reflectance spectroscopy data – PL effects

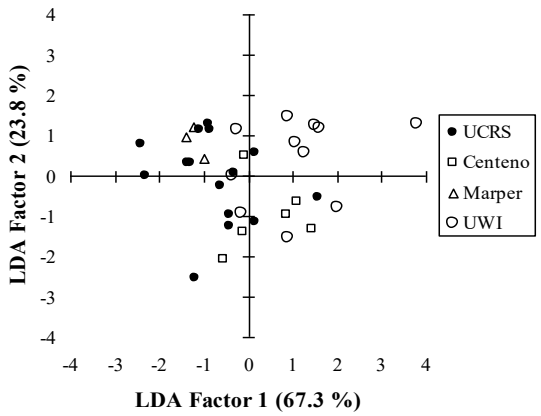
Spectral data were generated on all samples except ICS 84 and SCA 6, for which insufficient beans were available. Linear discriminant analysis was done on the first 10 PCs extracted from the PCA analysis of spectral data from ICS 1, IMC 67, CCL 200 and CCL 201 processed at the three locations. Results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

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

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

Growing environment effects

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



cocoa beans of the same clones from four growing environments and processed at a single location in Trinidad.

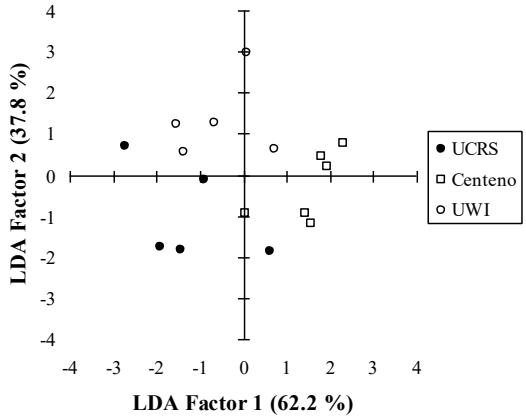


Figure 5b. Linear discriminant analysis of factors from spectral data on cocoa beans of the same clones from three growing environments and processed in a single location in Trinidad.

DISCUSSION CONCLUSIONS

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

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

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

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

dried at SJE were heaped at the centre of the tray for drying after heating up the drying tray for 2 hours. The differences in bean layer thickness for drying would change the surface area available for drying and thereby strongly affect the drying rate.

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

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

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

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

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

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

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

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

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

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DESARROLLO E IMPLEMENTACIÓN DE UN PROTOTIPO DE ACERO INOXIDABLE PARA EVALUAR EL PROCESO DE FERMENTACIÓN DE GRANOS DE CACAO.

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RESUMEN

En la post-cosecha del cacao se ha encontrado un gran potencial para implementar tecnologías que mejoren estos procesos, en particular el de fermentación de cacao, es así que se desarrolló un prototipo de acero inoxidable para llevar a cabo la fermentación de este proceso, con el propósito de generar alternativas de uso práctico para productores de este cultivo y obtener un producto con características deseables de calidad. El prototipo desarrollado se instaló en la Asociación de Productores de Banano y Cacao Orgánico (ASPROBO); consta de un sistema mecánico que realiza remociones de granos de cacao a velocidades bajas necesarias para el desarrollo del proceso fermentativo, además permite la recolección del mucílago que se segrega durante los primeros días de la fermentación, para ser utilizado en otras aplicaciones; también posee un sistema de aislamiento que evita pérdidas de calor y humedad en los granos, esto asegura la proliferación de los microorganismos responsables del proceso.

La recolección de datos de temperatura y de los sucesos que ocurren durante la fermentación, se lograron con sistemas embebidos, conectados a diversos sensores que forman parte del prototipo. El sistema adquiere los datos con un tiempo de muestreo igual a 10 segundos mostrándolos en tiempo real, mediante una interfaz con un entorno intuitivo y sencillo de utilizar. Este sistema permite tener acceso al proceso desde una página Web, para monitorear, activar y desactivar las principales funciones.

Para evaluar los resultados se fermentaron tres lotes de 100 Kg de granos de cacao en el prototipo construido y paralelamente 500 Kg de granos en cajas de madera, con la finalidad de comparar la temperatura, humedad y pH en ambos lotes. Al finalizar la fermentación se concluyó que las temperaturas en el prototipo de acero inoxidable fueron uniformes en los distintos niveles de la masa, hubo menor interferencia de la temperatura ambiente sobre la masa fermentada, y los valores de humedad y PH coincidieron con los rangos establecidos por diferentes autores internacionales. Por otro lado en los cajones de madera se notó un gradiente significativo de temperatura en los diferentes niveles de la masa en el proceso de fermentación y, también mayor influencia de la temperatura ambiente, provocando que los granos se enfríen notablemente por las noches, disminuyendo el porcentaje de granos fermentados y con ello la calidad del mismo.

INTRODUCCIÓN

La fermentación es una etapa muy importante en el procesamiento del grano de cacao, ya que se producen cambios bioquímicos que dan origen a los precursores de aroma y sabor [1].En la fermentación del cacao existen una serie de factores que contribuyen al resultado final de los granos fermentados; dentro de estos factores tenemos el tipo de cacao. Los diversos tipos de cacao tienen características físicas totalmente diferentes pero la forma de fermentar y los pasos

para la fermentación es la misma con diferencias en el tiempo de fermentación y la frecuencia de remoción.

Otro factor importante es la frecuencia con que se realizan las remociones y el tipo de fermentación que se utiliza, los cuales pueden variar de acuerdo al volumen de producción de la finca, entre los más usados y conocidos tenemos: sacos, montones, cajones y tambor giratorio [2].

Conociendo la gran importancia que tiene la etapa de fermentación surge la necesidad de desarrollar como alternativa un fermentador que permita tener un buen grado de granos fermentados, sin alterar la calidad final del cacao. La mejora e innovación está en diseñar un prototipo para fermentar cacao que funcione de forma automática, es decir, que cuente con sensores de temperatura, sistema de remoción que permita una fermentación más homogénea y un grado de fermentación mayor al obtenido con el proceso tradicional (fermentación en cajas de madera), Además que incluya un sistema de monitoreo y control que permita llevar un registro de los parámetros medidos (temperatura y tiempo de fermentación) durante todo el proceso, con la finalidad de mejorar la calidad y producción de cacao.

DESCRIPCIÓN Y FUNCIONAMIENTO DEL PROTOTIPO DISEÑADO

El diseño del prototipo de acero inoxidable se realizó en un software CAD (diseño asistido por computadora) para modelado mecánico en 3D (Solidworks), con la finalidad de hacer los estudios correspondientes y las simulaciones necesarias en cada parte del fermentador. El fermentador consta de cuatro partes principales (Figura 1)

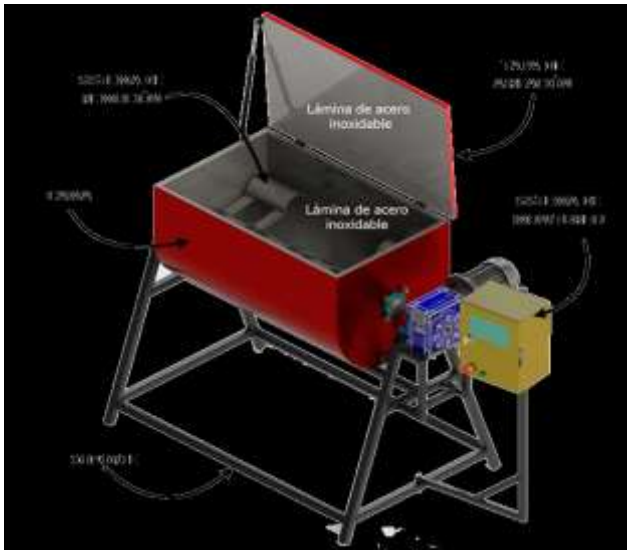

Figura 1 Partes del fermentador

El fermentador está en un inicio abierto (Figura 2 (a)), el cual se llena de granos de cacao frescos hasta el 50% de su volumen, luego se cierra la tapa para dar inicio a la fermentación; con la tapa cerrada el fermentador permanece estático para que se desarrolle la fase anaeróbica (Figura 2(b)), cuando es necesario realizar la remoción se activa un motor eléctrico que hace rotar lentamente un eje con un sistema de paletas, que permite reordenar la masa dentro de la cama del fermentador y homogenizar la temperatura en los granos; a la vez que se realiza este paso, la tapa del fermentador se levanta por un tiempo determinado para que ingrese aire fresco y expulse el CO_2, garantizando la aireación de la masa y dar inicio a la fase aeróbica (Figura 2 (c)). Luego se cierra la tapa y la masa vuelve a quedar en reposo hasta la próxima remoción (Figura 2 (d)).

Una vez concluida la fermentación, la cama debe girar 120° grados (Figura 2(e)), luego se abre la tapa y se activa el mecanismo de remoción para expulsar los granos de cacao fermentado (Figura 2(f)).

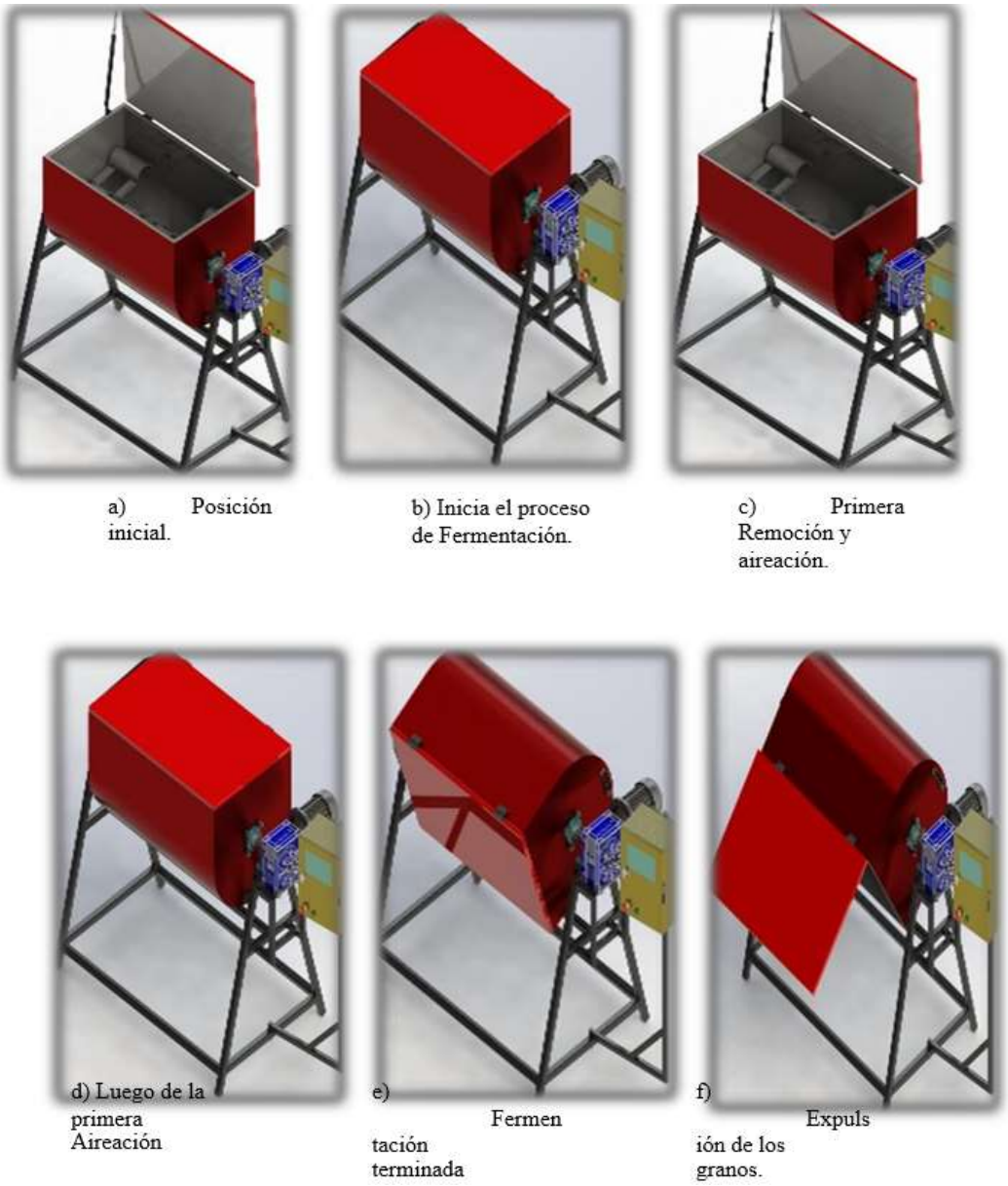

Figura 2 Funcionamiento y posiciones del fermentador.

MÉTODOS PARA OBTENER LOS PARÁMETROS A EVALUAR

Temperatura

Para medir la temperatura en la masa de cacao se utilizaron 4 RTD's PT 100 de cabezal con 4 hilos ubicados en diferentes niveles del cajón y del prototipo fermentador de acero inoxidable. En el cajón fermentador se ubicó una RTD en la parte inferior, en el centro y en la parte superior; la cuarta RTD se ubicó fuera del cajón para monitorear la temperatura ambiente (Figura 3). Para el fermentador de acero inoxidable la distribución de las RTD es similar al cajón y la cuarta RTD se ubicó en la parte vacía dentro de la cama para determinar la temperatura de los gases que ahí se almacenan (Figura 4).

Figura 3 Sistema de monitoreo de temperatura en cajones de madera.

Figura 4 Ubicación de las RTD's para el monitoreo de la temperatura en el fermentador

Humedad

Para determinar el contenido de humedad en los granos de cacao, se usa la norma técnica peruana ISO 2291:2006 [3, 4]. En esta investigación se utilizó un analizador de humedad Radwag PMC; este analizador sirve para indicar la humedad relativa de pequeñas muestras de diferentes materiales, para este caso es una muestra de 10 gramos de cacao (Figura 5).

Figura 5 Analizador de humedad Radwag PMC, utilizada para medir la humedad en los granos de cacao.

Para medir la humedad se tomó muestras una vez al día durante todo el proceso de fermentación, tanto en las cajas de madera como en el fermentador de acero inoxidable.

PH

Para medir el potencial de hidrógeno en el proceso de fermentación, se usa por lo general los métodos establecidos por la AOAC[1] [5], método N° 970.21; sin embargo existen otros métodos confiables como los usados por Pedro García [6] es su tesis doctoral.

Se tomó medidas del pH por separado en el mucílago y en el cotiledón, cada 24 horas durante el proceso de fermentación, las muestras se extrajeron del fondo, centro y parte superior del cajón y para el fermentador de acero inoxidable solo se extrajeron muestran del centro.

Para el mucílago se tomó aproximadamente 10 gramos de muestra y se diluyeron en 50 ml de agua destilada, se agitó con una licuadora, luego se filtró con papel Whatman #4 y se realizó la medición con un indicador de pH HANNA Instruments HI8510E (Figura 6).

Figura 6 Indicador de pH HANNA Instruments HI8510E, utilizado para medir el pH en los granos de cacao.

Para el cotiledón se precedió de forma similar que para el mucílago, tomando 50 gramos como muestra y se diluyeron en 100 ml de agua destilada.

EXPERIMENTACIÓN

El objetivo de este trabajo fue comparar los resultados de algunas variables que intervienen en el proceso de fermentación. Para ello se tomaron medidas de temperatura, humedad y pH de la masa en los cajones de madera (proceso tradicional) y en el fermentador diseñado (prototipo construido de acero inoxidable).

La fermentación tradicional se desarrolló en cajones de madera cúbicos con capacidad de 500 kilos de cacao en baba. Se realizaron tres remociones; la primera remoción se realizó después de 24 horas de iniciarse el proceso de fermentación, la segunda se hizo a las 52 horas y la tercera a las 120 horas, en total la fermentación duro aproximadamente 6 días.
Para los granos de cacao que se fermentaron en el prototipo de acero inoxidable desarrollado en la Universidad de Piura, se utilizaron lotes de 100 kilos de cacao en baba. Para poder determinar las condiciones adecuadas de fermentación en el nuevo diseño, se realizaron tres ensayos, en cada ensayo o lote fermentado se cambiaron los tiempos de remoción y las condiciones de aireación.

[1] Association of the Analytical Chemists - AOAC

- **Lote 1**: La fermentación duro 8 días y medio (204 horas). La primera remoción se realizó a las 24 horas, la segunda se realizó a las 72 horas, la tercera a las 120 horas y la última a las 192 horas. Durante las 120 primeras horas el fermentador de acero inoxidable permaneció cerrado y solo se abrió para hacer la remoción.
- **Lote 2**: La fermentación duró 7 días y las remociones se realizaron cada 24 horas, después de la primera remoción la tapa del fermentador permaneció abierta y se cubrieron los granos con hojas de banano, esto facilitó la fase aeróbica del proceso.
- **Lote 3**: En el tercer lote, la fermentación duro 7 días y las remociones se realizaron cada 24 horas, después de la primera remoción la tapa del fermentador permaneció abierta y se cubrieron los granos con hojas de banano y sacos de polietileno color negro.

Las remociones se realizaron a una velocidad de 7 rpm por un periodo de 3 minutos, con una frecuencia en el variador de 13 Hz.

RESULTADOS DE LA FERMENTACIÓN EN CAJAS DE MADERA

Medidas de temperatura

La temperatura se registró de dos formas; una de manera puntual, en la que se tomó la temperatura todos los días en los diferentes niveles del cajón (Figura 7) y la segunda forma, fue monitorear la temperatura de manera constante durante todo el proceso de fermentación (Figura 8) con un sistema de adquisición de datos desarrollado por el laboratorio de Sistemas Automáticos de Control (Universidad de Piura).

En ambas figuras las diferentes líneas de colores indican la temperatura en un nivel del cajón, tal como se indica en sus respectivas leyendas. En la Figura 8 la línea punteada de color azul indica el valor de la temperatura ambiente; esta tiene importancia en este estudio porque nos permite evaluar la interferencia que se tiene en el proceso de fermentación

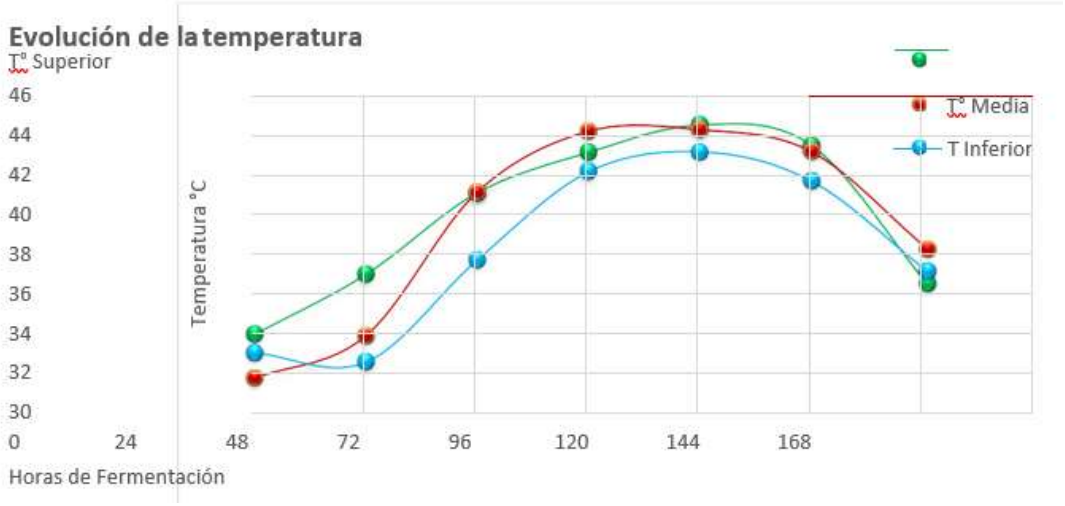

Figura 7 Evolución de la temperatura por días de fermentación en cajas de madera

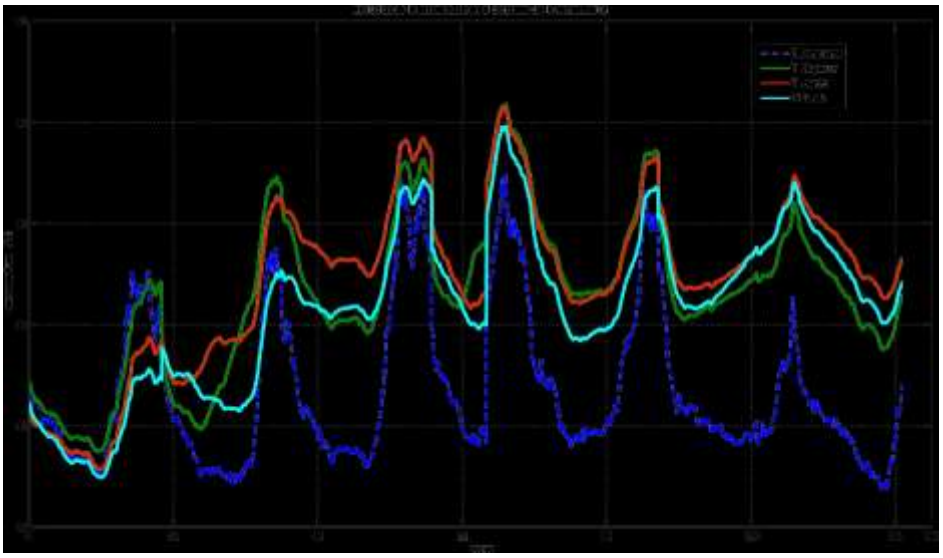

Figura 8 Evolución de la temperatura en tiempo real en cajas de madera

Medidas de humedad

La humedad se midió de forma puntual, una vez al día durante todo el proceso de fermentación, y cuyos datos de humedad obtenidos durante el proceso de fermentación en los cajones, se muestra en la Figura 99.

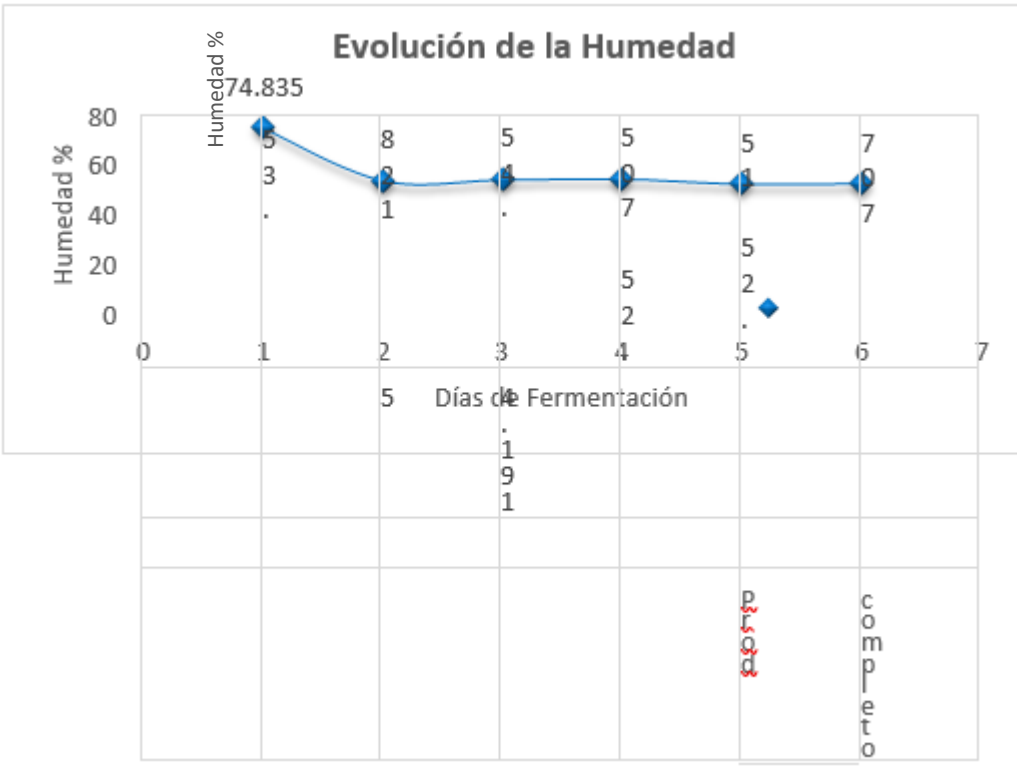

Figura 9 Evolución de la humedad en los cajones de madera.

Medidas de PH

Para las medidas de PH se extrajeron muestras de los tres niveles del cajón de madera. El pH de los granos del nivel medio y del nivel inferior solo fue posible tomarlo cuando se hizo remoción de la masa, en cambio el pH de los granos del nivel superior fue tomado de forma puntual todos los días del proceso de fermentación. Los resultados para cada nivel del cajón se muestran en las figuras 10, 11 y 12.

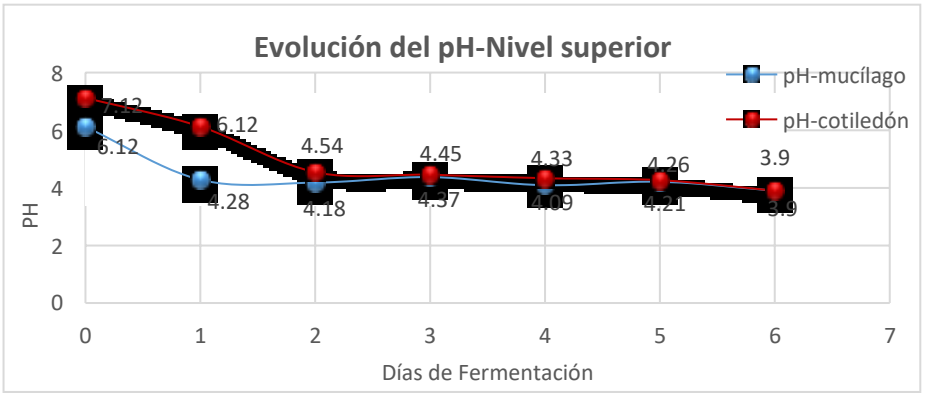

Figura 10 Medidas del pH en el nivel superior del cajón de madera.

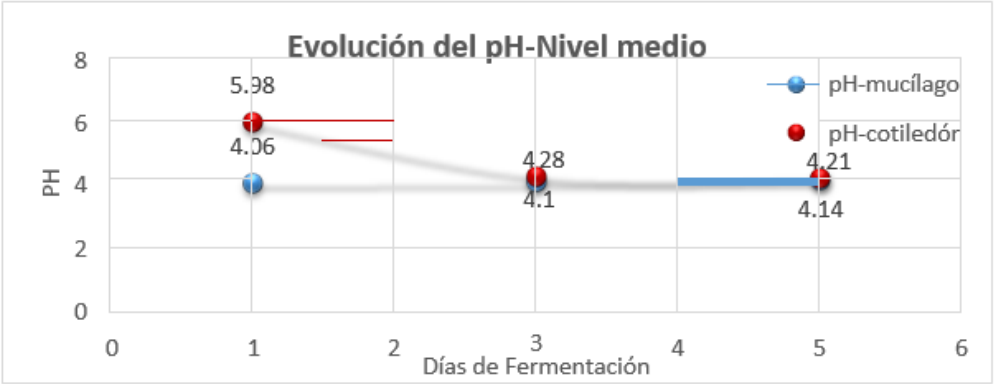

Figura 11 Medidas del pH en el nivel medio del cajón de madera.

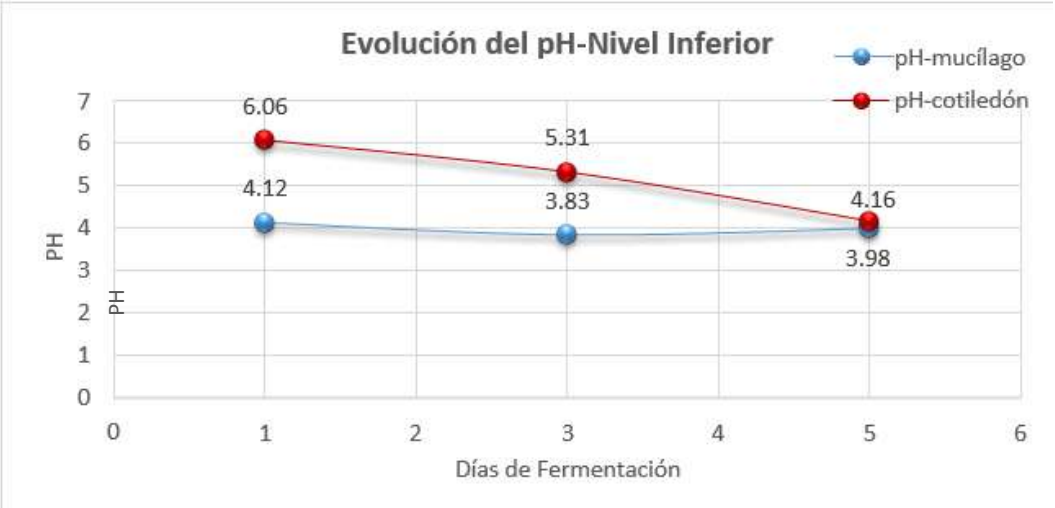

Figura 12 Medida de pH en el nivel inferior del cajón de madera.

RESULTADOS DE LA FERMENTACIÓN EN EL FERMENTADOR DE ACERO INOXIDABLE

Medidas de temperatura

Los resultados de la temperatura durante todo el proceso de fermentación de los tres lotes se muestran en las figuras 13, 14 y 15. Las diferentes líneas de colores de estas figuras indican la temperatura en los diferentes niveles del fermentador, tal como indican sus respectivas leyendas.

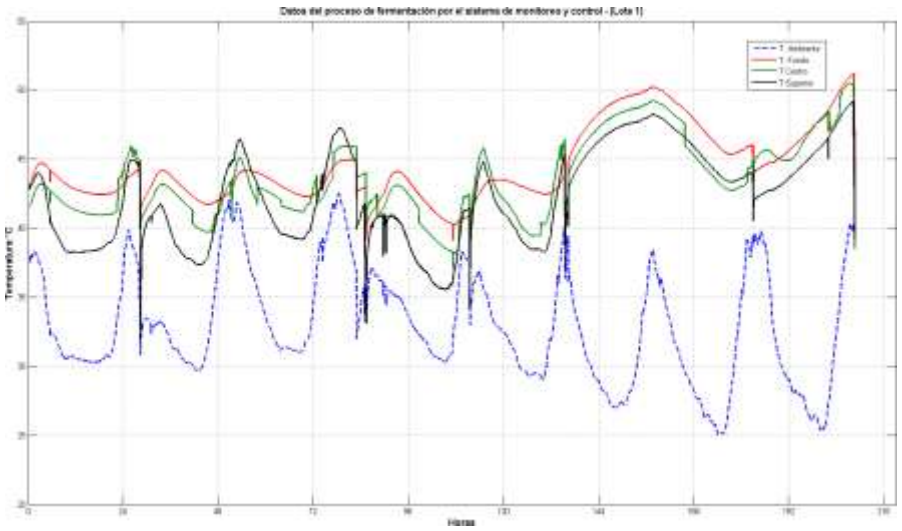

Figura 13 Evolución de la temperatura durante el proceso de fermentación – LOTE 1

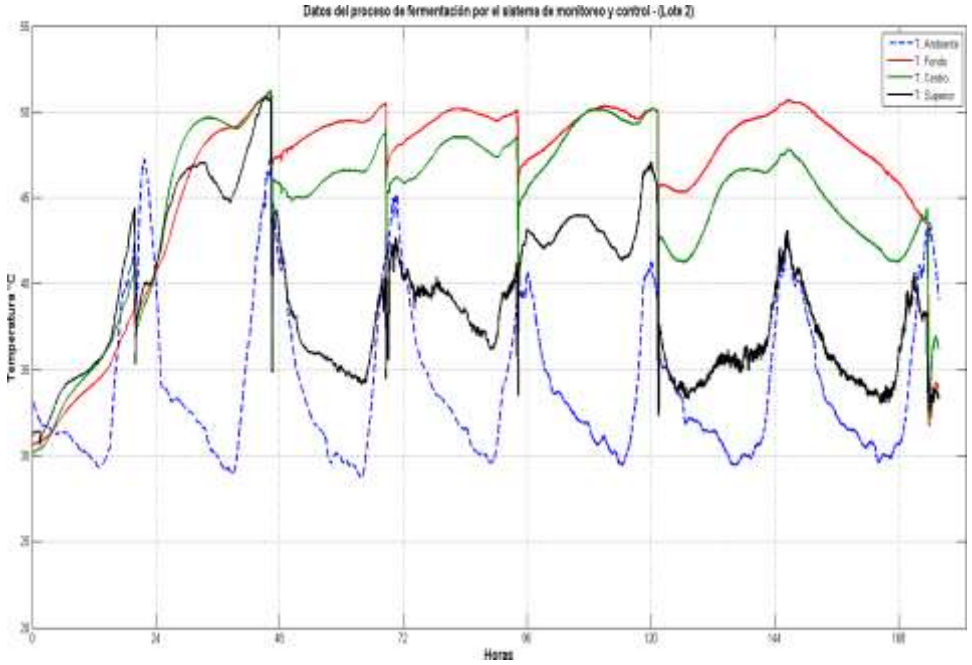

Figura 14 Evolución de la temperatura durante el proceso de fermentación – LOTE 2

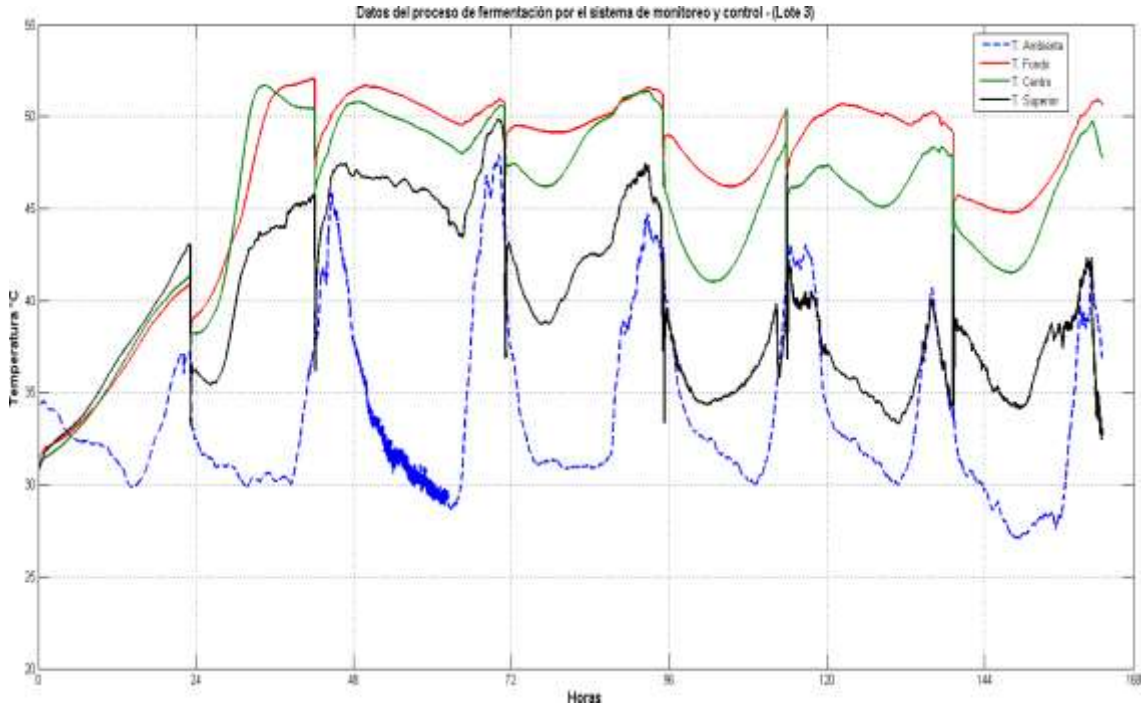

Figura 15: Evolución de la temperatura durante el proceso de fermentación – LOTE 3

Medidas de humedad

Para medir la humedad se extrajo granos del nivel medio del fermentador de acero inoxidable, estas medidas se hicieron de forma puntual una vez al día durante el proceso de fermentación. Los resultados de humedad se muestran en las figuras 16, 17 y 18.

Figura 16 Evolución del humedad durante la fermentación en el fermentador – Lote 1

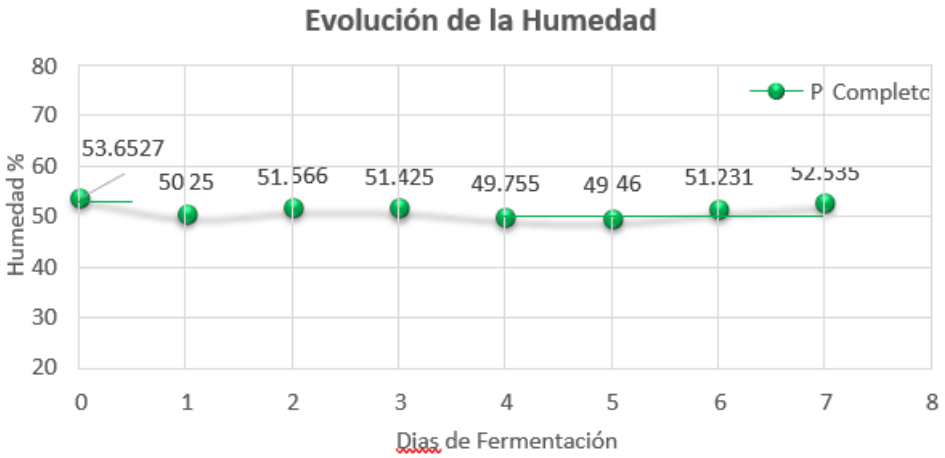

Figura 17 Evolución del humedad durante la fermentación en el fermentador – Lote 2

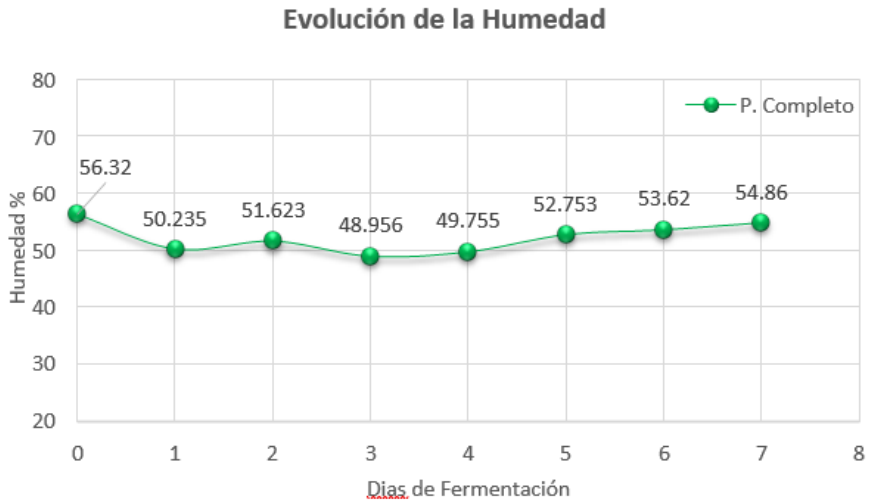

Figura 18 Evolución del humedad durante la fermentación en el fermentador – Lote 3

Medidas de PH

Para las medidas del pH se extrajeron muestras del nivel medio del fermentador, estas medidas también fueron tomadas de forma puntual todos los días. Los resultados del pH medido en los tres lotes se muestran en las figuras 19, 20 y 21.

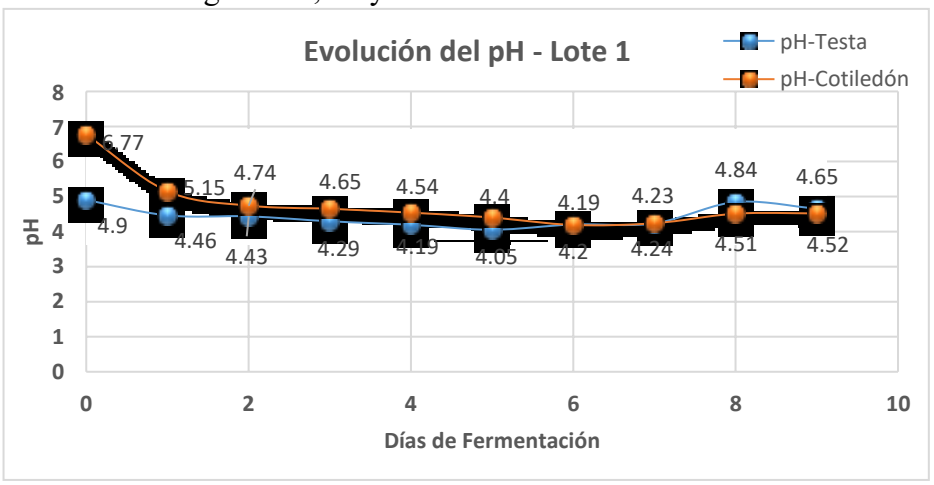

Figura 19 Evolución del pH durante la fermentación en el fermentador– Lote 1

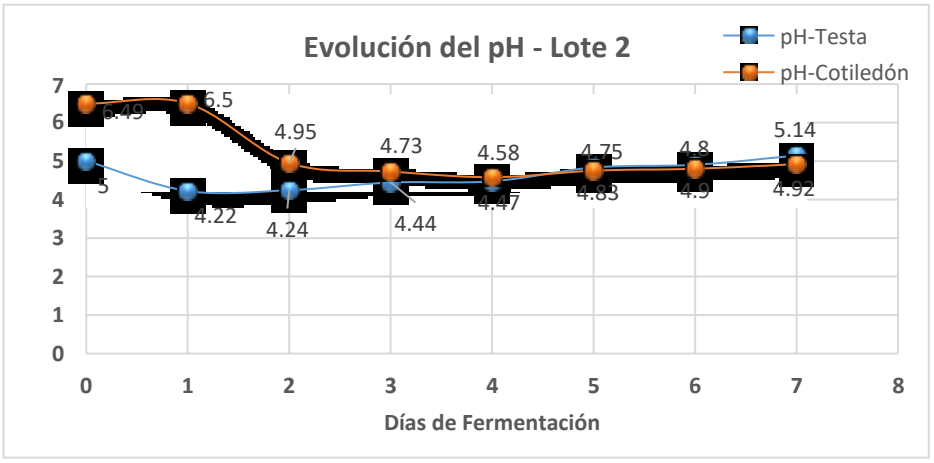

Figura 20 Evolución del pH durante la fermentación en el fermentador – Lote 2

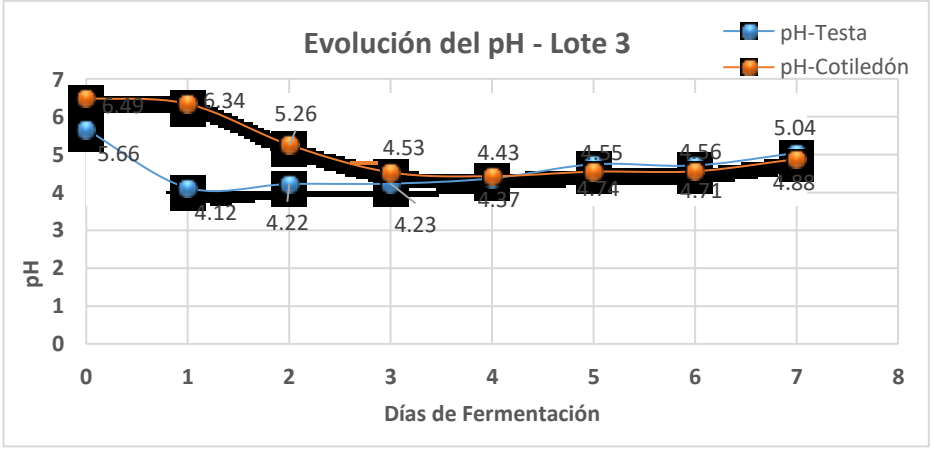

Figura 21 Evolución del pH durante la fermentación en el fermentador – Lote 3

DISCUSIÓN

Evolución de la temperatura durante la fermentación

En este trabajo la temperatura osciló entre los 28 °C y 46° C para cajas de madera, y para el fermentador osciló entre 31 °C y 51 °C aproximadamente; en ambos tipos de fermentadores (Cajón y fermentador de acero inoxidable) los rangos de temperatura coinciden con los reportados por [6], [7] y [8].

En la Figura 8 se observa que la temperatura ambiente influye de forma negativa sobre la fermentación, dado a que al disminuir la temperatura ambiente, la temperatura en la masa también disminuye notablemente en el nivel inferior y superior. Si analizamos la temperatura en los diferentes niveles del cajón de madera, existe una diferencia notable entre estos niveles, en algunos días se presentan gradientes de temperatura de 5 a 8 °C, y esto se debe a varios factores; uno de ellos es la falta de uniformidad en las remociones de los granos, también se le atribuye esta diferencia a las perforaciones que se encuentran en el fondo del cajón, por ello es que la temperatura en el fondo es menor que la temperatura en el centro (figura 8). La temperatura en la parte central de la caja es mayor que la temperatura en los demás niveles y la temperatura de la parte superior del cajón también se ve afectada por el intercambio de calor con los alrededores. Esta variación de temperatura en los diferentes niveles genera pérdidas económicas, dado que no permite una fermentación homogénea y se generan diferentes grados de granos fermentados, y en algunos casos granos sobre fermentados.

Para el lote 3 se siguió con el protocolo basado en los datos obtenidos del lote 1 y lote 2, se hizo remociones cada 24 horas a excepción del sexto día, para verificar la influencia en la frecuencia de remoción, se dejó la tapa del fermentador cerrada por 24 horas desde el inicio del proceso garantizando la fase anaeróbica; después de las 24 horas la tapa del fermentador se mantuvo abierta para facilitar el contacto del aire con los granos , en este lote 3 los granos en proceso de fermentación se taparon con hojas de plátano.

En este lote los granos inician la fermentación con una temperatura entre 32 y 33 °C, datos cercanos a los establecidos por [1]; cuando se hizo la remoción la tapa del fermentador se dejó abierta con la finalidad de evitar la condensación del vapor generado por la elevación de temperatura en los granos. En la figura 15, se observa que la temperatura superior tiende a seguir el perfil de la temperatura ambiente a partir de las 72 horas, este hecho se debe a que el sensor encargado de medir esta temperatura deja de estar en contacto con los granos a medida que transcurren los días de fermentación; por otro lado la diferencia de temperatura entre los granos del fondo y los del centro fue menor a 2 °C, hasta antes de las 96 horas, luego la temperatura del centro tiende hacer menor que la temperatura de los granos del fondo del fermentador.

Evolución de la humedad durante la fermentación

Los valores de humedad decrecieron en los primeros días desde 74.835 % a 53.821 %, a partir del segundo día la humedad osciló entre 53.821 y 52.707 hasta el final de la fermentación (figura 9). Estos valores a excepción del primero coinciden con los encontrados por [6] para el grano completo. Los valores de humedad encontrados en los granos fermentados en acero inoxidable se encuentran entre 56.32 y 54.86% con pequeñas variaciones alrededor de estos valores (figura 18); los valores de humedad encontrados en el prototipo de acero inoxidable, coinciden con los encontrado por [6] y[7], quienes afirman que los cambios en el contenido de humedad en los granos durante la fermentación son pequeños, comparados con los cambios que suceden en el secado.

Evolución del pH durante la fermentación

En la figura 10, se puede notar que el pH descendió de 7.12 a 3.9 en el cotiledón, y en el

mucílago el pH descendió 6.12 hasta 4.18 durante los dos primeros días, luego tiene un ligero aumento y finalmente desciende hasta 3.9; en general los valores del pH disminuyen y esto se debe en gran parte a que los ácidos producidos por las bacterias lácticas y acéticas se difunden al interior del grano [7]. Como resultado final en el cajón de madera se obtuvo un pH de 3.9 clasificándose como un cacao con presencia de ácidos no volátiles indeseables según [9].

Los valores de pH obtenidos en el fermentador de acero inoxidable, en el lote 3 presenta valores de pH en el cotiledón que descendieron desde 6.49 hasta 4.88, y en la testa el pH descendió desde 5.66 hasta 4.12 en el primer día y luego aumento hasta 5.04 al final de la fermentación; los valores de pH de la testa y el cotiledón coincidieron en el cuarto día, fenómeno que es común en el proceso de fermentación dado a que el pH de la testa asciende y el de los cotiledones desciende (figura 21).

Los valores de pH medidos en el lote 3, define a la fermentación como buena según [9] dado que este define que el pH óptimo para un cacao de calidad debe estar entre 5.1 y 5.4; por otro lado [7] indica que un cacao con pH entre 4.75 - 5.19 es considerado como cacao adecuadamente fermentado, y finalmente [10] indica que valores alrededor de 5.0 – 5.5 conducen a un aumento del potencial aromático del cacao. Por lo tanto los valores de pH obtenidos en el fermentador de acero inoxidable clasifican a la fermentación como buena y con un aumento del potencial aromático del cacao, aunque se debe tener en cuenta que estos valores pueden verse afectados por el tipo y calidad de cacao, así como la cantidad de granos.

CONCLUSIONES

Al monitorearse la temperatura en tiempo real en los cajones de madera, se notó que existe un gradiente de temperatura entre los diferentes niveles del cajón y que la temperatura ambiente influye en la temperatura de los granos, dado a que la temperatura de los granos disminuye notablemente en el transcurso de la madrugada y luego aumenta alcanzando los valores más altos en los picos de la temperatura ambiente. Para la fermentación en el prototipo de acero inoxidable el gradiente de temperatura en los diferentes niveles de la masa fue 5 veces menor en los diferentes niveles de la masa y la temperatura ambiente tuvo poca influencia en la temperatura de los granos.

En los cajones de madera se observó gran dificultad para mover la masa de granos de cacao de un cajón a otro. En el prototipo de acero inoxidable las remociones se realizan mediante paletas que giran a 7 RPM lo que facilita las remociones de grandes cantidades. Por otro lado se observó microflora en los cajones producto de la fermentación, esto origina malos olores que perjudican el proceso de fermentación, este hecho no se observó en el prototipo de acero inoxidable, además este prototipo tiene la ventaja de poder recolectar el mucilago segregado durante el proceso de fermentación, dado que tiene un sistema de drenaje que permite recolectarlo.

Los resultados obtenidos en el prototipo de acero inoxidable y en el cajón de madera, coinciden con los encontrados en las diversas bibliografías, por lo que el prototipo de acero inoxidable es considerado como una nueva alternativa para llevar a cabo el proceso de fermentación de cacao desarrollando el interés de usar prácticas de producción más higiénicas en bioprocesos industriales.

El prototipo de acero inoxidable contempla la parte de monitoreo y control, que está basada en sistemas embebidos y permite llevar una trazabilidad y adquisición de datos en tiempo real de todo el proceso de fermentación realizados por la asociación de productores de cacao. Este sistema también permite el monitoreo remoto del proceso de fermentación.

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DESARROLLO E IMPLEMENTACIÓN DE UN SOFTWARE UTILIZANDO SISTEMAS EMBEBIDOS PARA EL PROCESO DE FERMENTACIÓN DE CACAO Y SU MONITOREO REMOTO A TRAVÉS DE WEB

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RESUMEN

Se presenta el desarrollo de un software embebido aplicado al proceso de fermentación de Cacao. Se diseñó prototipos equipados con sensores que registran las variables de temperatura, oxígeno y dióxido de carbono durante el proceso y envían esta información a Internet. Finalmente se ha desarrollado un sistema Web que recibe la información y a través de gráficas monitorea y analizar el proceso en tiempo real.

INTRODUCCIÓN

Los sistemas embebidos cobran importancia al ser dispositivos electrónicos que pueden programarse para aplicaciones puntuales, entre sus características más importantes están: operar con pequeñas cantidades de memoria, poseer algún protocolo de comunicación y requerir un bajo consumo de energía.
En este trabajo el sistema implementado tiene la tarea de registrar algunos de los parámetros del proceso de fermentación de Cacao [4, 5]. A través de este sistema es posible evaluar el comportamiento durante el proceso y con ello permitir que los usuarios relacionados con este sector puedan tomar mejores decisiones. El objetivo es conectar a la nube equipos prototipo implementados con sensores [6] de temperatura, CO2, oxígeno, para medir variables que afectan al proceso de fermentación de Cacao.
Los equipos utilizados registran la información de las diferentes fases del proceso y la envían en tiempo real a un sistema Web [7] que permite mostrar los datos adquiridos al usuario final.

DISEÑO DEL SISTEMA EMBEBIDO

Para realizar el monitoreo de los parámetros durante el proceso de fermentación se implementaron dos prototipos; el primer prototipo es utilizado para monitorear el proceso de fermentación en un módulo de madera durante un periodo de siete días y el segundo prototipo permite monitorear la fermentación de Cacao en un fermentador de acero inoxidable (figura 1). A este prototipo se le dotó de mayor robustez para que soporte las condiciones adversas propias que presenta la sala de fermentación, entre ellas: altas temperaturas, presencia de insectos, ambiente corrosivo y húmedo.

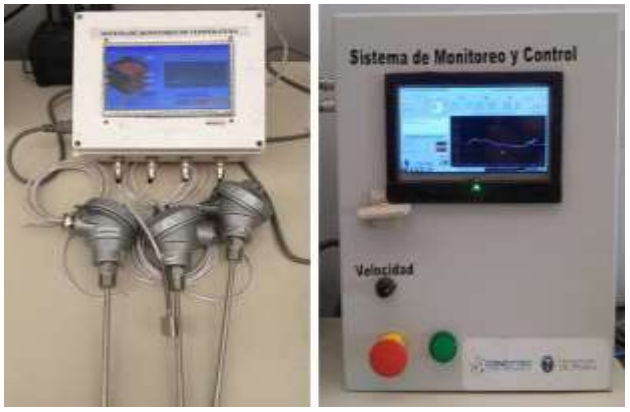

Fig. 1. Prototipos implementados

El prototipo para las cajas de madera está diseñado para monitorear la temperatura de forma local en la sala de fermentación y de forma remota desde un dispositivo electrónico con acceso a Internet.
Los sensores de temperatura están instalados en tres niveles del cajón de madera (figura 2), además también se registra la temperatura de la sala de fermentación para poder determinar la influencia que tiene la temperatura ambiente sobre la masa de Cacao durante el proceso, los valores son mostrados en la interfaz gráfica del software desarrollado y enviados al sistema Web para la supervisión remota a través de Internet.

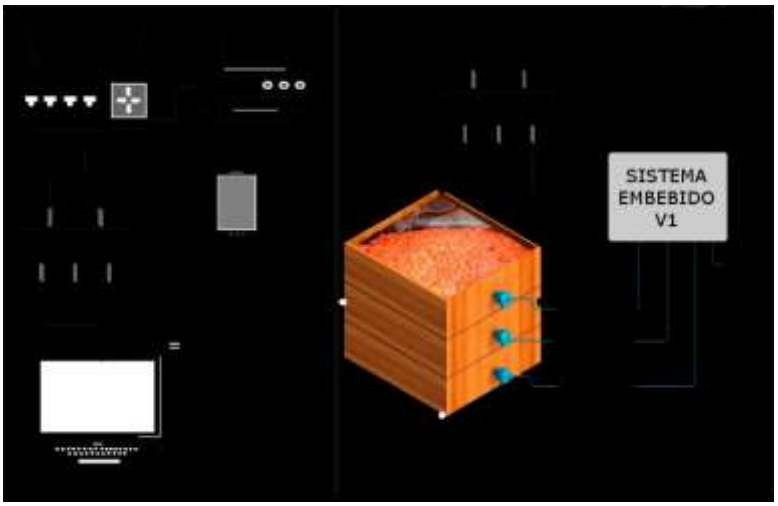

Fig. 2. Diseño del sistema para el prototipo en cajas de madera

El segundo prototipo está diseñado para trabajar junto a un módulo fermentador de acero inoxidable para Cacao, un tipo de fermentador distinto (figura 3). Este prototipo tiene como objetivo además de monitorear la temperatura en los tres niveles de la masa de Cacao, medir también los niveles de dióxido de carbono (CO2) y de oxígeno (O) dentro del fermentador llevando un registro de estas medidas.
En este prototipo se incluyó un variador de frecuencia dentro del tablero, ya que es necesario que el fermentador realice remociones periódicas a los granos de Cacao, esto se logra a través de un eje conectado a un moto-reductor eléctrico que gira a revoluciones muy bajas.
Durante algunos experimentos con una masa de Cacao de 100 kg las remociones se realizaron cada 24 horas a una frecuencia de 13 Hz, lo que equivale a una velocidad de 7 RPM. Además cada remoción tuvo una duración de 3 minutos.

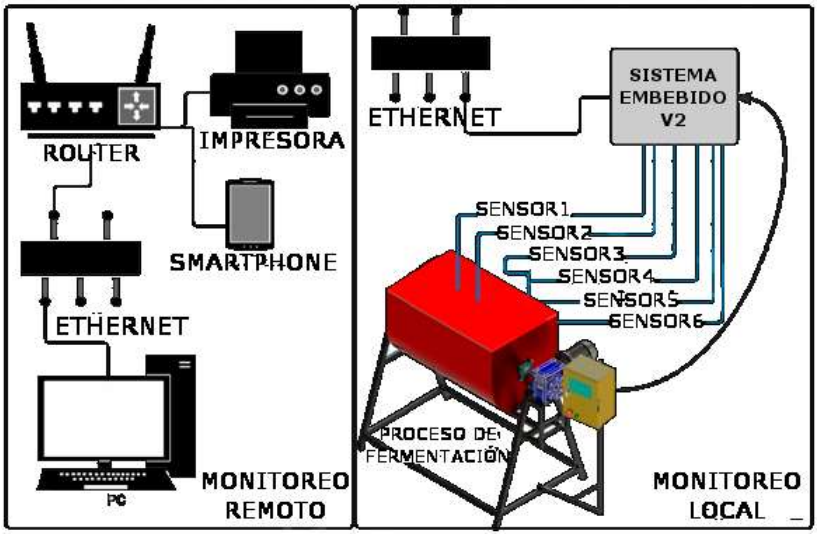

Fig. 3. Diseño del sistema para el prototipo en el fermentador de acero inoxidable

DISEÑO DE LA INTERFAZ GRÁFICA DEL SOFTWARE EMBEBIDO

Se ha desarrollado un software en el lenguaje de programación Java [10] (aplicación cliente) para el monitoreo local de la fermentación. La interfaz gráfica de esta aplicación tiene un panel diseñado para mostrar en tiempo real la temperatura ambiente y las temperaturas en los tres niveles de los módulos de fermentación (figura 4).

Fig. 4. Panel de monitoreo que muestra los valores de temperaturas.

La figura 5 muestra algunas opciones de configuración que ejecutan las acciones de inicio y finalización del registro de datos del proceso de fermentación.

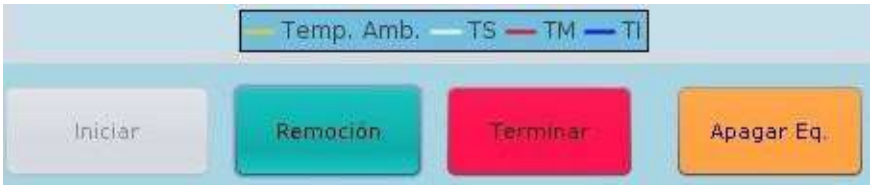

Fig. 5. Panel de monitoreo que muestra los valores de temperaturas.

Asimismo se puede visualizar el comportamiento en el tiempo de las distintas temperaturas (figura 6).

Fig. 6. Interfaz gráfica de la aplicación Cliente

DESARROLLO DE APLICACIÓN WEB

Se ha desarrollado un sistema Web con formato adaptable para una correcta visualización de la página (figura 7) en distintos dispositivos electrónicos, con esto, el usuario pueda monitorear el proceso de fermentación desde la laptop, PC, tablet, Ipad, o smartphone.

Fig. 7. Vista principal del sistema Web

Esquema de funcionamiento: El sistema de monitoreo embebido se conecta con el sistema implementado en la Web a través de Internet (figura 8), para poder enviar los datos del proceso de fermentación y que el sistema Web pueda mostrarlos al usuario final.

Fig. 8. Vista principal del sistema Web

Menú del sistema Web: El menú del sistema Web (figura 9) está formado por los siguientes ítems:

- Grupos de Fermentación: Donde se muestran los registros de los grupos que han pasado por el proceso de fermentación.
- Monitoreo en tiempo real: Donde se elige la caja para ver los valores (figura 10) y su gráfica de temperaturas en el tiempo (figura 11).
- Configuración del sistema: Donde se configura los parámetros a medir, el tipo de sensores utilizados en el sistema y el número de cajas (módulos de fermentación) existentes.

Fig. 9. Menú del sistema Web

Fig. 10. Visualización de parámetros en tiempo real en el sistema Web La figura 11 muestra la gráfica de temperaturas generadas por el sistema Web.

Fig. 11. Gráfica en tiempo real generada desde el sistema Web

PRUEBAS EXPERIMENTALES

En la figura 12 se muestra el registro de temperatura en un experimento, las diferentes líneas de colores indican la temperatura en un nivel del cajón; la línea punteada de color azul indica el valor de la temperatura ambiente; esta tiene importancia en este estudio porque nos permite evaluar la interferencia que tiene en el proceso de fermentación.

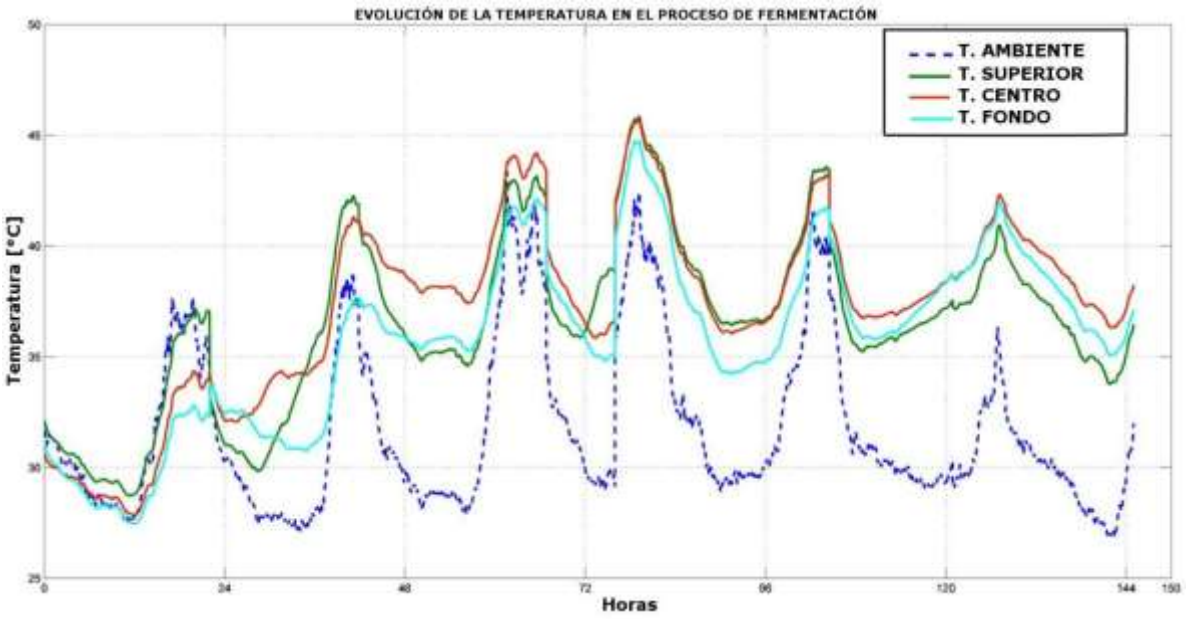

fig. 12. Evolución de la temperatura en tiempo real.

El oxígeno (O) y el dióxido de carbono (CO2) fueron monitoreados (figura 13) para determinar indirectamente el crecimiento de ciertos microrganismos que intervienen en el proceso de fermentación.

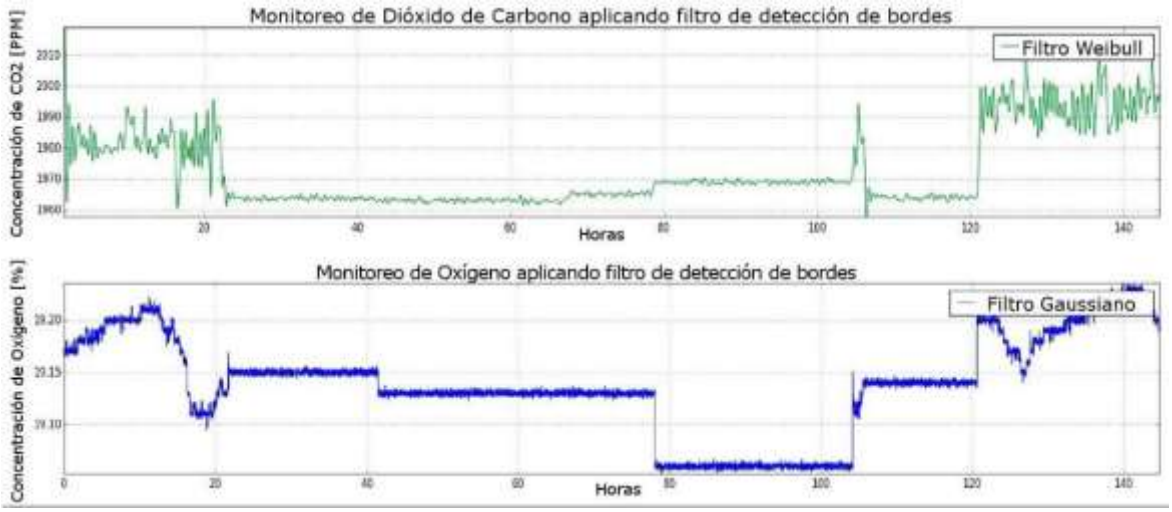

Fig. 13. Evolución del dióxido de carbono y oxígeno

Para la adquisición de estos datos se utilizó la plataforma basada en sistemas embebidos desarrollada en el Laboratorio de Sistemas Automáticos de control de la Universidad de Piura. Este sistema está instalado en la sala de fermentación (figura 14) y permite reportar los datos obtenidos por medio de comunicación TCP/IP hacia Internet para almacenar los datos durante todo el proceso.

Fig. 14. Prototipo instalado en sala de fermentación

CONCLUSIONES

Se logró el objetivo de monitorear el proceso de fermentación de Cacao con los dos prototipos desarrollados. El primer prototipo es usado para monitorear el proceso en cajones de madera y el segundo prototipo para monitorear el proceso dentro de un fermentador de acero inoxidable. Además también se ha desarrollado un sistema Web para adquirir los datos del proceso local y mostrarlo a usuarios finales a través de Internet. Tanto el software embebido desarrollado como el sistema Web están hechos en el lenguaje de programación Java por la facilidad de este lenguaje de programación para interconectar aplicaciones diferentes.

Se ha logrado programar una aplicación embebida con un entorno intuitivo, se ejecuta de manera local y también tiene opciones para guardar la fecha de inicio, el número de remociones, y la fecha de finalización del proceso.

El software embebido se comunica con un sistema Web que se encarga de pedir la información de las temperaturas para poder mostrarlas a un usuario final a través de una página Web.

Se logró hacer un sistema con formato adaptable a pantallas de diversos dispositivos eléctronicos con acceso a Internet. Esta comunicación es posible es posible gracias a la arquitectura “cliente-servidor” que es un patrón muy común para enlazar dos sistemas independientes.

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AISLAMIENTO E IDENTIFICACIÓN DE MICROORGANISMOS PRESENTES DURANTE EL PROCESO DE FERMENTACIÓN DE *THEOBROMA CACAO* L., VARIEDAD "CHUNCHO" DEL CUZCO

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RESUMEN

Los granos de cacao (*Theobroma cacao* L.) son la materia prima principal para la producción de chocolate. La fermentación de los granos de cacao es un proceso post cosecha esencial para el desarrollo de precursores del sabor del chocolate la cual viene dada por su genotipo. El proceso de fermentación del grano involucra microorganismos como levaduras, bacterias ácido lácticas y ácido acéticas, las cuales producen reacciones bioquímicas que impactan en el sabor y aroma del chocolate. El conocimiento y control de estos, ayudaría a la mejora del producto final. Perú posee una alta diversidad de genotipos del árbol de cacao. Entre ellas tenemos La variedad de cacao Chuncho la cual posee una superioridad en calidad organoléptica. En este trabajo se reporta el estudio microbiológico del proceso de la fermentación del cacao "Chuncho" del cultivar "Común Cáscara de Huevo".

Se realizó una prueba de fermentación durante 4 días, en Quillambamba, Cusco. Se tomaron muestras de 20 gr de los granos en fermentación, cada 24 horas. Para el análisis microbiológico, se emplearon medios para conteo y aislamiento de levaduras (Sabouraud con cloranfenicol); bacterias ácido lácticas (agar MRS) y bacterias ácido acéticas (agar GYC). Posteriormente se realizaron pruebas bioquímicas para su identificación.

Durante el análisis microbiológico se observó una sucesión de microorganismos. Las levaduras identificadas fueron: *Saccharomyces cerevisiae*, *Kloeckera apiculata, Candida* sp. Entre las bacteria ácido lácticas se aisló e identificó a *Leuconostoc mesenteroides ssp, Lactobacillus plantarum y Lactobacillus brevis.* En cuando a las bacterias ácido se determinó que pertenecían a los géneros *Acetobacter*, *Gluconobacter* y *Gluconacetobacter.*

INTRODUCCIÓN

El cacao (*Theobroma cacao* L.) tiene su centro de origen en el Perú. Sus granos constituyen la materia prima para la industria del chocolate. El cacao se clasifica desde el punto de vista botánico o genético en: criollo, trinitario y forastero. En Perú existen 5 grupos genéticos de acuerdo a la clasificación propuesta por Lachenaud, en 1997: Criollo, Forastero del Alto Amazonas o Amazonas, Forastero del Bajo Amazonas o Guyanas, Nacional y Trinitario (2). El Perú está clasificado según el Convenio Internacional del Cacao 2010 de la ICCO, como el segundo país productor y exportador de cacao fino (3).

El potencial para desarrollar sabores específicos depende principalmente del genotipo (4). Sin embargo, las intensidades pueden variar dependiendo de las condiciones de cultivo. Las semillas frescas de cacao no contienen los precursores necesarios para la formación del sabor del chocolate (4,5). Por lo tanto, se someten a un proceso posterior a la cosecha, que consiste en la fermentación y el secado (6).

Durante la fermentación, la pulpa de la fruta se degrada y los precursores del sabor a chocolate se desarrollan en las semillas de cacao. La intensidad de las notas amargas y astringentes disminuye y se reduce aún más durante el secado (4,7). Se pueden resaltar diferentes atributos del potencial del sabor del mismo cacao fresco a través de la fermentación (4,5).

El proceso de fermentación requiere una acción secuencial compleja de diferentes microorganismos:
En primer lugar aparecen las levaduras, posteriormente las condiciones anaeróbicas y micro-aeróbicas facilitan el establecimiento de bacterias ácido lácticas y finalmente, bajo condiciones aerobias, aparecen las bacterias ácido acéticas (8).

Cacao Chuncho
Perú posee una diversidad única con respecto a los genotipos del árbol de cacao (1). Entre ellas tenemos la variedad de cacao Chuncho del cultivar “Común Cáscara de Huevo”. El cacao Chuncho se encuentra ubicado geográficamente en los valles de La Convención de la Región Cusco (3). Pertenece al grupo genético Forastero del Alto Amazonas. Las principales características organolépticas de este cacao son de nota floral, frutal y nuez; con baja astringencia y amargor.

La importancia del estudio de esta variedad de cacao radica en su superioridad en calidad organoléptica. Un buen manejo de los procedimientos post cosecha asegura que las cualidades de sabor y aroma (determinadas por su genotipo) se expresen (9).

En las fermentaciones espontáneas tradicionales, los microorganismos necesarios para la fermentación entran aleatoriamente en la masa de fermentación (8). Esto hace que la fermentación y, por ende, el desarrollo del perfil del sabor sean escasamente controlables. La selección de la variedad del cacao y el manejo influye en la diversidad de especies de microorganismos aislados impactando la calidad de la fermentación del cacao. El conocimiento de los microorganismos presentes y el control de algunas especies de bacterias durante la fermentación podría permitir el control de una producción de alta calidad de chocolate y la generación de un pre cultivo para obtener aromas deseados en el chocolate (10,11).

En este trabajo se reporta el estudio microbiológico (caracterización e identificación de microorganismos) del proceso de la fermentación del cacao “Chuncho” del cultivar “Común Cáscara de Huevo”.

MATERIALES Y MÉTODOS

Recolección de frutos de la variedad de cacao chuncho, cultivar "cáscara de huevo"

La recolección de frutos fue llevada a cabo en el distrito de Echarate, en la provincia de La Convención, en la ciudad de Quillabamba ubicada en el departamento de Cusco a 1400 m.s.n.m. La cosecha fue realizada utilizando un machete cortando el pedúnculo. El tiempo de cosecha fue de 3 horas.

Proceso de fermentación

El proceso de fermentación se realizó de acuerdo al estudio hecho por Rojas et al 2015 en el libro "Estudio del proceso poscosecha y caracterización morfológica-sensorial-molecular de 3 variedades de cacaos nativos de Cusco, Junín y Piura. Lima" (9).

Recolección de las muestras

Se recolectó alrededor de 30 gramos de masa de cacao en fermentación, desde el inicio del proceso (tiempo 0) y cada 24 horas hasta el cuarto día. La toma de muestra se realizó utilizando bolsas de polietileno con cierre hermético, tomando porciones en la superficie y en 5 puntos distintos distribuidos en la mitad de la caja de fermentación. Se midió la temperatura de la masa así como el pH.

Tratamiento de la muestra

Las muestras se transportaron en refrigeración hasta el lugar de análisis microbiológico ubicado a 7 minutos. Una vez ahí, se pesaron asépticamente 20 gramos de muestra y se lavaron en 180 ml de agua peptonada al 0.1%, agitando las botellas por cinco minutos y dejando reposar por 30 minutos. Se tomaron 0.3 ml de la fase liquida y se le agregaron a viales que contenían 2.7 ml de agua peptonada al 0.1% para realizar diluciones seriadas. Posteriormente se sembraron alícuotas de 0.1ml por extensión en agares selectivos para los microorganismos que se iban a aislar. Una vez sembradas las muestras en las placas fueron transportadas al laboratorio de Biotecnología Ambiental en la Universidad Peruana Cayetano Heredia para el aislamiento y caracterización.

Siembra en medios diferenciales

Para el aislamiento de levaduras se utilizó el medio de cultivo agar Sabouraud de (HIMEDIA) (caseína enzimática hidrolizada 0.5%, peptona 0.5%, glucosa 4% y agar 1.5%). Para el aislamiento de bacterias ácido lácticas se utilizó el medio de cultivo agar MRS (marca HIMEDIA) (peptona 1%, extracto de carne 1%, extracto de levadura 0.5%, glucosa 2%, tween 80 0.1%, citrato de amonio 0.2%, acetato de sodio 0.5%, sulfato de magnesio 0.01%, sulfato de manganeso 0.005%, fosfato dipotásico 0.2% y agar 1.2%). Para las bacterias ácido acéticas se empleó el medio de cultivo GYC (2% glucosa, 1% extracto de levadura, 0.3% $CaCO_3$, y 1.5% agar) suplementado con 5% de etanol para las bacterias ácido acéticas**.**

Aislamiento e identificación

Cada una de las muestras obtenidas al ingresar al laboratorio de Biotecnología Ambiental fue sembrada en medios de cultivos diferenciales e incubados a 28 ° C. Se obtuvieron cultivos axénicos para continuar con la caracterización morfológica y la caracterización bioquímica. Para levaduras se utilizó el método de identificación API 20C AUX (Biomérieux, Francia). Además se realizaron pruebas complementarias como son: crecimiento en agar maíz,

crecimiento a 37 °C y crecimiento en tiamina. Para las bacterias ácido lácticas se realizaron las pruebas bioquímicas de catalasa y oxidasa además de la tinción Gram, para luego realizar las pruebas de fermentación de azúcares mediante API 50 CHL Medium (Biomérieux, Francia). Para las bacterias ácido acéticas se realizó una serie de pruebas bioquímicas: prueba de la catalasa y oxidasa; oxidación de acetato; crecimiento a pH 3, 3.5, 4, y 6.8; crecimiento en 30% de glucosa; prueba de motilidad y crecimiento en 10% de etanol.

RESULTADOS

Sucesión de poblaciones microbianas

En el Gráfico 1 se muestra la variación en las poblaciones microbianas. Estas variaciones están representadas mediante el crecimiento en agar Sabouraud para levaduras, agar MRS para bacterias ácido lácticas y levaduras; y agar GYC para bacterias ácido acéticas y levaduras.

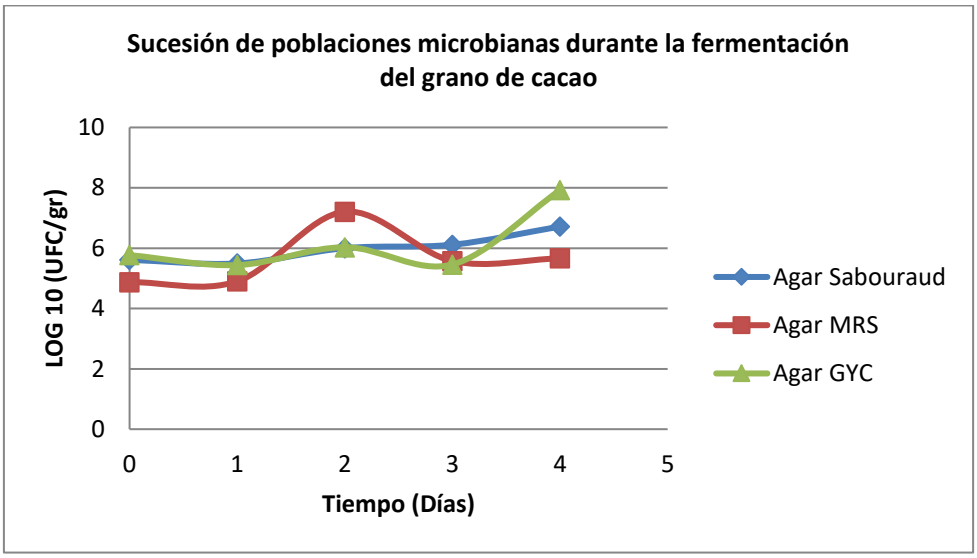

Gráfico 1. Variación en las poblaciones microbianas durante el proceso de fermentación de cacao

Durante el análisis microbiológico se observó una sucesión de microorganismos. A partir del segundo día se evidenció mayor número de microorganismos en el medio de cultivo MRS. Posteriormente hubo un incremento marcado de crecimiento en el medio Sabouraud. A partir del cuarto día se incrementó el crecimiento en el medio GYC, conservando este crecimiento hasta el final de la fermentación (Gráfico 2).

IDENTIFICACIÓN DE LEVADURAS

Se seleccionaron 10 morfotipos para realizar la prueba de asimilación de azúcares con el cual se determinó que correspondían a 3 especies: *Saccharomyces cerevisiae*, *Kloeckera spp.* (anamorfo de *Hanseniaspora*) y *Candida sp.* Con la prueba de crecimiento en tiamina y ausencia de crecimiento a 37 °C se determinó que la especie perteneciente al género *Kloeckera sp.* fue *Kloeckera apiculata* (anamorfo de *Hanseniaspora uvarum*). Las características morfológicas se presentan en la tabla 1.

	Características Morfológicas	
	Macroscópicas	**Microscópicas**

Saccharomyces cerevisiae	colonias con forma circular, con bordes enteros, convexa, opaca, color blanco con 1 cm de diámetro	células de globosas a elipsoidales grandes
Hanseniaspora uvarum	colonia de forma circular, con borde ondulado, plana, opaca, color beige con 1.3 cm de diámetro	células con forma de limón
Candida sp	colonia con forma circular, con borde entero, convexa, opaca, color beige con 5 mm de diámetro	células globosas a elipsoidales pequeñas

Tabla 1. Características morfológicas de las levaduras aisladas.

IDENTIFICACIÓN DE BACTERIAS ÁCIDO LÁCTICAS

A 10 de las colonias aisladas que fueron de tipo bacilos Gram positivos, catalasa negativas y oxidasa negativas se les realizó la prueba de fermentación de azúcares con la cual se pudo determinar la presencia de *Leuconostocmesenteroidesssp, Lactobacillus plantarum y Lactobacillus brevis*. Las características morfológicas se presentan en la tabla 2.

	Características Morfológicas	
	Macroscópicas	**Microscópicas**
Leuconostoc mesenteroides ssp	colonia con forma circular, 3 mm de diámetro, convexa, brillante, cremosa y color blanco	cocobacilos Gram positivos
Lactobacillus plantarum	colonia convexa, 6 mm diámetro, forma circular con borde irregular, opaca y cremosa	bacilos alargados Gram positivos
Lactobacillus brevis	colonia convexa con forma circular, 4 mm de diámetro, opaca y cremosa	bacilos Gram positivos

Tabla 2. Características morfológicas de las bacterias ácido lácticas aisladas.

IDENTIFICACIÓN DE BACTERIAS ÁCIDO ACÉTICAS

Se obtuvieron 12 colonias que presentaban halos de aclaramiento en el agar GYC y que eran bacilos Gram negativos. Las pruebas fisiológicas y bioquímicas realizadas a las bacterias ácido acéticas aisladas. permitieron determinar a qué género pertenecen las bacterias aisladas y las posibles especies a las cuales podrían corresponder. La mayoría de aislados fueron catalasa positiva y oxidasa negativo. Dos de los aislados dieron resultados positivos para la oxidación

de acetato. Ninguno creció en concentraciones altas de glucosa. Se evaluó el crecimiento de los aislados a diferentes pH, tres de los aislados crecieron en pH 3 y ocho crecieron en la prueba de crecimiento a una concentración de 10% de etanol. Las características morfológicas se presentan en la tabla 3.

	Características Morfológicas	
	Macroscópicas	**Microscópicas**
Gluconobacter sp	colonia con forma circular, 5 mm de diámetro, convexa, brillante, cremosa y color beige	bacilos Gram negativos
Acetobacter spp	colonia con forma circular, 2 mm de diámetro, convexa, brillante, cremosa y color beige oscuro	bacilos con forma de curva Gram negativos

Tabla 3. Características morfológicas de las bacterias ácido acéticas aisladas.

DISCUSIÓN

Durante el análisis microbiológico se observó una sucesión de microorganismos. En general, durante el proceso de fermentación, el recuento total de microorganismos aumenta durante las primeras 24-36 horas y luego se estabiliza o se reduce gradualmente (13). En este estudio se encontró que las bacterias ácido lácticas cumplían con esta generalidad, mientras que las levaduras y las bacterias ácido acéticas al cuarto día aumentaron su población. Esto se puede deber a la frecuencia con la que se realizó el proceso de volteado, cada 24 horas, permitiendo mayor oxigenación de la masa, ayudando a la prevalencia de microorganismos aerobios.
Las poblaciones iniciales microbianas son variables, dependen de factores como el cultivar de cacao, la calidad de la mazorca y la calidad del grano, por lo que la dinámica de la comunidad microbiana varía entre lugares de fermentación y tipos de fermentación (10,13,14).

Se identificó durante el proceso de fermentación la presencia de *Saccharomyces cerevisiae, Hanseniaspora uvarum* y *Candida sp* (la cual aún se mantiene en estudios). Dos de estas especies aisladas se encuentran dentro del grupo de especies de levaduras más frecuentemente aisladas (*Saccharomyces cerevisiae*, *Hanseniaspora guilliermondii*, *Hanseniaspora opuntiae*, *Hanseniaspora uvarum*, *Pichia kudriavzevii*, *Pichia membranifaciens*, *Pichia. fermentans*, *Pichia anomala* y *Kluyveromyces marxianus*) (15–17).

Tanto en las investigaciones dependientes de cultivos como en las independientes se ha demostrado el papel dominante de *H. guilliermondii*, *H. opuntiae*, *H. uvarum*, *P. membranifaciens* y *S. cerevisiae* en varios procesos de fermentación del grano de cacao (16,17). Sugiriéndonos así que al haber aislado estos microorganismos (*S. cerevisiae* y *H. uvarum)* podrían también tener un papel de dominancia en la fermentación llevada a cabo con cacao Chuncho.

La importancia de la presencia de levaduras radica en provocar la reducción de la viscosidad y el drenaje de la masa de la pulpa de cacao, debido a la secreción de enzimas pectinolíticas que descomponen las paredes celulares de la pulpa (8,18) esto permite la entrada de aire, lo que promueve el crecimiento de bacterias, en particular BAL y BAA.

Se identificó la presencia de *Leuconostoc mesenteroides ssp, Lactobacillus plantarum y Lactobacillus brevis*. Este aislamiento se ve corroborado con estudios previos donde han encontrado que *Lactobacillus brevis*, *Lactobacillus plantarum* y *Lactobacillus fermentum* son las as especies aisladas con más frecuencia (10,11,13,14,18–20).

La importancia de la presencia de *L. plantarum* en la fermentación radica en que junto con *Leuc. Pseudomesenteroide* y *L. fermentum* consumen el citrato en las primeras etapas de la fermentación del cacao bajo condiciones de pH bajo evitando así la competencia con las levaduras (citrato - negativas) que se encuentran degradando anaeróbicamente azúcares a etanol (13).

Dos de los aislados de bacterias ácido acéticas dieron positivo para la prueba de oxidación de acetato, confirmando la presencia del género *Acetobacter* y/o *Gluconoacetobacter*; mientras que el resto de las 10 colonias fueron identificadas como *Gluconobacter.* La incapacidad de crecimiento de los 12 aislados en una concentración de 30% de glucosa confirma la pertenencia a los géneros de *Acetobacter, Gluconoacetobacter* y *Gluconobacter*.

El crecimiento en 10% de etanol es una característica diferencial entre algunas especies del genero *Acetobacter*, de los aislados que dieron positivo a la prueba de la oxidación de acetato ambos dieron positivo para la prueba de crecimiento en 10% de etanol (A17 y A90). *A. ghanensis, A. oeni, A. nitrogenifigens*y *A. pasteurianus*son son las especies que toleran tales condiciones (21). Por lo tanto es probable que los aislados pertenezcan a alguna de estas especies.

En general, los miembros del género *Acetobacter* se encuentran con más frecuencia que los de *Gluconobacter* (14,22) sin embargo en este estudio se ha encontrado lo contrario, de 12 aislamientos solo 2 pertenecieron al género de *Acetobacter*. El mayor aislamiento de *Gluconobacter* en este estudio, puede indicar una fermentación diferente de la masa de la pulpa con producción de otros ácidos orgánicos y otros productos finales de la fermentación, que podrían ser responsables del sabor diferente del grano fermentado del cacao Chuncho.

CONCLUSIONES

Las levaduras identificadas fueron: *Saccharomyces cerevisiae*, *Hanseniaspora uvarum, Candida sp.* Las bacterias ácido lácticas (y relacionadas) aisladas fueron: *Leuconostoc mesenteroides ssp, Lactobacillus plantarum y Lactobacillus brevis.* En cuanto a las bacterias ácido acéticas se determinó que pertenecían a los géneros *Acetobacter*, *Gluconobacter* y *Gluconacetobacter.*

Se continúa realizando pruebas bioquímicas para la determinación de las especies de *Candida* y otras levaduras más, involucradas en el proceso de fermentación.

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