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Enhancing cacao fermentation for small-scale farmers: quality comparison with traditional process

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Abstract

The fermentation of cacao beans as a postharvest process yields necessary metabolites, which are flavor precursors of the unique cocoa flavor and aroma developed during drying and roasting. Quality cacao fermentation is governed by various requirements, crucial of which is the amount of wet beans necessary to achieve the temperature of fermenting mass between 45-50°C, starting on the third day until the end of fermentation. To achieve this, it is necessary to use large amounts of cacao wet beans (25 kg – 500 kg) placed in fermentation boxes. For small-scale farmers with a minimal amount of harvested beans, this volume requirement poses a challenge. This study compared a pod husk-enhanced small-scale fermentation set-up [use of 3 kg wet beans with 4 kg cacao pod husk (3FB+4CPH)] with a large-scale fermentation set-up [use of 25 kg wet beans (25FB)] by evaluating physicochemical parameters [pH of pulp, pH of beans, temperature of beans, temperature of cacao pod husk (CPH), titratable acidity (TA), and fermentation index (FI)] relevant to cacao fermentation. Also, dried samples from the fermented beans were compared with dried samples of unfermented beans in terms of FI. For the fermenting beans, the temperature, pH (pulp and beans), and TA were not significantly different between the small-scale and large-scale set-ups. The FI values for the small-scale and large-scale set-ups were comparable, exceeding 1.0, indicative of well-fermented beans. For the dried beans, there was a significant difference between the unfermented dried (UD) and fermented dried (FD_3FB+4CPH; FD_25FB) beans in terms of FI. The findings suggest that the small-scale fermentation is a viable, cost-effective alternative to the traditional fermentation set-up. This study demonstrates that small-scale fermentation can achieve comparable quality to traditional methods, addressing a critical need for smallholder farmers.

1. Introduction

As a net importer of cacao, the Philippines' average annual cocoa consumption is 50,000 MT (Department of Agriculture, 2021). Having been considered as one of the high-value crops in the Philippines, the Department of Agriculture instituted certain programs that will alleviate the cacao production in the country. In comparison with large, industrialized crops, around 80% to 90% of cacao comes from small, family-run farms, with approximately five to six million cocoa farmers worldwide (World Cocoa Foundation Update, 2014).

The geographical advantage of the Philippines makes it a viable source of the global supply of cacao. Research and development efforts are being exerted by the government and private research institutions on improving cacao yield or mitigating cacao pests and diseases. However, there is a lack of research that focuses on improving postharvest processes, specifically the fermentation process and flavor assessment and quality. The price of beans in the market is dictated by the quality of the harvested cacao and its subsequent postharvest processing.

In cacao postharvest processes, fermentation is the vital step that brings out the desired chocolate flavor in the beans. The chocolate flavor is achieved once essential flavor precursors obtained during fermentation and drying are fully developed during the subsequent roasting and conching processes. To achieve proper cacao fermentation, it requires at least 25 kg of beans to attain the required fermentation temperature (45-50°C) in the set-up (Schwan and Wheals, 2004; Afoakwa *et al.*, 2008).

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Cacao farmers are aware that fermented beans are of better quality than unfermented beans. However, there are reasons why many of them skip fermentation in their postharvest processing. Among these are inadequate amounts of fermentable wet beans, the cost of fermentation set-up, and a lack of technical knowledge regarding fermentation. Fermentation adds value to cacao beans. This can be realized if a technology to ferment a small volume of wet beans is available, resulting in quality fermented beans comparable to those from traditional fermentation. In this study, the pod-husk enhanced small-scale fermentation set-up was used to compare quality parameters with the traditional fermentation set-up. The use of cacao pod husk in the fermentation process alleviates the waste problems in cacao farms, thus addressing environmental sustainability.

This study sought to compare fermentation and drying parameters between an optimized small-scale fermentation set-up from the previous study of Bobiles *et al.* (2022) and the traditional fermentation set-up. This alternative cacao fermentation set-up, using a small volume of cacao wet beans enhanced with CPH, can alleviate the fermentation challenges of small-scale cacao farmers with limited volume of cacao harvests.

2. Materials and methods

This research used a previously established optimized small-scale fermentation set-up (Bobiles *et al.,* 2022). The optimization in the mentioned study identified the best combination of amount of wet beans and CPH that achieved 45-50℃ fermentation temperature from day 3 to day 5 in a 5-day fermentation. For the small-scale fermentation set-up, it utilized 3 kg wet beans (3FB), which were volume-enhanced with 4 kg CPH (4CPH). It was fermented for 5 days to avoid over-fermentation. The mixing of beans and CPH was done at Days 2, 3 and 4. It was compared with a large-scale fermentation set-up, which utilized 25 kg wet beans (25FB) only and fermented for 5 days also.

2.1 Source of samples

Fresh, ripe cacao fruits were sourced from local cacao farms in Guinobatan (13°11′N 123°36′E) and Camalig (13°08′N 123°40′E), Albay, Bicol Region, Philippines. The variety of cacao fruits used was a limitation of this study, as most farms have no single variety being cultivated in the area. Cacao fruits were sorted to eliminate diseased, overripe, unripe, and spoiled pods. Also, fruits with damage due to rats and microbial infection were eliminated.

2.2 Pod preconditioning and pod breaking

After harvesting, pods were stored in a cool and dry place, under cover from rain, for five to ten days. Pods are ready to ferment when a moving sound is produced by the beans when shaken. This indicates moisture loss from the pod, which will allow for good fermentation. Then, the pods were broken, and the wet beans were scooped out of the pods, leaving the placenta in the pod. The beans were weighed and transferred to a container prior to being weighed and set up for fermentation.

2.3 Experimental treatments

The cacao bean samples were divided into two treatments: unfermented (U) and fermented (F). The fermented beans were further subdivided into small-scale fermentation and large-scale fermentation. For each treatment/sub-treatment, there were three replicates (R) made: R1, R2 and R3.

For clarity in the succeeding parts, the “3FB+4CPH” refers to the small-scale fermentation set-up, which means that it used 3 kg wet beans with 4 kg CPH (CPH), “25FB” refers to the large-scale fermentation set-up, which means it used 25 kg cacao wet beans and “UB” for beans from the unfermented set-up. Beans sampled for each fermentation day (Fd) and dried (D) from each fermentation set-up (3FB+4CPH, 25FB) were subjected to analysis together with beans from the unfermented (UB) set-up. For fermented samples, labels used were “d0” for Day 0, “Fd1_3FB+4CPH”, “Fd1_25FB” for fermented samples from day 1 and so on. For the fermented dried (FD) samples, labels used were “FD_3FB+4CPH” and “FD_25FB”.

2.4 Unfermented treatment

The cacao wet beans for the unfermented treatment were gathered after pod breaking. A 50 g cacao wet beans were placed in a ziploc container and stored in a freezer for further analysis. The rest of the wet beans were immediately placed in the sun drying beds. After drying for 7 days, the beans were placed in a ziploc container and stored in a cool, dry room. From the dried beans, around 20 g samples were gathered for extraction. Another set of dried samples (20 g) was roasted using a portable home cocoa roaster (China OEM). The beans were roasted for 25 min at 120℃. This was based on the standard roasting condition for the Trinitario variety (Cocoa Quality, 2015), and the roasted sample was then stored for extraction.

2.5 Fermented treatment

The samples for the fermentation treatment were weighed and prepared for the fermentation set-ups

https://doi.org/10.26656/fr.2017.9(6).241

specified per set-up. The small-scale fermentation set-up is based on the set-up designed in the optimization process. For the large-scale fermentation (25 kg), the wet beans were placed in a Styrofoam box large enough to be filled by 25 kg of wet beans. Approximately 50 g of cacao bean samples were collected daily from each set-up throughout the fermentation process, then placed in a ziploc container and frozen for further analysis (Figure 1).

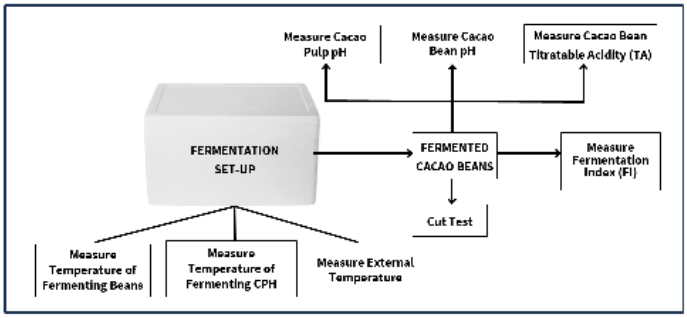

Figure 1. Flowchart of the experimental design and analysis.

The CPH, after pod breaking, was chopped into small cube-like pieces (approximately 0.5 × 0.5 × 0.5cm) to reduce size and increase surface area. A measured amount of chopped CPH (4 kg CPH) was then placed at the bottom of a Styrofoam ice chest. Once inside the box, it was covered with cheesecloth to separate it from the beans and prevent undesirable mixing and possible flavor effect on the beans. Wooden slats were placed on top of the cheesecloth to further prevent contact of beans with CPH, but still allowing heat transfer and aeration. The beans were put in a mesh bag and then placed on top of the wooden slats. It was covered with another cheesecloth to prevent heat loss. Thermometers were readily inserted from the outside through the holes on the side of the boxes, ensuring that the ends are placed at the middle of the fermenting mass of cacao beans and the CPH mass. This was done to monitor temperature changes during fermentation. The boxes were then covered. Perforations/holes at the bottom and sides of the box were made to allow the release of fermentation liquid and prevent its accumulation throughout the duration of the fermentation period, and allow aeration when needed.

The fermentation set-ups were placed in a room free from rain or exposure to sunlight. Mixing of the cocoa beans and CPH was done manually at 48, 72 and 96 h. Fermentation was done for up to 5 days.

2.6 Analysis of pulp pH

The bean samples collected daily throughout the whole fermentation process were analyzed for pulp pH. The pulp pH analysis was based on the AOAC Official Method 970.21 (AOAC INTERNATIONAL, 2023) with slight modifications (Ardhana and Fleet, 2003; Nazaruddin *et al.,* 2006, Fahrurrozi, 2015). Approximately 20 g of sample was placed in a clean ziploc container and 20 mL of distilled water was added. This was massaged for 5 min to physically separate the pulp from the beans. The pulp fraction was recovered by decanting to a beaker. Approximately 10 g of this fraction was weighed in a beaker and 40 g of hot, distilled water was slowly added while stirring. It was then centrifuged for 5 min. The supernatant liquid was decanted in a 10 mL beaker and the pH was measured using a pH meter (Mettler Toledo SevenCompact S220, Switzerland).

2.7 Determination of bean pH and total acidity

Samples for pH and total acidity (TA) analyses were obtained from the beans used for the analysis of pulp pH. The beans were allowed to air dry for 24 h. The bean seed coat/testa was removed by a scalpel and the cotyledons were ground using a laboratory mill (FOSS Knifetec: KN 295, PRC). Non-volatile acidity of the cocoa beans was determined according to AOAC Official Method 967.21 (AOAC INTERNATIONAL, 2023) and expressed as % acetic acid by titrating the samples with 0.1N NaOH. A total of 5 g of the beans were homogenized for 30 s in 100 mL of hot distilled water and vacuum filtered through Whatman filter paper No. 4. A 25 mL aliquot was pipetted into a beaker and the pH was measured using a pH meter (Mettler Toledo SevenCompact S220, Greifensee, Switzerland). A further 25 mL aliquot was titrated to an end point pH of 8.1 with 0.1N NaOH and the values reported were expressed as % acetic acid. The results were calculated and the mean values reported.

2.8 Sun drying and moisture determination

The fermented and unfermented cacao samples were all subjected to sun drying for seven days, approximating the moisture content of the beans around 6-7%. For the fermented treatments, after the 5-day fermentation, the remaining samples were immediately placed in sun drying beds and were dried for seven days. A customized sun dryer was made for the purpose of this study (Figure 2). The standard (CAOBISCO/ECA/FCC, 2015) moisture content of dried cacao beans that can be stored prior to secondary processing is less than 7%. The moisture content of the samples was determined using a moisture analyzer (Shimadzu Moisture Balance MOC – 120H, Japan). After drying, the beans were placed in ziploc containers and stored in a cool, dry place. From the dried beans of each fermentation treatment, around 20 g samples were gathered for extraction.

2.9 Fermentation index

A change in the color of cocoa bean cotyledon can

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Figure 2. Sun drying of fermented (left) and unfermented (right) cacao beans.

determine the degree of fermentation index (FI). The FI was measured based on the method of Racine *et al.* (2019) with some modifications. From the freeze-dried and sun-dried samples, approximately seven to ten randomly selected cocoa beans were ground to fine powder using a blade grinder (Foss Knifetec: KN 295, PRC). A 0.10 g sample of the resulting powder was weighed in a 15 mL centrifuge tube and mixed with 10 mL methanol: HCl (97:3 v/v). The mixture was vortexed for 2 min and allowed to stand at 4°C for 16-18 h. Then the mixture was centrifuged for 3 min at 3500×*g*, and the supernatant was collected in test tubes. The absorbance was measured using a UV-Vis spectrophotometer (G10S UV-Vis, Thermo Scientific, Madison, WI, USA) at 460 nm and 530 nm. These wavelengths can show structural properties and distribution through fermentation. The general λ_{max} for anthocyanin spectra is at 530 nm, and the 460 nm reflects the glycoside distribution (Harborne, 1958). The fermentation index was reported based on the ratio of the absorbance at 460 nm and 530 nm.

$$FI = \frac{A_{460}}{A_{530}}$$

Fermented cocoa beans with FI values of less than one (FI < 1.0) indicate under fermentation, while FI values equal to or more than one (FI ≥ 1.0) are considered well and adequately fermented.

2.10 Statistical and data analysis

Three replicates were used through independent experiments. Data were collated, and a descriptive summary was established and expressed as mean ± standard deviation (SD). It was analyzed using T-test assuming equal variances (α = 0.05) to identify significant differences among treatments. For the relationship among quality parameters, Pearson's correlation coefficient (r) was calculated among interactions between variables. All statistical calculations were done using MS Excel.

3. Results and discussion

Since the fermentation quality of the small-scale set-up was compared with the traditional set-up, the internationally accepted qualitative method to compare the quality of fermented beans through the cut-test method cannot guarantee an accurate result. In the cocoa industry, fermentation status is assessed based on ISO 2451, the International Standard for Cocoa Beans – Specifications and Quality Requirements (ISO 2451:2017). According to this specification, the cut-test is used to define fermentation qualities such as fair-fermented and well-fermented by using visual inspection of a percentage of slaty and defective beans (Kumari *et al.,* 2018). The visual inspection method was not used to compare the 3FB+4CPH against the 25FB, as the amount of beans in the 3FB+4CPH set-up was not sufficient for the number of bean samples required in the cut test, which is 100 beans per sampling (Wood and Lass, 2001). But there are methods that describe the use of 300 bean samples for the cut test (Guehi *et al.,* 2007). Besides, in the cut-test method, the industry uses dried fermented beans.

3.1 Temperature

The daily mean temperature of the fermenting cacao beans and CPH for the 3FB+4CPH and 25FB are shown in Figure 3. The mean external temperature during the course of fermentation ranged from 28.8 to 29.7°C. This observed range of environmental temperature was almost constant, which may have less effect on the variations in the fermenting beans' temperature. In cases where the beans are sourced from different farms and set-ups will be exposed to varying environmental conditions and geographical variations, the result on fermentation temperature may vary.

For the small-scale set-up, the temperature of the fermenting beans ranged from 27.1 to 50°C. The temperature drastically rose from Day 1, peaked at Day 4, and exhibited a small decline by Day 5. The same trend was observed for the fermenting CPH in which the temperature ranged from 27.8 to 50.8°C. The rise in temperature started at Day 1, peaked at Day 4 and slightly declined by Day 5. For the 25 kg set, the temperature ranged initially from 27.4°C, increased gradually as fermentation progressed, and peaked at 46.9 °C on Day 4 and Day 5.

As shown in Figure 3, in the small-scale set-up, both beans and CPH mass have higher temperature generated during the course of fermentation compared with the large-scale set-up. It can be observed that the beans of the 3FB+4CPH set up reached 50°C, an indication of a well-fermented bean. The t-test result for two-sample assuming equal variances [P(T≤t) = 0.57; α ≤ 0.05)] for

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the mean temperature of the fermenting beans of 3FB+4CPH and 25 kg gave no significant difference. This showed that the small-scale set-up is comparable in cacao fermentation with the large-scale set-up in producing well-fermented cacao beans in terms of fermentation temperature.

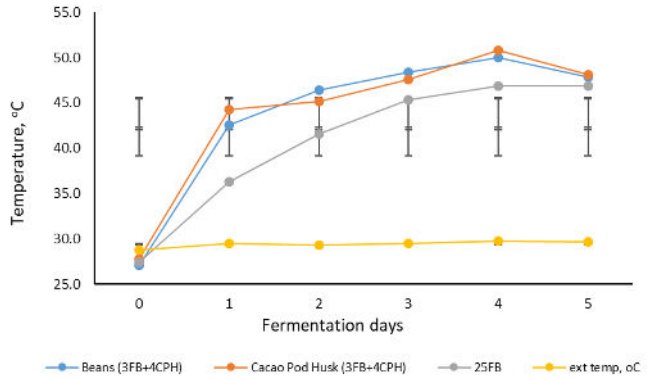

Figure 3. Daily external temperature, temperature of cacao beans and cacao pod husk (CPH) in the fermentation set-ups (3FB+4CPH – 3 kg beans with 4 kg CPH and 25FB – 25 kg beans).

At the start of fermentation until around the second day, the condition of the fermenting mass is anaerobic due to the close packing of the pulp covering the beans. The initial microorganisms that thrive in this condition are yeasts, which are favored by the low oxygen level and a low pH due to the citric acid content of pulp. Yeasts utilize the sugar-rich, acidic pulp by producing ethanol in the presence of some lactic acid bacteria (LAB). The primary activity of fermentative yeasts is to convert sucrose, fructose and glucose to ethanol and CO_2 (Schwan and Wheals, 2004). At this stage, the action of yeast and to some extent, LAB, raises the temperature of the mass to 30-35°C after 24 h and to 35-45°C after 48 h (Roelofsen, 1958; Schwan and Wheals, 2004). As the air starts to penetrate the beans due to pulp degradation and sweating, and the yeast population is declining, LAB population increases. The LAB population peaks around 36 h after the start of fermentation. Aeration of the fermenting mass is enhanced by means of manual mixing, to aid penetration and ensure uniformity in the fermenting mass. As the air increases in the mass, the temperature rises typically around 45°C after 72 h (Day 3), remaining at 45-50°C until fermentation is complete (Afoakwa *et al.*, 2008). A high temperature (45-50°C) beyond 48 h with the presence of ethanol and acetic acid was responsible for cacao bean death in the fermentation set-up (Biehl *et al.,* 1977). Bean death is crucial to flavor precursor development as it is accompanied by the loss of cellular integrity and vacuolization, which allows the contact of substrates and enzymes, leading to reactions that produce the flavor precursors (Amin *et al.,* 1998).

3.2 Pulp pH

The result of the analysis of pulp pH during the course of fermentation of the 3FB+4CPH and 25FB set-ups is shown in Figure 4. The pulp pH of the two setups started at 3.99 at Day 0. At Day 0, the wet beans were freshly scooped out from the cocoa pods right after pod breaking. Ardhana and Fleet (2003); Schwan and Wheals (2004); Thompson *et al.* (2007); Afoakwa *et al.* (2013) reported that the pH range of unfermented cocoa pulp ranges from 3.3 to 4.0. This is primarily due to the high concentration of citric acid. By Day 1, the pulp pH decreased to 3.80 and 3.56 for 3FB+4CPH and 25FB, respectively.

By Day 2, the pulp pH started to increase until the end of fermentation. For 3FB+4CPH, the pulp pH rose from 3.95±0.27 to 4.53±0.37 at the end of fermentation, while that of the 25FB, it increased from 3.80±0.29 to 4.36±0.21 at the end of fermentation. In Figure 4, it shows the same trend of pulp pH readings for the two setups throughout the course of fermentation. The pH reading for the 3FB+4CPH is slightly higher than that of the 25FB. To know if the result is statistically different, a t-test for two samples assuming equal variance for the mean pulp pH of the fermenting beans of the two set-ups was done. Based on the t-test [$P(T \leq t) = 0.35$; $\alpha = 0.05$], there was no significant difference among the means of pulp pH of the two set-ups. This means that the changes in the pulp pH of the fermenting beans of the 3FB+4CPH set-up are comparable with the fermenting beans of 25FB.

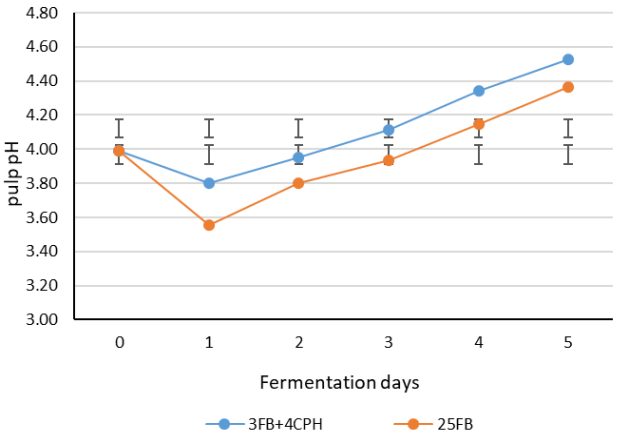

Figure 4. Daily pulp pH of fermenting cacao beans in the 3FB+4CPH (3 kg beans with 4 kg CPH) and 25FB (25 kg beans) fermentation set-ups.

The rich cocktail of organic substances in the pulp is favorable to the growth of microorganisms, producing a diversity of metabolites, such as organic acids. Production of organic acids in the pulp is a crucial process in cacao fermentation. These acids diffuse into the beans through the testa and subsequently induce the

https://doi.org/10.26656/fr.2017.9(6).241

important biochemical reactions, like hydrolysis of proteins in the cotyledons, leading to well-fermented cacao beans (Schwan and Wheals, 2004). However, high acid production in the pulp is detrimental as it leads to excessive acid diffusing into the beans, resulting in the production of acidic beans. Changes in acidity during fermentation of cacao are crucial in the final bean quality (Afoakwa *et al.,* 2013).

3.3 Bean pH

The pH of beans for the duration of fermentation for the 3FB+4CPH and 25FB are shown in Figure 5. For the 3FB+4CPH, a slight increase occurred from Day 0 (5.74) to Day 1 (5.90) and eventually decreased from Day 2 (5.79) until Day 5 (5.25). For the 25 kg, it increased from Day 0 (5.74) to Day 2 (5.88) and eventually declined by Day 3 (5.40) until Day 5 (5.08).

Generally, as shown in Figure 5, the same trend of cacao bean pH readings for the two setups was observed throughout the course of fermentation. The pH reading for the 3FB+4CPH is slightly higher than that of the 25FB. To know if the result is statistically different, a t-test for two samples assuming equal variances for the mean pulp pH of the fermenting beans of the two set-ups was done. Based on the t-test [$P(T \leq t) = 0.54$; $\alpha \leq 0.05$)], there was no significant difference between the means of pulp pH of the two set-ups. This means that the changes in the pH of the fermenting beans of the 3FB+4CPH set-up are comparable with the fermenting beans of the 25FB set-up.

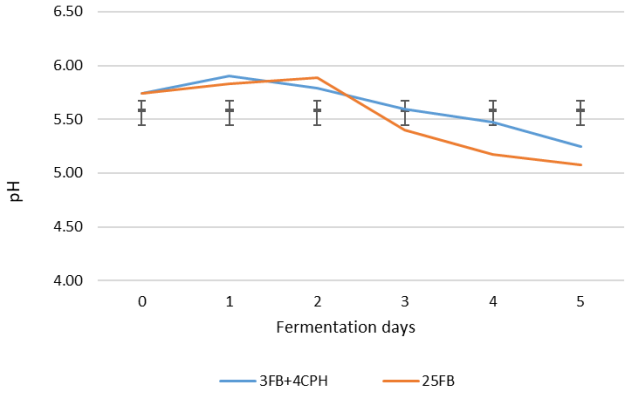

Figure 5. Daily pH of fermenting cacao beans in the 3FB+4CPH (3 kg beans with 4 kg CPH) and 25FB (25 kg beans) fermentation set-ups.

According to Biehl and Voigt (1999), as mentioned in Afoakwa *et al*. (2008), the rate of diffusion of organic acids produced in the external fermentation (pulp/ mucilage) into the cotyledons, timing of initial entry, duration of the period of optimum pH, and the final pH are crucial for optimum flavor formation. When the pulp fermentation metabolites (ethanol, lactic acid, and acetic acid) brought about by the succession of microorganisms (yeasts, LAB and acetic acid bacteria) that inoculate cocoa pulp spontaneously start to penetrate the bean through the seed coat, it generates a decrease in bean pH. Together with this decrease in pH, the rise in temperature around 45-50°C due to the exothermic reaction during metabolite formation caused the death of the seed embryo. Upon the death of the embryo, it triggers the breakdown of subcellular structures that allow contact of enzymes and substrates and the start of production of flavor precursors upon the hydrolysis of macromolecules such as proteins and carbohydrates (Santander Muñoz *et al*., 2019). Also, the diffusion of acids in the bean provides an acidic environment for enzymatic reaction to occur (Biehl *et al*., 1977) and has the protective function against the attack of putrefactive bacteria, which causes off-flavors (Quesnel, 1972).

The penetration of acid metabolites from the pulp to the beans caused an increase in the pulp pH and a decrease in bean pH as fermentation progressed. In the study of Jinap and Dimick (1990), they found that cocoa beans of lower pH at the end of fermentation (4.75 – 5.19) were scored lower for chocolate flavor and higher pH (5.50 – 5.80) were scored for off-flavor notes. Chocolate from intermediate bean pH (5.20 – 5.49) was scored more highly for the cacao flavor attribute. Similarly, Voigt and Lieberei (2014) specified that bean pH during fermentation affects the developed cocoa-specific flavor. According to them, fermentation at pH 5.5 – 5.0 gives strong cocoa-specific flavor while at pH 4.5 – 4.0 it produces weak cocoa-specific favor. When the pH is greater than 5.8, off-flavor notes (ham-like flavor) are formed.

3.4 Titratable acidity

The result of the TA analysis of the beans (expressed as % acid) for the two setups is shown in Figure 6. For the 3FB+4CPH set-up, TA from Day 0 (0.1432) up to Day 2 (0.1485) increased slightly and peaked at Day 3 (0.2050). It decreased again by Day 4 (0.1939) and yielded a final value of 0.1978. In contrast, the 25FB set-up has TA values increasing from Day 0 (0.1432) up to Day 5 (0.2758). Comparing the TA of the two set-ups at the end of fermentation, the acidity of the 3FB+4CPH set -up was lower than the 25FB. Although statistically, based on the t-test of means of TA for the two set-ups, they are not significantly different [$P(T \leq t) = 0.316$, $\alpha = 0.05$].

The total acid concentration in food is measured through TA. Organic acids such as citric, malic, lactic, tartaric, and acetic acid are the most common food acids. These acids, when present in foods, influence the flavor (tartness), color (impact of anthocyanins and other pH-influenced pigments), microbial stability (via pH-sensitivity characteristics of organisms), and food quality

https://doi.org/10.26656/fr.2017.9(6).241

(varying chemical sensitivities of food components to pH). While organic acids may be naturally present in food, they also may be formed through fermentation, or they may be added as part of a specific flavor formulation. Though related, however, TA is not a good predictor of pH, since pH is a combined function of titratable acid and conjugate base (Tyl and Sadler, 2017).

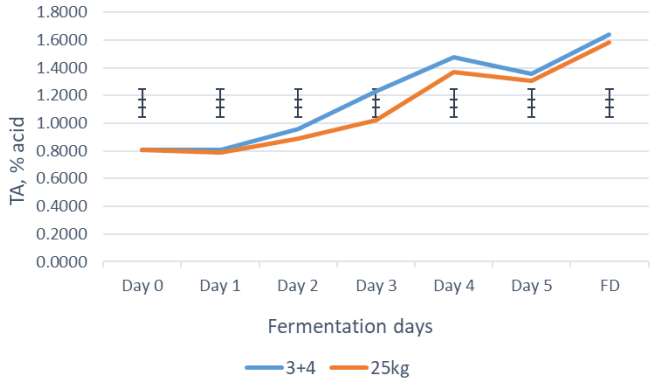

Figure 6. Daily titratable acidity (% acid) of fermenting beans in the 3FB+4CPH (3 kg beans with 4 kg CPH) and 25FB (25 kg beans) fermentation set-ups.

In the study of Jinap and Dimick (1990) where they studied 39 dried and fermented cacao bean samples from 13 countries, the TA ranged from 0.083 to 0.198 meq NaOH/g. The high acidity beans (0.17 – 0.20 meq NaOH/g) came from Malaysia, Brazil, and the Solomon Islands. Nazaruddin *et al*. (2006) looked into the effect of pod storage prior to fermentation on pH and TA. The results indicated a decreasing TA at the end of fermentation with longer pod storage (15 days). The same trend was shown in the study of Khairul Bariah and co-workers (2017) where they looked into the effect of pod storage on the temperature and physical changes during shallow box fermentation. For a duration of 5-day fermentation, the TA peaked at days 3 and 4 before decreasing at Day 5. The values of TA at day 5 for various pod storage ranged from 0.205 meq NaOH/g (6 days pod storage) to 0.333 meq NaOH/g (0 day of pod storage). These values are higher compared with the results of Nazarrudin *et al*. (2006).

From these various studies, it can be noted that the TA results can be variable depending on several factors like geographical origin of beans, method of fermentation, cacao variety, pod storage, and even drying procedure of fermented beans. Generally, TA values increase from Day 0 of fermentation and have a peak value around Day 3 to Day 4 of fermentation and eventually decrease up to the end of fermentation. It decreases further upon drying of beans. Bean quality on the basis of acidity is one factor that is considered by cocoa product manufacturers and, eventually consumers of cocoa products. In the study of Jinap and Dimick (1990), the high correlation between acetic acid and both pH and TA indicated that this acid could be primarily responsible for high acidity in cacao beans.

3.5 Fermentation index

The result of the FI analysis for the fermented beans in the two set-ups is shown in Figure 7. Generally, the trends for the FI of both setups were the same. By Day 3, FI values were already greater than one (> 1.0), which means that beans could be considered as well-fermented. FI values peaked at Day 4 with 1.4783 for the 3FB+4CPH set-up and 1.3673 for the 25FB. There was a decrease in FI values by Day 5 for both setups.

Considering the results, the 3FB+4CPH set-up had higher FI values than the 25FB. However, the t-test for the mean FI values for the two set-ups showed no statistical difference [$P(T \leq t) = 0.68$, $\alpha \leq 0.05$]. This suggests that 3FB+4CPH is comparable with 25FB in attaining well-fermented brown beans.

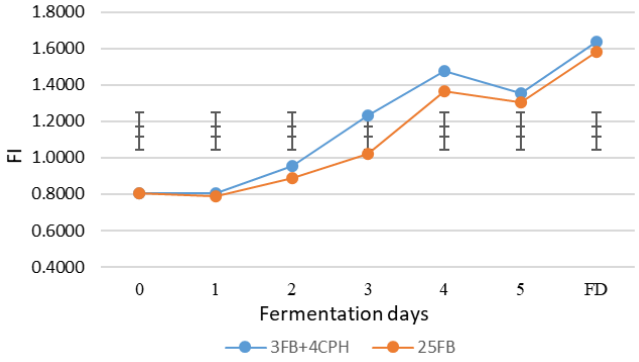

Figure 7. Fermentation index of fermenting and dried cacao beans in the 3FB+4CPH (3 kg beans with 4 kg CPH) and 25FB (25 kg beans) fermentation set-ups.

Fermentation index is usually measured using dried beans. The FI of dried unfermented and fermented beans were also measured (Table 1). There was a significant difference between the FI of the fermented and unfermented dried beans. While the FI of fermented beans increased, that of the unfermented beans decreased. For the fermented dried beans, FI values increased from 0.8037 (Day 0) to 1.6383 and 1.5817 for the 3FB+4CPH and 25FB set-ups, respectively, while the FI values for the unfermented dried beans decreased from Day 0 (0.8037) to 0.467.

Anthocyanins usually disappear rapidly during

Table 1. Comparison of the fermentation index of fresh and dried beans from 3FB+4CPH (3 kg beans with 4 kg CPH) and 25FB (25 kg beans) fermentation set-ups and unfermented set-ups

	Mean ± SD		
	3+4	25 kg	U
d0	0.8037±0.025	0.8037±0.025	0.8037±0.025
Dried	1.6383±0.142	1.5817±0.155	0.4670±0.097

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fermentation. These anthocyanins are hydrolyzed into anthocyanidins during fermentation and eventually polymerize along with simple catechins to form complex tannins (Ziegleder, 2009). In the review article by Wollgast and Anklam (2000) on polyphenols in *Theobroma cacao*, they reported that around 93% of anthocyanins were lost after four days of fermentation, and there was a change in color from purple to brown.

Indicators of well-fermented beans and dried cacao beans include a good brown color, low astringency and bitterness, and absence of off-flavors such as smoky notes and excessive acidity (Afoakwa *et al.*, 2012). The fermentation index (FI), which indirectly measures anthocyanin content, is considered a reliable indicator of cocoa bean fermentation levels (Romero-Cortes *et al.*, 2012). This color change due to anthocyanin content has been considered as a good index for the determination of the degree of fermentation (Pettipher, 1986). A sample cut test of beans from Day 0 to Day 5 of fermentation is shown in Figures 8-13. Fermented beans with fermentation index (FI) values less than one (<1) indicate under-fermented beans while FI values one or greater (≥1) indicate well-fermented beans. Fermentation index is determined spectrophotometrically as the ratio of absorbance of cacao pigments at 460 nm to that of 530 nm. These wavelengths can show structural properties and distribution through fermentation. The general λ_{max} for anthocyanin spectra is at 530 nm, and the 460 nm reflects the glycoside distribution (Harborne, 1958).

3.6 Correlation of temperature, pH, total acidity, and fermentation index

As presented in Table 2, strong to almost perfect linear relationships, either positive or negative, can be observed. For the 3FB+4CPH, an almost perfect positive (r = 0.99) relationship between the temperature of beans and the temperature of CPH exists. This substantiates the complementing effect of CPH in the rise in temperature of the fermenting beans, which is necessary in the death of the beans and commencing the cascade of biochemical reactions in the cotyledon.

The temperature of beans for both 3FB+4CPH and 25FB have similar trends for relationships with pH of pulp (weak positive, r = 0.45), pH of beans (moderate to strong negative, r = -0.45 and r = -0.71, respectively), TA (moderate to strong positive, r = 0.61 and r = 0.83, respectively) and FI (strong positive, r = 0.72 and r = 0.79, respectively). Notably, the rise in bean temperature

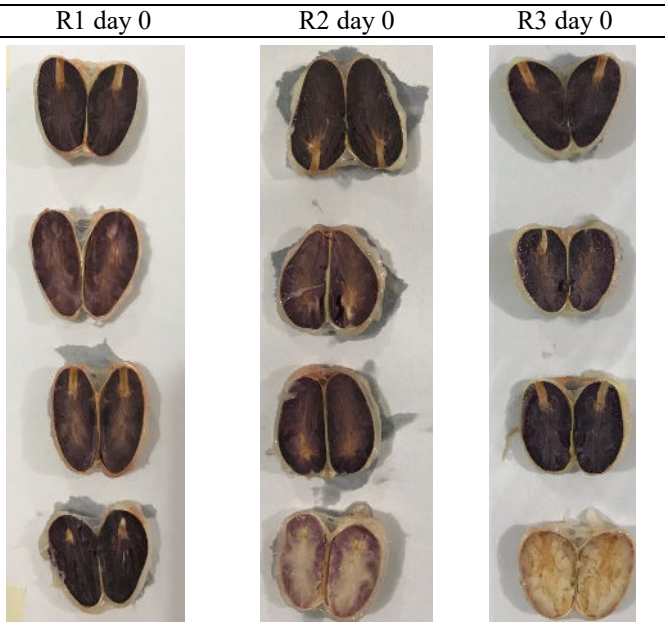

Figure 8. Cut test of beans at day 0 of fermentation.

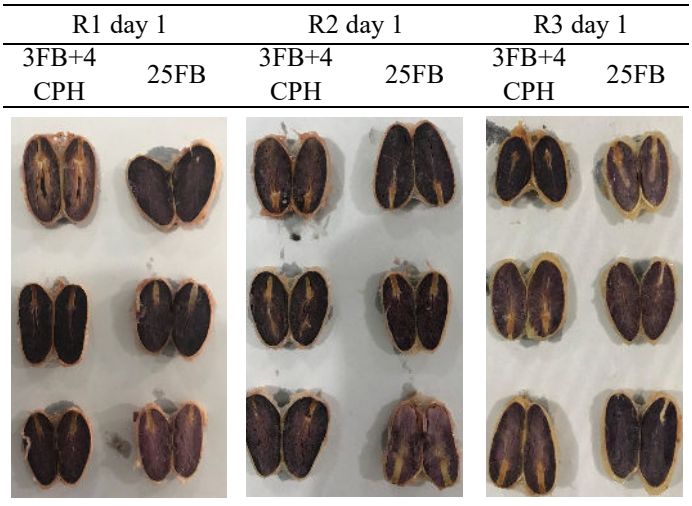

Figure 9. Cut test of beans from small-scale set-ups (3 kg beans with 4 kg CPH) and large-scale set-ups (25 kg beans) at day 1 of fermentation.

Table 2. Correlation coefficient (r) of various fermentation parameters in the 3FB+4CPH (3 kg beans with 4 kg CPH) and 25FB (25 kg beans) fermentation set-ups.

Parameters	Temp. beans, °C		Temp. CPH, °C		pH, pulp		pH, beans		TA		FI	
	3FB+ 4CPH	25FB	3FB+ 4CPH	25FB	3FB+ 4CPH	25FB	3FB+ 4CPH	25FB	3FB+ 4CPH	25FB	3FB+ 4CPH	25FB
Temp. beans, °C	1	1	0.99	-	0.45	0.45	-0.45	-0.71	0.61	0.83	0.72	0.79
Temp. CPH, °C	0.99	-	1	-	0.45	-	-0.45	-	0.59	-	0.72	-
pH, pulp	0.45	0.45	0.45	-	1	1	-0.99	-0.86	0.83	0.84	0.88	0.82
pH, beans	-0.45	-0.71	-0.45	-	-0.99	-0.86	1	1	-0.85	-0.98	-0.86	-0.94
TA, %acid	0.61	0.83	0.59	-	0.83	0.84	-0.85	-0.97	1	1	0.92	0.95
FI	0.72	0.79	0.72	-	0.88	0.82	-0.86	0.94	0.92	0.95	1	1

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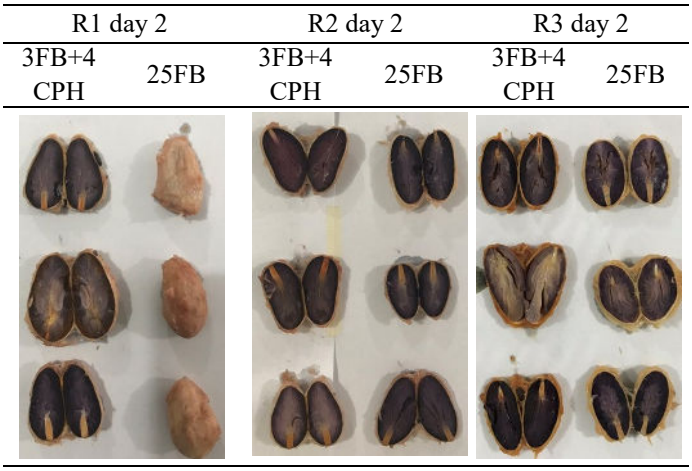

Figure 10. Cut test of beans from small-scale set-ups (3 kg beans with 4 kg CPH) and large-scale set-ups (25 kg beans) at day 2 of fermentation.

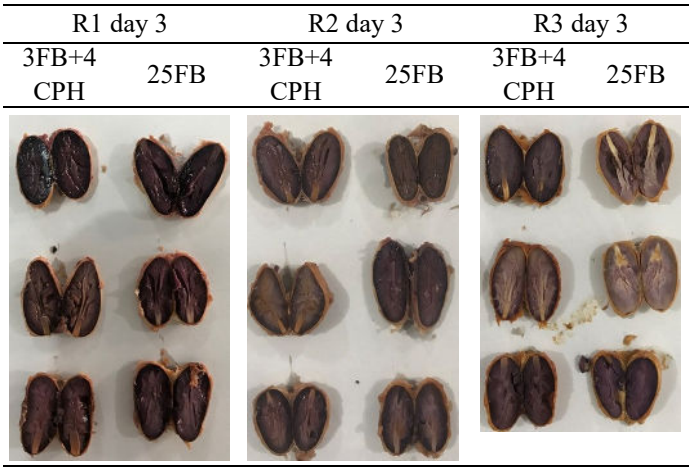

Figure 11. Cut test of beans from small-scale set-ups (3 kg beans with 4 kg CPH) and large-scale set-ups (25 kg beans) at day 3 of fermentation.

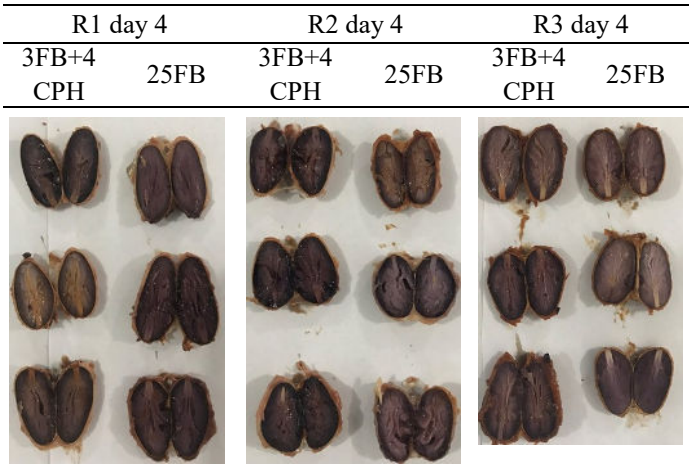

Figure 12. Cut test of beans from small-scale set-ups (3 kg beans with 4 kg CPH) and large-scale set-ups (25 kg beans) at day 4 of fermentation.

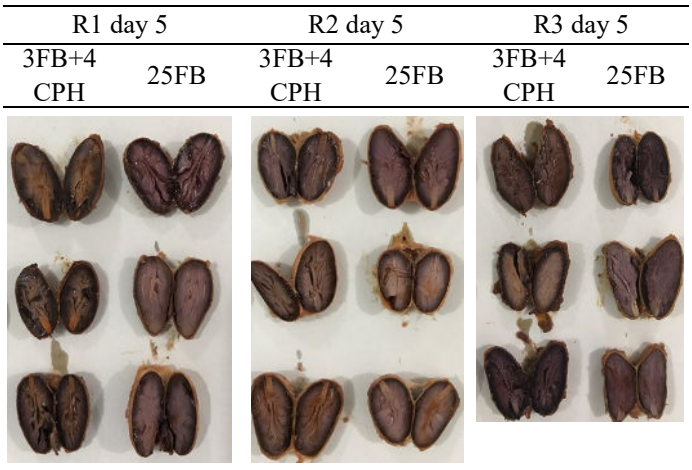

Figure 13. Cut test of beans from small-scale set-ups (3 kg beans with 4 kg CPH) and large-scale set-ups (25 kg beans) at day 5 of fermentation.

during fermentation is related to the conversion of pulp substrates to metabolites that will penetrate the beans (citric acid and sugars to lactic and acetic acid). The temperature of fermenting cacao bean mass influences the succession of microorganisms that will thrive, utilize substrates, and produce various metabolites in the fermenting mass. While the fermentation temperature rises, the simultaneous entry of these metabolites in the beans decreases the pH and subsequently increases TA. As this occurs, the underlying biochemical processes in the beans progress into completion.

For the pH of pulp and pH of beans, a strong negative relationship exists, both for the 3FB+4CPH and 25FB ($r = -0.99$ and $r = -0.86$, respectively). As expected, the increase in pH of pulp occurs once the lactic acid and acetic acid start to penetrate the beans, which in turn causes a decrease in the pH of cotyledons. This decrease in pH of beans means an increase in TA of beans. This is the reason for the strong negative relationship between the pH of beans and TA, and the strong positive relationship between the pH of pulp and TA.

Considering the foregoing results, the quality of beans fermented using the small-scale set-up are not significantly different from the quality of beans fermented using the large-scale set-up. Fermentation creates a value addition in the cacao beans that will be significant to farmers. Having comparable fermentation quality can alleviate the fermentation challenges of small -scale cacao farmers with limited volume of cacao harvests, thus improving the value addition in their products. This will give them market access and increase profit.

4. Conclusion

The study demonstrated that the optimized small-scale cacao fermentation set-up (3 kg beans + 4 kg cacao pod husk) performed comparably to the conventional large-scale set-up (25 kg) in key physicochemical parameters, including pulp and bean pH, bean and cacao pod husk temperature, titratable acidity, and fermentation index ($\alpha \leq 0.05$). Both fermentation set-ups yielded significantly higher fermentation index values than unfermented samples, which exhibited FI < 1.0,

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confirming inferior quality. These findings indicate that the small-scale set-up can effectively replicate large-scale fermentation performance, making it a viable and practical option for smallholder farmers and cacao research applications. Moreover, the incorporation of cacao pod husk as a fermentation substrate promotes waste reduction and environmental sustainability. When optimized conditions are strictly followed, the small-scale system enables farmers with limited bean volume to produce high-quality, fermented cacao beans with greater market competitiveness.

Conflict of interest

The authors declare no conflicts of interest.

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