International Journal of Microbiology and Biotechnology
2023; 8(1): 10-18
http://www.sciencepublishinggroup.com/j/ijmb
doi: 10.11648/j.ijmb.20230801.12
ISSN: 2578-9678 (Print); ISSN: 2578-9686 (Online)

Comparison of Cocoa Bean Quality Produced with Different Starter Cultures and Fermentation Methods

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To cite this article:

Levai Lewis Dopgima, Fru-Cho Jerome, Tatsinkou Fossi Bertrand, Nanganoa Lawrence Tatanah, Afoh Rauwitta Omabit, Tah Yannick, Monono Ekwa Yawa, Tang Erasmus Nchuaji, Enow Lohr Lewis Fombang, Titanji Vincent Pryde Kehdinga, Akoachere Jane-Francis Tatah Kihla. Comparison of Cocoa Bean Quality Produced with Different Starter Cultures and Fermentation Methods. *International Journal of Microbiology and Biotechnology*. Vol. 8, No. 1, 2023, pp. 10-18. doi: 10.11648/j.ijmb.20230801.12

Received: January 26, 2023; **Accepted**: February 16, 2023; **Published**: February 27, 2023

Abstract: *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Acetobacter tropicalis* and *Shah* (maize-based fermented broth) was used as starter cultures in fermentations and the quality of cocoa bean produced assessed. This was a cross sectional study. Experiments were performed at JPJ Biotechnology Laboratory at IRAD Ekona, Cameroon from March – October, 2020. Fermentations of 10kg were performed in buckets and heaps. These were inoculated with 10^6CFU/mL per kg of cocoa bean for Composed Starter (CS) and 100 mL per kg of cocoa for *Shah*. Samples were collected every day for 5 days. Dried beans from Day 3 onwards of fermentation were assessed using cut-test and Equivalent per cent Fully Brown Score (EB_{Score}) while the Fermentative Index (FI) was carried out on dried beans of the 5 days. Means were compared using Analysis of Variance and a p - value < 0.05 was considered significant. Good cocoa bean quality (CBQ) with a cut-test of 66% and 62% was respectively obtained with CS and *Shah* after 3 days in bucket. Natural fermentation attained 62% fully brown beans after 4 days. The CBQ from heap fermentation with CS reached 64% after 4 days while all others remained moderate. Calculated EB_{scores} were not significantly different except for heap fermentations with values of Day 4 and 5 being higher. By Day 3, CS (FI=1.06) and *Shah* (FI=1.01) had FI values >1 suggesting that these bucket fermentations could be terminated. In the heap fermentation, good CBQ with CS (FI=1.02) was achieve on Day 4. *Shah* went on to D5 though the value of D4 (0.96) was not significantly different from that of D5 (1.03). An affordable method for cocoa fermentation was proposed using *Shah*. Good CBQ was attained faster with use of starter cultures and fermentation time was reduced to 3 days. This is the first study to demonstrate that *Shah* can be used as a starter culture with comparable results to CS for cocoa fermentation.

Keywords: Cocoa Bean, Starter Culture, Fermentation, Quality, Time, Affordable

1. Introduction

The physical and chemical attributes of Cocoa Bean Quality (CBQ) have been documented to encourage the cocoa community towards the production of better-quality cocoa [1] These quality characteristics include flavour, purity or wholesomeness (e.g., free from bacteria, infestation, allergens, mycotoxins, heavy metals and pesticide residues), physical characteristics (e.g., consistency, yield of edible

material bean, bean size and uniformity, shell content, fat and moisture content) and cocoa butter characteristics (e.g., free fatty acid content) [1]. Quality bean has to be free of particles, having a loose shell that is not moldy and with fully brown nibs.

The flavour profile of beans is a key quality measure in cocoa. Several factors such as location and soil type of farms, the age of trees and post–harvest treatments (poor handling, bad fermentation, inadequate drying and roasting) also affect cocoa bean flavour [1, 2, 3]. Good harvesting and post–harvest practices are key to upholding many of the CBQ descriptors that have been mentioned earlier. The selection of suitable planting materials or the desired genetic background for cultivation, is necessary to maintain the required flavour, yield, bean size and colour, and cocoa butter content [4]. With regards to post-harvest practices, controlled fermentation and drying of the fermented cocoa beans is a crucial step to avoid development of off-flavours that may affect CBQ.

The microbiological dynamics of cocoa fermentation is very important and researchers in many countries have carried out studies on it [2, 5-9]. Several methods are used for cocoa bean fermentation. All methods depend on removing the beans from the pods and piling them in heaps or putting them in boxes, baskets, bags and trays though heaps placed on and covered with banana or plantain leaves is by far the most dominant method [10, 11]. In the South West Region of Cameroon, after the pods are opened, some farmers place the beans in 50 kg rice-bags for fermentation. Once in the bags, they are easily transported from the farms (on motorbikes and push-trucks) to the houses or ovens where the beans will be dried after fermentation. Another method used, which is often claimed to give a better cocoa quality, is fermentation in stacked trays (20–100 kg of beans per tray), giving a series of thin layers of cocoa beans with air circulating between each layer [12].

In all of these models, a natural fermentation of the pulp sugars is allowed to go on for 4–6 days [13]. This process is spontaneous, not controlled, takes a relatively long time, with a high risk for failure and may lead to variable and/or poor-quality cocoa beans [14]. Failure of fermentation processes can result in spoilage and/or the survival of pathogens, thereby creating unexpected health risks in food products [15, 16]. During fermentation, yeasts break down mucilaginous pulp and increases aeration, favouring the establishment of acetic bacteria [2, 5, 9]. Bacteria, especially lactic acid bacteria then oxidise the ethanol to lactic acid, carbon dioxide and water and then acetic acid bacteria actively oxidises the alcohol to acetic acid [6-8].

Heat and acetic acid activity cause the death of the seed embryos, with consequent loss of the membrane's selective permeability [17, 18]. Following the death of the embryo, seed enzymes (such as proteases, polyphenol oxidases), and substrates (such as anthocyanins, flavanols, phenols and storage proteins), which were previously separated in specialized cells and/or compartments, come together, interact and react in a specific manner aided by the heat produced and the decrease in pH [9, 19, 20]. These end products (ethanol, acetate and lactate) and the heat produced can directly affect the bean components, causing important biochemical changes that influence the development of typical chocolate colour, flavour, and aroma precursors [21, 22].

Too much pulp will reduce cocoa bean quality, slowing down fermentation rate leading to excess production of lactate and ethanol [23]. This leads to a decrease in the oxidation of acetate to carbon dioxide and water by acetic acid bacteria. It will thus increase the residual acidity of the beans. This has however been overcome by aiding the aeration through periodically turning the heap, thus greatly reducing the levels of acetate and lactate in fermented beans [10, 23-25]. Aeration can also be obtained by partial reduction of excess pulp before fermentation. Some farmers practice carrying out fermentation in jute bags after pressing to remove excess pulp. Seed centrifugation or washing has also been used to give similar results [6].

Many yeast species have been identified from indigenous cocoa fermentation [26]. Species that have been found to show pectinolytic activity such as *Saccharomyces cerevisiae* var. *chevalieri*, *Kluyveromyces marxianus*, *Kluyveromyces thermotolerans* and *Candida rugopelliculosa* have been isolated [26]. However, it has always been observed that the insufficient activity of these wild pectinolytic strains in natural fermentation limits the amount of drained pulp sweating, and has been attributed to the fact that the starting inoculum is always small [23].

The use of inoculums has been shown to minimize the lag phase during fermentation [27]. A 10% inoculum accelerated microbial activity associated and reduced fermentation duration from 7 to 3 days [28]. The quality of the processed cocoa beans was confirmed to be good and consistent. In this study, we have used two types of starter culture to control fermentation of cocoa. The first is a consortium of identified microbes (*Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Acetobacter tropicalis*) and the second *Shah* (a maize-based fermented broth).

Shah is a product of fermented maize mostly consumed by the people from the North West Region of Cameroon, especially those from Bui and Donga Mantung Divisions. It is made following the principal steps in beer making in an artisanal fashion [29]. Briefly, malting is achieved by soaking the grains and allowing them to germinate followed by drying of the germinated grains in the sun. The dried grains are then brewed by milling, pasting, decorticating, filtering and cooking. When the mash is cooled a starter (usually a portion from the previous batch) is added and allowed to ferment for 12 – 24 hours. This maize alcoholic drink is a live beer as it contains active microbes including those used to constitute the consortium. It could be interesting if *Shah* produces fermentation results comparable to those of a composed starter culture. Since it is relative cheaper and available, *Shah* could be recommended for use.

This study also evaluates the effects of different types of fermentations on the quality of beans produced. These fermentations have been conducted on heaps and in buckets.

2. Methodology

2.1. Study Design

This was a cross sectional study carried out ripe Forastero cocoa pods (*Theobroma cacao*) harvested from the research plot at IRAD Barombi-Kang in Kumba, South West Region. After sorting, fresh ripe pods were placed in sacks and taken to the JP Johnson Biotechnology Laboratory at IRAD Ekona, Fako Division, South West Region of Cameroon. The climatic conditions in this area is principally equatorial with mean annual temperature of 22.8°C and annual rainfalls averagely 2719 mm. Its geographical coordinates are 4^{o} 14′ 0″N and 9^{o} 20′ 4″E.

2.1.1. Preparation of Inoculums

Saccharomyces cerevisiae, *Lactobacillus plantarum* and *Acetobacter tropicalis* which are microbes playing a lead role in cocoa fermentation [30] had been earlier identified and composed in a consortium for use as a starter culture. *Saccharomyces cerevisiae* was propagated in 10mL of Potato Dextrose Broth on a shaker and *Lactobacillus plantarum* and *Acetobacter tropicalis* were grown on MRS broth and CAAR broth respectively, at 30°C for 24–48h in a water bath. After propagation, the cells were harvested by centrifugation at 8000G for 10mins. The supernatant was discarded and cells re-suspended in sterile distilled water. The cells were washed twice and counts adjusted by microscopy to 10^6 CFU/mL.

The second inoculum investigated was shah, a maize-based fermented broth. *Shah* is a live beer as it contains active microbes. This was made in an artisanal fashion based on the methodology described by Djoulde DR *et al*., [29]. *Shah* was chosen because of its ready availability in the community which cultivates corn and the fact that it contains all the micro-organisms selected for use in the starter culture amongst other micro-organisms.

2.1.2. Fermentation Models

About one thousand (1000) ripe Forastero cocoa pods were used for this study. These were cracked open with the aid of a wooden bat to avoid injury of the beans. The beans as well as pulp were retrieved with gloved hands and the content poured into clean basins for measurement. This pulp was divided into 6 groups of 10kg each. Half served for heap fermentation and the other half for bucket fermentation. Fifteen (15) litre buckets perforated at the bottom were used for bucket fermentations and the heaps were placed on and covered with freshly harvested plantain leaves.

The separate fermentations were as such; heap inoculated with CS, heap inoculated with shah, heap with no inoculum, bucket inoculated with CS, bucket inoculated with shah and bucket with no inoculum. The fermentations with no inoculum will be referred to as natural fermentation.

2.1.3. Inoculation

The CS and *Shah* were all added at the beginning of both heap and bucket fermentations and mixed thoroughly with a sterile glass rod. The CS was inoculated as per the methodology of Massawe and Lifa [31] at a rate of 10^6 CFU/mL per kg of cocoa mash while *Shah* was inoculated at 100mL/L of cocoa mash (this corresponded to a 10%v/v). The mash was turned daily using a sterile wooden rod for aeration. During fermentation, samples were collected daily. The beans from these samples were dried.

2.1.4. Drying of Seeds After Fermentation

Samples were collected every day from the fermentations and the beans were sun-dried in the screen house by spreading on the drying platform at one layer thickness and turned every three hours during the day to ensure uniformity in drying. When these beans were properly dried, sampling using quartering tools [32] was performed until each quarter contained about 250 grams. These beans were assessed for quality by investigating the cut test, Equivalent per cent Fully Brown Score (EB_{Score}) and the Fermentation Index (FI) [32-34].

Whole dried beans from the third day of fermentation were cut lengthwise and observed for cut test and EB_{score} analyses. For Fermentation Index analysis, beans were collected daily for all five days of fermentation, milled to a fine flour and used. The temperature and time to attain good quality bean was also monitored and used to compare the different methods of fermentation carried out.

2.2. Quality Assessment of Dried Cocoa Bean

2.2.1. Cut Test

Cut test is an evaluation of sanitary and fermentation quality of beans. It is a determinant of the percentage of defective beans and the level of fermentation of the beans. It was performed according to Hii, CL *et al*., [35]. A total of 100 dried fermented beans were randomly taken out from the created quarters as described in 2.1.4. The beans were cut lengthwise into halves for a maximum surface of exposure and inspected for surface colour under the bright light of a lamp stand. They were then placed in one of the following categories based on the Malaysian Standard of Cocoa Beans – Specification MS 2672: 2017 [33]: Fully Brown (FB); Partly Brown (PB), Partly Purple (PP); Fully Purple (FP); slaty; insect damaged; moldy; or germinated. This was repeated thrice.

2.2.2. Equivalent Percent Fully Brown Score (EB_{Score})

The EB_{Score} was calculated from the cut test results using the following equation as described [32, 34].

$$EB_{Score} = \{(1 \times \%FB) + \big(0.7 \times (\%PB + \%PP)\big) + (0.5 \times \%FP) + (0.3 \times \%slaty)\}$$

Where, EB_{Score} is the Equivalent per cent Fully Brown Score; FB = Fully Brown; PB = Partly Brown; PP = Partly Purple and FP = Fully Purple.

2.2.3. Fermentation (Browning) Index

Fermentation Index (FI) was measured following methodology described by Bariah, K [32]. This was carried out on dried beans from all the 5days of fermentation. The shells of dried cocoa beans samples were removed to obtain

nibs. The nibs were ground into powder using a commercial blender (SOVIO Model: BLW-03). About 0.4g of the powders were homogenized in 40mL Methanol: Hydrocloric Acid (97:3) solution and incubated at 4^{o}C overnight. The homogenate was filtered through a muslin cloth and the absorbance of the filtrate was determined using a spectrophotometer (UV800 Spectrophotometer, Biometrics) at 460nm and 530nm. The FI was calculated as follows:

$$Fermentation\ Index\ (FI) = \frac{Absorbance\ at\ 460nm}{Absorbance\ at\ 530nm}$$

The values were recorded and the mean FI of the triplicate samples calculated. Results were interpreted as follows; under-fermented if the value of FI was below 1.000, fully fermented if FI value was between 1.000 to 1.599 and over-fermented when the FI was more than 1.600 [33, 34].

2.3. Proposition of an Efficient and Affordable Method for Cocoa Fermentation

After analysing the results (time taken to achieve an FI=1), a methodology was proposed for cocoa fermentation. This methodology would easily be adopted by field extension officers for use in Farmer Field Schools and by farmers when carrying out fermentation if the methodology is seen as easy to use, affordable and efficient in contributing to producing good quality bean.

2.4. Analyses of Data

The responses were keyed into an MS excel spread sheet and analysed using the Statistical Package for Social Sciences (SPSS) version 26 software. Results have been presented as frequency and percentages in tables and figures. Means were compared using Analysis of Variance (ANOVA). A two-sided p – value; $p<0.05$ was considered significant for all analyses.

3. Results

Samples of bean seeds were collected from the third day of fermentation, dried and analysed for been quality. Physical observation was done by the cut-test, and the EB_{score} and the Fermentation Index calculated. Results obtained are as shown below.

3.1. Cut-Test

After observing the cotyledons of 100 beans derived from quarters, and cut test score calculated, beans with cut-test score of above 60% are generally regarded as being good. The following was observed;

Table 1. *Cut-test analysis (% of 100 beans) of fermented beans from the third day.*

Days	Parameters	Heap			Bucket		
		Natural	Composed Starter	*Shah*	Natural	Composed Starter	*Shah*
3	Fully Brown	30	42	43	51	66	62
	Brown - Purple	40	42	35	37	28	30
	Fully Purple	13	8	14	12	6	8
	Slaty	17	8	8	6	0	0
4	Fully Brown	44	64	58	62	78	70
	Brown - Purple	38	27	24	28	18	24
	Fully Purple	11	9	14	7	4	6
	Slaty	7	0	4	3	0	0
5	Fully Brown	54	78	66	70	87	78
	Brown - Purple	26	15	28	20	10	18
	Fully Purple	12	7	6	8	3	4
	Slaty	8	0	0	2	0	0

Table reveals that from the third day, the beans were readily well fermented. This is supported with cut test values above 60%.

The fermentations in buckets (84.7, 88.6 and 87.0) were significantly better than those in heaps (69.6, 77.8, 76.9) from the third day. No significant difference was seen in either of the fermentations by the fifth day. However, *Shah* inoculated samples showed significant better results for fermentations in buckets (87.0 and 89.8) than on heaps (76.9 and 83.0) on Day 3 and 4 of fermentation.

By the fifth day sample inoculated with CS (95.5 and 92.0) fermented best as compared to *Shah* inoculated samples (92.6 and 88.6) and natural fermentation samples (88.6 and 80.6). *Shah* inoculated samples showed better fermentations than natural fermented samples throughout the 3 days of observation. On Day 3 and 4, the *Shah* inoculated samples presented better fermentation than CS inoculated samples. However, the reverse is observed on the fifth day where the CS induced samples (92.0 and 95.5) presented better fermentation than fermentations inoculated with *Shah* (88.6 and 92.6).

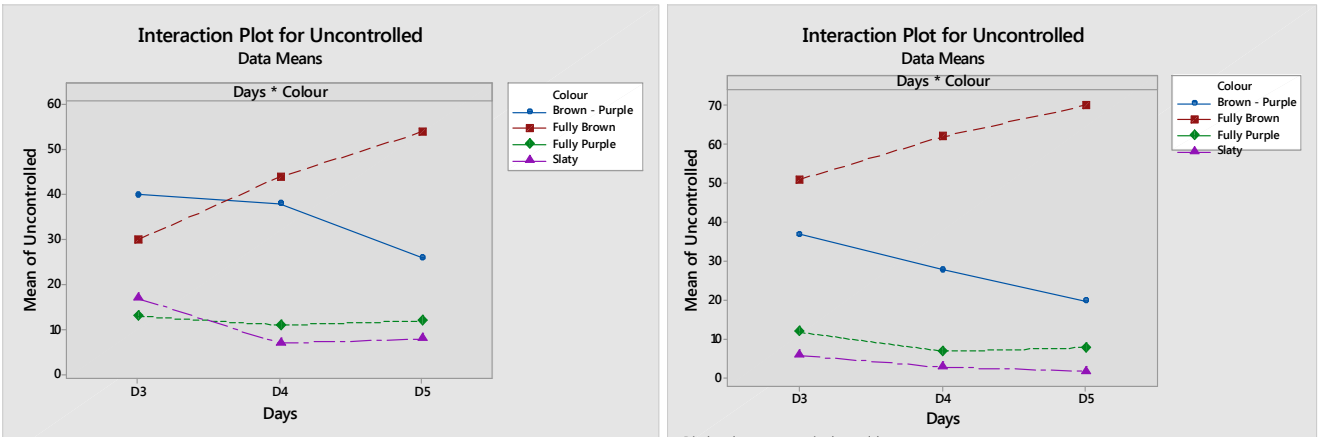
The interaction plots for the parameters (colours) were done with the types of fermentation that were carried out and the results are shown in Figures 1, 2 and 3.

In all fermentations, it was generally observed that as the fermentations progressed, the %FB increased while the %PB, %FP and % slaty beans dropped. This is an indication of progress of fermentation.

It was generally observed that, the composed starter inoculated fermentations yielded best %FB as compared to the other 2 fermentations. Shah inoculated fermentations yielded better %FB than natural uncontrolled fermentations throughout the three days.

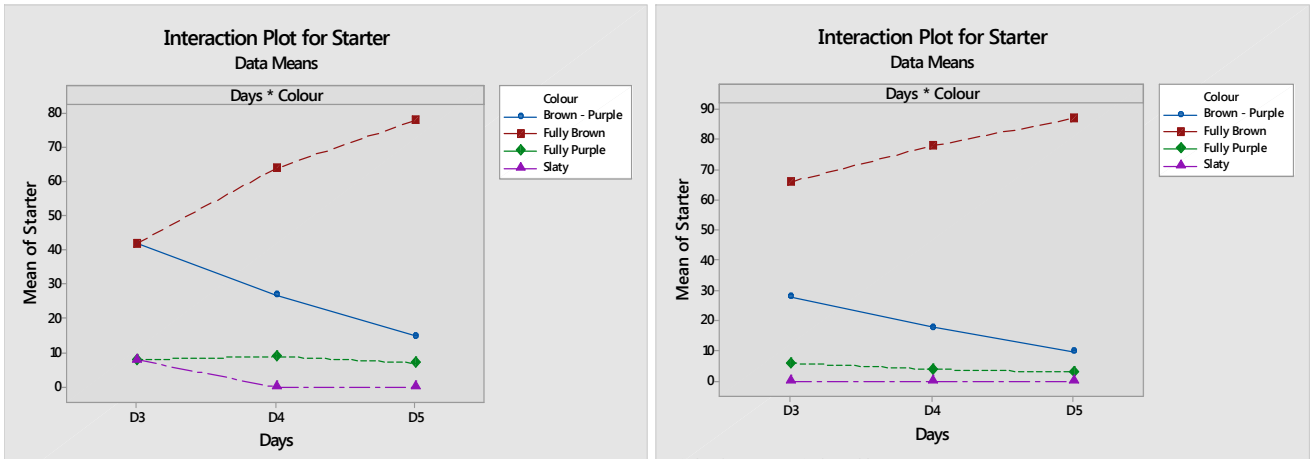
In bucket fermentations, Shah and composed starter inoculated fermentations presented no slaty beans whereas

the uncontrolled natural fermentation possessed some slaty beans. However, this occurrence of slaty beans was lesser for fermentations in the buckets (6%, 3% and 2%) than for heap fermentations (17%, 7% and 8%).



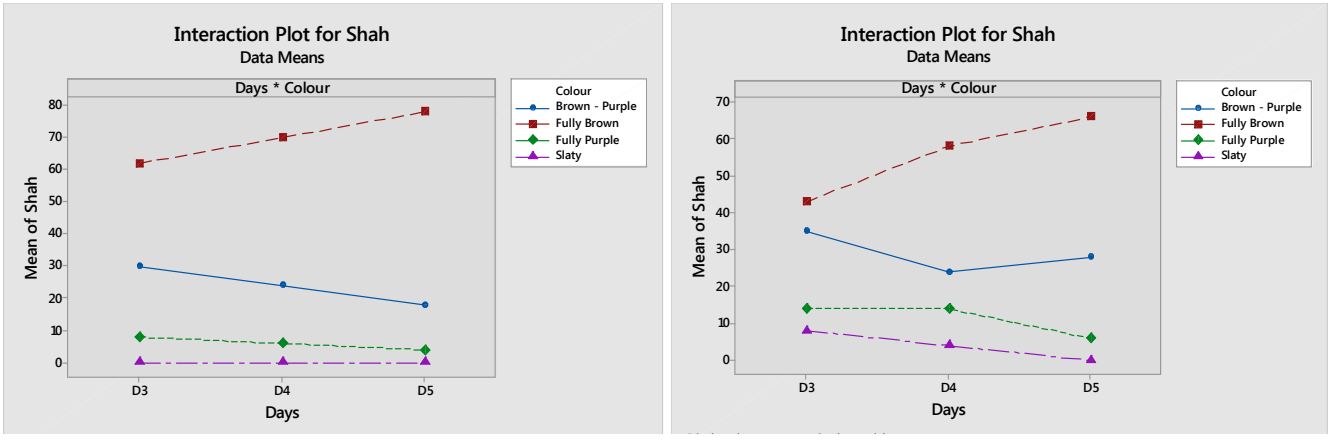
A. Heap B. Bucket

Figure 1. *Interaction of colors in the natural fermentation (uncontrolled) with the type of fermentation conducted. (A = Heap and B = Bucket).*



A. Heap B. Bucket

Figure 2. *Interaction of colours in the Composed Starter (CS) inoculated fermentation with the type of fermentation conducted. (A = Heap and B = Bucket).*



A. Heap B. Bucket

Figure 3. *Interaction of colors in the shah inoculated fermentation with the type of fermentation conducted. (A = Heap and B = Bucket).*

3.2. Equivalent Percent Fully Brown (EB) Score

Following the formula enumerated in the methodology, the mean EB Scores calculated are presented in Table 2.

Table 2. *Equivalent percent Fully Brown (EB_{Score}) based on cut-test analysis of beans from third day of fermentation.*

Days	Heap			Bucket		
	Natural	**Composed Starter**	***Shah***	**Natural**	**Composed Starter**	***Shah***
D3	69.60 ± 02.48^{b}	77.78 ± 02.52^{b}	76.90 ± 04.85^{b}	84.70 ± 05.72^{a}	88.60 ± 06.48^{a}	87.00 ± 05.00^{a}
D4	78.20 ± 04.07^{a}	87.40 ± 02.57^{a}	83.00 ± 03.94^{ab}	86.00 ± 06.44^{a}	92.60 ± 06.66^{a}	89.80 ± 04.45^{a}
D5	80.60 ± 04.70^{a}	92.00 ± 06.04^{a}	88.60 ± 02.67^{a}	88.60 ± 06.54^{a}	95.50 ± 06.54^{a}	92.60 ± 06.66^{a}

Values (Mean EB_{Score}±SD) within column followed by the same letter are not significantly different at p=0.05

Similar to the cut test, EB_{Score} of heap fermentations on day 3 were significantly lower than all other samples. The EB_{Score} increased gradually as fermentations progressed through the days with highest values on the fifth day. Comparatively, CS inoculated samples presented highest EB_{Score} values, second by shah inoculated values. Also, bucket samples presented higher EB_{Score} than heap samples.

The best score is however recorded for bucket CS inoculated sample on day 5 (95.5) while the lowest was for heap uncontrolled fermentation on day 3 (69.6).

3.3. Fermentation (Browning) Index, FI

Due to the fact that the cut-test and EB_{score} are somewhat subjective, the FI which is calculated by the ratio of the absorbance at 460nm and 530nm, is more reliable.

Table 3. *Mean of Fermentation Index of beans samples collected during fermentation.*

Colour	Heap			Bucket		
	Natural	**Composed Starter**	**Shah**	**Natural**	**Composed Starter**	**Shah**
D1	0.66 ± 0.04^{a}	0.67 ± 0.06^{c}	0.67 ± 0.01^{c}	0.68 ± 0.05^{c}	0.68 ± 0.08^{d}	0.69 ± 0.05^{d}
D2	0.75 ± 0.13^{a}	0.76 ± 0.04^{c}	0.74 ± 0.05^{c}	0.81 ± 0.07^{bc}	0.88 ± 0.06^{c}	0.85 ± 0.04^{c}
D3	0.83 ± 0.12^{a}	0.84 ± 0.05^{bc}	0.84 ± 0.07^{bc}	0.95 ± 0.10^{ab}	1.06 ± 0.10^{b}	1.01 ± 0.05^{b}
D4	0.94 ± 0.23^{a}	1.02 ± 0.18^{ab}	0.96 ± 0.09^{ab}	1.03 ± 0.10^{a}	1.19 ± 0.11^{b}	1.11 ± 0.08^{b}
D5	0.94 ± 0.23^{a}	1.14 ± 0.15^{a}	1.03 ± 0.16^{a}	1.08 ± 0.07^{a}	1.56 ± 0.10^{a}	1.23 ± 0.08^{a}

Values (Mean FI±SD) within column followed by the same letter are not significantly different at p = 0.05

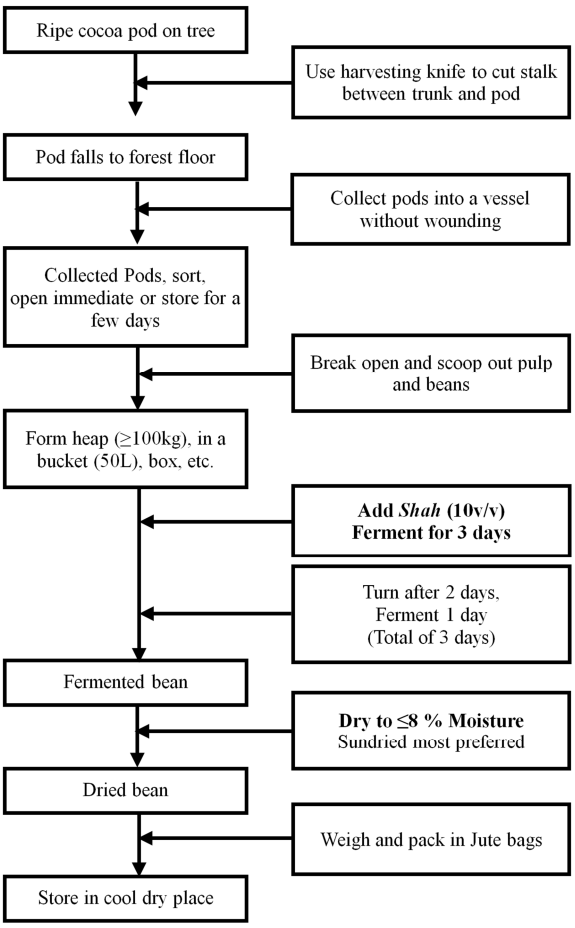


Figure 4. *Schematic representation of cocoa fermentation.*

The FI increases constantly with the highest mean value observed for CS inoculated sample in the bucket and smallest curve seen for uncontrolled heap fermentation sample.

Bucket samples generally produced better bean FI values than heap samples. The CS inoculated heap samples yielded beans with better FI values than uncontrolled bucket samples.

Fermentations of 2 days are too short to attain FI values of 1.0. By day 3, the desired FI values are reached by CS inoculated bucket samples (1.06) and *Shah* bucket sample (1.01). By day 4, uncontrolled natural bucket sample (1.03), CS inoculated heap sample (1.02). By day 5, *Shah* inoculated heap sample (1.03). Even at Day 5, uncontrolled natural heap sample did not attain an FI value of 1.0.

3.4. Proposed Efficient and Affordable Method for Cocoa Fermentation

Based on the results obtained, the following method for cocoa fermentation has been proposed (Figure 4).

4. Discussions

In this experiment, the objective was to evaluate the effects of inoculum (CS and *Shah*) and the effect of fermentation model (heaps and buckets) on the quality of beans produced. The control experiment was natural uncontrolled fermentation. Bean quality has often been defined in relation to the degree of fermentation. And degree of fermentation is analyzed chemically as cut test, EB_{Score} and Fermentation index.

Fermentation of cocoa achieves to major results. First,

oxidations cause heat production. This heat kills the germinating material in the beans, killing it, hence stopping germination and causing a change in the structure of the cotyledon tissue and increasing permeability of the seed coat [2, 33, 36, 37]. Next, the metabolites produced, seep through the permeable seed coat and inter react to develop the desired chocolate flavour [23, 37, 38].

The cut test is a qualitative test, based on visual inspection of the colour of nibs of sliced dried beans under artificial white light. Normally, unfermented beans are grey (slaty), under-fermented beans are partly purple and fully-fermented beans are fully brown in colour [34, 39]. The results of this test is based on the ocular quality of the evaluator. This creates a bias in its results, hence the need for clarification via the fermentation index (FI) test. The FI test is a measure of the level of breakdown of anthocyanins (colour pigments) during fermentation.

The cut test results show more than 60% FB nibs are obtained after 3 days of fermentation for bucket fermentations with CS inoculum (66%) and shah inoculums (62%) correlate with other works [28, 33]. Based on the Malaysian Standard for categorizing cocoa beans, these cocoa beans are good. The use of inoculum reduced fermentation time yet yielded good quality beans.

In our previous study in 2015, it was realized that more than 85% of the farmers in this region ferment their cocoa pulp for at least four days [13]. Using an inoculum therefore implies that the farmer will gain 24 hours. It was equally observed that there were no slaty beans produced with the use of inoculums. This is added advantage for farmers.

With the natural fermentation, at day 3. a cut test value of only 51% was registered, classifying he beans only as moderately good. As the fermentation progressed towards the fourth day, the natural fermentation recorded 62% of fully brown beans but with 6% slaty beans.

By the fifth day all bucket fermentations had cut test results of at least 70%. These cut-test results are similar to those obtained by Tee, TK *et al* [34]. In Malaysia, cocoa farmers are already adopting boxes, trays or containers for improved fermentation to substitute the conventional banana heaps fermentation [24].

During the heap fermentation, beans fermented with composed starter were categorized as good (64% fully brown) after 4 days. The fact that the beans from the natural heap remained moderately good even after 5 days could be attributed to the size of the heap. The constituted heaps were 10 kg each and this might have been too small to build up the temperature required to kill the bean germinating tissue, render the seed coat permeable for chemicals to seep in and start the breakdown of anthocyanins which causes browning of the nibs.

However, in the bucket, this heat and chemicals might have been easy to be created and retained respectively for the browning process to happen fast. With the heaps, this heat concentration is not very possible and chemicals leach out. This dispersal of heat is seen on the banana leaves which withered out like they had been cooked. Usually, farmers who observe good agricultural practice in this region carry out cocoa fermentation for at least 4–6 days [13]. From our results, 04 days of natural fermentation in heaps might not be good enough as only moderately good cocoa was produced. If fermentation has to be natural, there is need to increase heap size. The production and conservation of heat during cocoa fermentation is paramount. It has been observed that more farmers are using jute bags for fermentation. In such cases, it is recommended that the beans be poured out from one bag into another daily for aeration purposes.

When the EB_{scores} were calculated, highest scores were obtained with CS after 3 days of bucket fermentation and 5 days with heap fermentations (Table 2). Though relatively higher, the EB_{scores} were not significantly different from each other. The exception was with heap fermentations where values of Day 4 and 5 of fermentation were significantly higher than those of Day 3. These EB_{scores} results correlated with those of the cut test. With scores above 85% as described by Tee, TK *et al.,* [34], beans from Day 3 onwards of bucket fermentation with starter cultures could be classified as good while from heap fermentations, on Day 4 beans with composed starter was good.

The cut test and EB_{scores} are qualitative tests which depend on the quality of acuity of the ebaluator. This gives room for error. It is for this reason that the FI test is done to supplement the understanding of the results of the experiment. The FI test makes use of a spectrophotometer. Normally, unfermented beans are grey (slaty), under-fermented beans are partly purple and fully-fermented beans are fully brown in colour [34, 39] The absorbance ratio 460/530 nm of a cocoa-methanol-acid extract is a reliable indicator of Fermentation Index, and this presents a non-linear relationship with the cut-test [40].

The results of the FI show that bucket fermentations which contained inoculums should be stopped at the third day (FI=1.01 and FI=1.06 for shah and composed starter respectively). This is not significantly different for natural bucket fermentations at Day 5 (FI=1.08). If the inoculated bucket fermentations were left to continue after Day 3, the beans would be over fermented (FI=1.23 and FI=1.56 for shah and composed starter culture respectively on Day 5). Therefore, farmers would gain in 2 days as the duration would be significantly reduced if they fermented in buckets with inoculum, as suggested [24].

5. Conclusion

With regards to our fermentation experiments, the use of starter cultures in bucket fermentations reduced fermentation time from 4-6 days to 3 days. This is the first study to demonstrate that *Shah*, a maize-based broth can be used as a starter culture for cocoa fermentation with comparable results to artificially Composed Starter. The beans produced from both bucket and heap fermentations attained good quality faster when starter cultures were used. After 3 days, beans fermented with starter cultures already attained an FI>1.0 in bucket fermentations and the values were not significantly

different from those of Day 5. This leaves and extra day for the farmer to carry out other activities and guarantees that the bean produced from every fermentation batch will be of good quality. It might be important to standardise production of Shah for this purpose and for large scale fermentation of cocoa bean.

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