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FULL PAPER

Small-scale cacao (*Theobroma cacao* L.) fermentation process utilizing cacao pod husk

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Abstract

Cacao fermentation is a postharvest process that yields cacao flavour precursors which eventually develop into distinct cacao flavour and aroma upon roasting. A standardized procedure to achieve quality fermented beans is the utilization of a large amount of cacao wet beans (25 kg – 500 kg) placed in fermentation boxes to achieve desired fermentation temperature, posing a challenge to small-scale farmers with a minimal amount of harvested beans. This study aimed to optimize a small-scale cacao fermentation treatment by enhancing volume requirement by utilizing cacao pod husk (CPH) in the fermentation matrix. Fermentation parameters such as bean temperature and pulp pH were factors considered during optimization. The optimization process used 9 treatments with varying ratios of beans and CPH. The temperature of CPH was also considered a factor that affected the temperature of beans. The treatments with CPH were observed to have significantly higher daily mean temperatures than those without ($p = 0.01529$). Test for correlation between the temperature of beans and CPH for treatments with CPH revealed 3 kg wet beans with 4 kg CPH (3FB+4CPH) have the highest correlation at 0.9806. It is based on this result that the treatment identified as the best small-scale set-up was the 3FB+4CPH. The high-temperature readings of CPH in the treatments with CPH (40°C or higher) indicate a complementing effect to the temperature of fermenting beans above it. It also suggests that added CPH in the treatments have the same effect in increasing the temperature of the fermenting beans. Results also showed that there was no significant difference in pulp pH among treatments ($p = 0.9464$). Based on the result of the pH reading for the various optimization treatments and considering results from different studies, the day 5 pH readings gave the best range of pH that is expected at the end of fermentation. The findings proved that the small-scale fermentation (3FB+4CPH) can be a practical and low-cost treatment compared with the traditional fermentation treatment.

1. Introduction

Recently, much interest has been given to cacao production due to a possible shortage in cacao supply as forecasted by the chocolate industry (Ferdman, 2014; Ford *et al*., 2014). Worldwide, the demand for cacao has nearly tripled since 1970 with leading countries like China and India with an annual growth rate of 3% and 7.9%, respectively. In the Philippines, the average annual cocoa consumption is 50,000MT according to the Department of Agriculture, Philippines (2017). Currently, the Philippines is a net importer of cacao. In 2014, the Philippines has exported $24.3 M worth of various cacao items while imported $102.3 M worth of various cacao items (Ramos, 2016).

Compared 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, 2014). Cacao is considered a high-value crop in the Philippines. Through the Department of Agriculture, the government has instituted policy measures by passing RA 7900 or the High Value Crop Development Program Act to boost

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high value crops such as cacao in the country (Philippine Republic Act 7900, 1995)

The Philippines is positioned to be a viable source of the global supply of cacao given its various advantages. Though much effort is being exerted by the government, private and research institutions, most of these actions are focused on improving cacao yield or mitigating cacao pests and diseases. Very few or almost none of these researches in the Philippines have been made or published that focus on improving postharvest processes, specifically fermentation process and flavour assessment and quality. The quality of the harvested cacao and its subsequent postharvest processing will dictate the price of the beans that can be sold in the market.

Among the postharvest processes, fermentation is the most crucial that brings out the sought-for chocolate flavour in the beans. Fermentation is a process necessary to get good cacao flavour from the beans. Later in the fermentation stage, acetic acid bacteria produce organic acids in the pulp which is a crucial process in cacao fermentation. These essential flavour precursors obtained during fermentation and drying are fully developed during the subsequent roasting and conching processes.

It is established that the quality of fermented beans is influenced by various factors. These include heap size (amount), postharvest pod age, fermentation time, drying time, and number and timing of turns/mixing during fermentation (Camu *et al*., 2008). These factors in turn affect the microbiota involved in the natural fermentation of cacao beans. The external manifestation that is easily measured as a result of the fermentative action of the succession of these microbiotas is the temperature of the fermenting mass, pH of pulp and oxygen tension (Camu *et al*., 2008). Among these factors, one crucial parameter in cacao fermentation that is a common problem among small-scale farmers is the heap size or volume of cacao wet beans to be fermented. In the various studies of farm -scale fermentation, the smallest volume needed to achieve a good fermentation is 25 kg of wet beans (Schwan and Wheals, 2004). Fermentation requires at least 25 kg of seeds to attain the required temperature (45 -50^{o}C) for proper fermentation to occur (International Cacao Organization, 1973; Afoakwa *et al*., 2008). This amount is large for most small-scale farmers leaving no other option but to skip fermenting their beans and instead, sell freshly harvested wet beans (Ramos, 2016).

In cacao farms, farmers are aware that fermented beans are better. However, there are reasons why many of them skip fermentation in their postharvest processing. These are inadequate amounts of fermentable wet beans, less significant financial incentives to take on added costs including labour and the desire of farmers to earn immediate income as the fermentation process takes between five to six days plus several more days for drying (Ramos, 2016). For these reasons and to assure uniformity in commercial beans, cocoa traders, agents or buyers generally prefer to buy wet beans from farmers. Small-scale farmers tend to sell wet beans to processors who have sufficient beans thus left with few opportunities for value addition. The value addition of selling fermented beans will be significant to these farmers if the technology to ferment a small volume of wet beans will be available. Since the volume of fermentable material is one requirement, this research tried to look into the possibility of combining chopped/ ground pod husk in the fermentation matrix to add fermentable volume. Pod husks are waste materials in the cacao industry. The pectin content of the pod husk is a polymer composed of a monosaccharide called galacturonic acid which has the possibility of being a fermentable material. As far as literature shows, this optimization for small-scale cacao fermentation is a novel and low-cost approach to seeking fermentation technology that can be adopted by small-scale farmers to enhance bean quality and marketability.

This study intended to present an alternative cacao fermentation set-up by establishing the use of a small volume of cacao wet beans enhanced with cacao pod husks (CPH) that can help small-scale cacao farmers in fermenting limited volume of cacao harvest.

2. Materials and methods

2.1 Source of samples

Fresh, ripe cacao fruits were sourced from local cacao farms in Guinobatan and Camalig, Albay, Bicol Region, Philippines. The variety of cacao fruits used was a limitation of this study as most farms in the area have no single variety being cultivated. Cacao fruits were sorted to eliminate diseased, overripe, unripe and spoiled pods. Also, damaged pods due to pests and fungal infestation were removed. A total of 1,138 cacao pods were used for the optimization process.

2.2 Sample preparation and treatment

2.2.1 Pod husk preconditioning and pod breaking

After harvesting, pods were subjected to storage in a cool and dry place, under cover from the rain, for 5 days. After 5 days, pods are ready to ferment when the moving sound is produced by the beans when shaken. This indicates moisture loss from the pod to allow good fermentation. Then the pods were broken using a clean machete and the beans were scooped manually out of the pods leaving the placenta in the pod. The beans were weighed and transferred to the Styrofoam boxes that were used for fermentation.

2.2.2 Fermentation optimization treatments

The optimization of the small-scale fermentation treatments was based on the 25 kg wet beans minimum amount in industrial fermentation (Schwan and Wheals, 2004). With this amount as a basis, a pre-trial fermentation was done using 5 kg of wet beans. These 5 kg wet beans were added with 5 kg chopped cacao pod husk (CPH). The daily temperature was measured throughout the fermentation process. Since the temperature of the fermenting mass reached 43^{o}C, it was deduced that this amount of wet beans can be considered for the small-scale set-up.

With the good result of the pre-trial set-up, optimization treatments were done starting with the 5 kg wet beans + 5 kg CPH (5FB+5CPH) and compared with 5 kg wet beans only (5FB+0). All optimization treatments were done in triplicates. The combination of cacao wet beans with CPH is shown in Table 1. All treatments of wet beans with CPH were compared with treatments of wet beans only. The cacao pod husks, after pod breaking, were chopped into small cube-like pieces (approximately 0.5 × 0.5 × 0.5cm) to reduce the size and increase surface area. For the treatments with CPH, a measured amount of chopped CPH was then placed at the bottom of Styrofoam iceboxes. For the treatments 2FB+3CPH, 2FB+4CPH, 3FB+0, 3FB+3CPH, 3FB+4CPH, 4FB+0, 4FB+4CPH, the dimension of the Styrofoam ice boxes used was 28 cm L × 20 cm W × 28 cm H × 2 cm T, while for treatments 5FB+0, 5FB+5CPH it was 42 cm L × 25 cm W × 32 cm H × 3 cm T. Once inside the box, the chopped CPH was covered with cheesecloth to separate it from the beans and prevent undesirable mixing and possible flavour effect on the beans. A wooden slat was placed on top of the cheesecloth to further prevent contact of beans with CPH while still allowing heat transfer and aeration. The beans were put in a cloth mesh bag and then placed on top of the wooden slats. It was covered with another cheesecloth to prevent heat loss and finally covered with the Styrofoam box cover.

Table 1. Variation in weight of beans and weight of CPH in various fermentation optimization treatments

Treatment	Weight of beans (kg)	Weight of CPH (kg)	Abbreviations
1	5	5	5FB+5CPH
2	5	0	5FB+0
3	4	4	4FB+4CPH
4	4	0	4FB+0
5	3	4	3FB+4CPH
6	3	3	3FB+3CPH
7	3	0	3FB+0
8	2	4	2FB+4CPH
9	2	3	2FB+3CPH

FB: Fermented beans, CPH: Cacao pod husks

2.3 Measurement of temperature

A calibrated thermometer was inserted from the outside through the holes on the side of the boxes and the mercury end was ensured to be in the middle of the fermenting mass of cacao beans and the CPH mass (Figure 1). This was done to monitor temperature changes during the entire fermentation process. Perforations/holes at the bottom and sides of the boxes were made to allow the release of fermentation liquid and prevent its accumulation throughout the fermentation period and allow aeration when it is needed.

Figure 1. The fermentation set-up in ice boxes

The fermentation treatments were placed in a ventilated room not directly exposed to sunlight. Mixing of the cocoa beans and CPH was done manually after 48, 72 and 96 hrs. Fermentation was done for six (6) consecutive days. The temperature of the fermenting mass and CPH were measured daily for the whole duration of fermentation. The environmental temperature and relative humidity were also obtained daily.

2.4 Analysis of pulp pH

Approximately 50 g bean samples were taken before fermentation (0 hr), and every 24 hrs during fermentation until 144 hrs. Bean samples were analyzed after sampling. In some cases the samples were not analyzed immediately, they were placed in clean plastic containers and stored in a freezer at around -18^{o}C (de Brito *et al*., 2000) for subsequent analysis of pulp pH.

The pH of the bean pulp was analyzed every 24 hrs throughout the fermentation period. The pulp pH analysis was based on AOAC method 970.21 (AOAC, 2012) with slight modifications (Ardhana and Fleet, 2003; Nazaruddin *et al*., 2006; Fahrurrozi, 2015). A total of 20 g of the sample was placed in a clean plastic zip lock and 20 mL of distilled water was added. This was massaged for five minutes to physically separate the pulp from the beans. The pulp fraction was recovered by decanting it into 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 mins. The supernatant liquid was decanted in a 10 mL beaker and the pH was measured

using a calibrated pH meter (Horiba D-54 pH and EC Meter, Japan).

2.5 Data analysis

Data were collated, and a descriptive summary was established and expressed as mean ± standard deviation (SD). It was analyzed using one-way ANOVA to identify significant differences among treatments (α = 0.05). To know which among the nine treatments are significantly different from each other, a test using the least significant difference (LSD) was done. Correlation test was performed to measure the relationship between the temperature of fermenting beans and the temperature of CPH. All statistical analyses were done using Microsoft Excel 2013.

3. Results and discussion

3.1 Fermentation temperature

The initial temperature of the fermenting cacao beans for all treatments ranges from 28.5 to 29.3°C (Table 2 and Figure 2). After 24 hrs, there was a difference between the mean temperatures of the treatments with CPH and those without CPH. For the treatments without CPH, the mean temperature ranged from 30.6 to 33.0°C while for those with CPH, the mean temperature ranged from 37.8 to 41.0°C. By the time the fermentation reached 48 hrs, those without CPH had a mean temperature ranging from 35.0 to 37.3°C and the treatments with CPH has a mean temperature ranging from 43.1 to 44.4°C. The first mixing of the beans and the CPH was done after 48 hrs. Mixing enhances the aeration of the beans and CPH. Mixing was subsequently done after 72 hrs and 96 hrs. After 72 hrs, only the treatments with CPH were able to reach a fermentation temperature of around 45°C and above. In Table 2, the temperature of the treatments with CPH after 72 hrs ranged from 44.4 to 48.0°C.

The treatment that gave the highest temperature of fermenting mass at 72 hrs was the 3FB+4CPH. From 96 hrs until 144 hrs, the 3FB+4CPH treatment was the only one that gave a consistent temperature of 45-50°C. The treatments with CPH that were able to reach 45-50°C at 72 hrs were 2FB+3CPH, 2FB+4CPH, 3FB+3CPH, and 3FB+4CPH. Treatments 4FB+4CPH and 5FB+5CPH attained a temperature range of 45 – 50°C at 96 hrs.

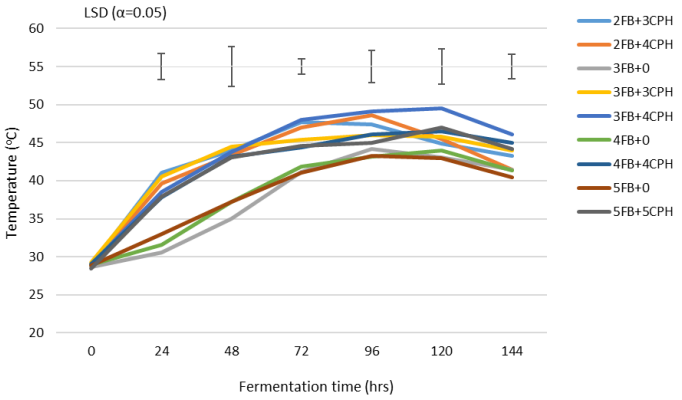

Figure 2. Changes in temperature of fermenting beans (FB) in the various optimization treatments with and without cacao pod husk (CPH)

The temperature of the chopped cacao pod husk added to the six treatments with CPH was monitored during the duration of the fermentation. Figure 3 shows the daily mean temperature of fermenting CPH mass of the various treatments with CPH used for optimization. After 24 hrs, the mean temperature range of the fermenting CPH was from 41.6°C to 51.0°C and after 48 hrs, the mean temperature range of the fermenting CPH was from 42.2°C to 49.7°C. At 72 hrs, almost all of the temperatures of CPH in the treatments reached 45.0°C, ranging from 44.6°C to 49.0°C except for 5FB+5CPH which was 43.7°C. This temperature was maintained for up to 120 hrs. At 144 hrs, only 3FB+3CPH, 3FB+4CPH and 4FB+4CPH had CPH temperature at 45°C or higher.

The trend for the temperature of fermenting beans for all treatments was similar though the treatments with CPH were observed to have higher daily mean temperatures. At 48 hrs, the mixing of beans and CPH was done. When mixing the mass of beans, there is a temporary cooling followed by a more rapid rise in temperature (Roelofsen, 1958). Aeration affects the succession of microorganisms involved in fermentation. From the start of fermentation until around 48 hrs, the condition of the fermenting mass is anaerobic due to the

Table 2. Changes in temperature of fermenting bean (FB) mass of the various treatments with cacao pod husk (CPH) used for optimization

Fermentation Treatment (kg beans + kg CPH)	Temperature (°C) at various fermentation time (hrs)								
	0	24	48	72	96	120	144	Mean	SD
2FB+3CPH[a]	29.0	41.0	44.0	47.7	47.4	44.8	43.3	44.7	2.3
2FB+4CPH[a]	29.0	39.6	43.3	47.0	48.6	45.5	41.5	44.3	3.1
3FB+3CPH[a]	29.3	40.6	44.4	45.3	46.0	45.7	44.0	44.3	1.8
3FB+4CPH[a]	29.0	38.5	43.7	48.0	49.1	49.5	46.1	45.8	3.8
4FB+4CPH[b]	29.1	37.8	43.2	44.4	46.1	46.5	45.0	43.8	2.9
5FB+5CPH[b]	28.5	38.0	43.1	44.6	45.0	47.0	44.2	43.6	2.8

Treatments with different superscript are significantly different (α = 0.05)

close packing of the pulp covering the beans. The initial microorganisms that thrive in this condition are yeasts, which are favoured with the low oxygen level and a low pH due to the citric acid content of the pulp. Yeast utilizes the sugar-rich, acidic pulp by producing ethanol with the presence of some lactic acid bacteria. 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, lactic acid bacteria, raises the temperature of the mass to 30-35°C after 24 hrs and to 35 -45°C after 48 hrs (Roelofsen, 1958; Schwan and Wheals, 2004). As the air starts to penetrate the beans due to pulp degradation and sweating, and the yeast population declines lactic acid bacteria (LAB) population increases. At around 36 hrs after fermentation, the population of LAB is expected to peak. Aeration of the fermenting mass is enhanced by manual mixing to aid penetration and uniformity in the fermenting mass. As air increases in the mass, the temperature rises typically around 45°C after 72 hrs, remaining at 45-50°C until fermentation is complete (Afoakwa *et al*., 2008). In the study of Biehl *et al*. (1977), a high temperature (45-50°C) beyond 48 hrs with the presence of ethanol and acetic acid was responsible for cacao bean death in the fermentation set up. Bean death is crucial to flavour 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 flavour precursors (Amin *et al*., 1998).

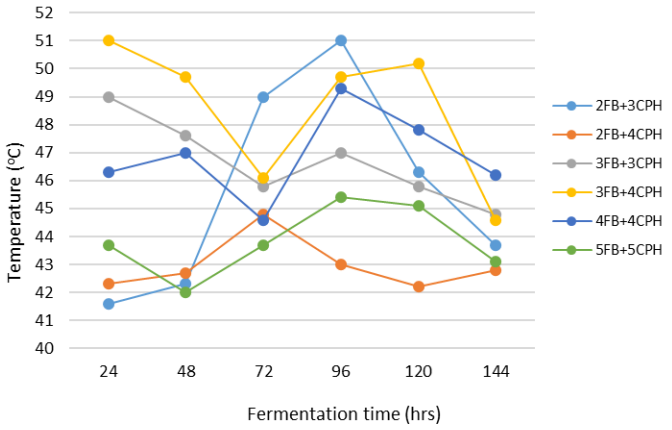

Figure 3. Changes in temperature of cacao pod husk (CPH) of the various treatments with CPH

From 96 hrs until 144 hrs, the 3FB+4CPH treatment was the only treatment that gave a consistent temperature of 45-50°C. According to Hatmi *et al*. (2015), the fermentation process will go well if, at the end of fermentation, the temperature is over 40°C and less than 50°C. This is consistent with the study of Afoakwa *et al*. (2008) that recommends the temperature remain at 45-50°C until fermentation is complete. The treatments with CPH that were able to reach 45-50°C at 72 hrs were 2FB+3CPH, 2FB+4CPH, 3FB+3CPH and 3FB+4CPH. Treatments 4FB+4CPH and 5FB+5CPH attained a temperature range of 45-50°C at 96 hrs. Noting these results and the recommendation for good fermentation based on temperature, it is worth considering that all treatments with CPH can be used for small-scale fermentation.

One-way ANOVA ($\alpha = 0.05$) was done to identify significant differences across treatments and it was found that there is a significant difference across treatments ($P = 0.01529$). Using LSD, treatments with CPH had significantly different daily mean temperatures compared to those without CPH.

Considering Figure 2, treatments with CPH have all attained significantly higher temperatures than the set-ups without CPH. The high daily mean temperature (45-50°C) of fermenting beans from the treatments with CPH suggests that the CPH was contributory to the markedly higher temperature of fermenting beans. Likewise, the high-temperature readings of CPH in the treatments with CPH (40°C or higher) indicate a complementing effect to the temperature of fermenting beans above it. On the contrary, the daily mean temperature of fermenting beans from treatments without CPH did not even reach the recommended minimum temperature of 45°C for fermentation.

The size of the iceboxes has an effect on the attainment of desired fermentation temperature. It can be noted that the fermentation box for the 4FB+4CPH was almost filled up with the beans and CPH restricting aeration which lowers temperature increase. On the other hand, the 5FB+0 and 5FB+5CPH treatments used a bigger box dimension as the amount of beans and CPH for these treatments would not fit in the same box used for the other treatments. The bigger box would require a bigger mass to attain the necessary temperature for fermentation. The size of the fermenting container may be a factor in the fermentation process.

The LSD test result showed that the treatment 3FB+4CPH gave the highest mean difference from treatments without CPH (3FB+0, 4FB+0 and 5FB+0). It is based on this result that the optimization treatment identified as the best small-scale treatment was the 3FB+4CPH. Also, it can be concluded, based on this study, that cacao pod husk (CPH) complements the amount of wet beans to reach the recommended temperature of the fermenting mass during the course of fermentation.

An ANOVA analysis was done to differentiate the temperature of beans compared with the temperature of CPH in all treatments with CPH (2FB+3CPH, 2FB+4CPH, 3FB+3CPH, 3FB+4CPH, 4FB+4CPH,

5FB+5CPH). The result showed that there is no significant difference between the temperature of fermenting beans and CPH across all treatments with CPH. To show statistically the complementing effect of CPH on the temperature of fermenting beans, a correlation test was done (Table 3). Among the treatments with CPH, the 3FB+4CPH treatment gave the highest positive correlation at 0.9806 followed by 2FB+4CPH at 0.9791. This shows that the temperature of the fermenting beans is positively correlated with the temperature of the added CPH in the fermentation treatment. Also, the best effect on the temperature of wet beans during fermentation is that of 3 kg wet beans added with 4 kg CPH. The correlation values were subjected to a test of significance ($\alpha = 0.05$) and the result showed that the correlation values were not significantly different.

3.2 Pulp pH

The pulp (mucilage) is the basic substrate for the growth of microorganisms (yeasts and bacteria) during the fermentation process. It is a well-defined layer around the seeds containing 84.4% water and 15.6% dry mass which is broken down into 11% total sugars (fructose- 4.5% and glucose- 4.5% as predominant sugars), 1.6% fat, 0.2% protein-N, 1-3% citric acid, 1-1.5% pectins, 2-3% pentosans (Pettipher, 1986). The low pH of around 3.0 – 4.0 of unfermented cacao pulp is mainly because of citric acid (Roelofsen, 1958; Ardhana and Fleet, 2003; Schwan and Wheals, 2004; Thompson *et al*., 2007; Guehi *et al*., 2010; Afoakwa *et al*., 2013). This rich cocktail of organic substances in the pulp is favourable to the growth of microorganisms that produces a diversity of metabolites like organic acids which are crucial in cacao fermentation. These acids diffuse into the beans through the testae and subsequently induce 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 the fermentation of cacao are crucial in the final bean quality (Afoakwa *et al*., 2013).

Generally, the trend for the pulp pH for all fermentation treatments was increased with hrs of fermentation (Figure 4). From 0 hr to 24 hrs, there was a slight decrease in pH among all treatments except for the 2FB+3CPH and 2FB+4CPH followed by a slight increase in the pH after 48 hrs of fermentation. The trend continued to rise until the end of fermentation at 144 hrs. At 144 hrs, the pulp pH ranged from 5.14 to 6.76.

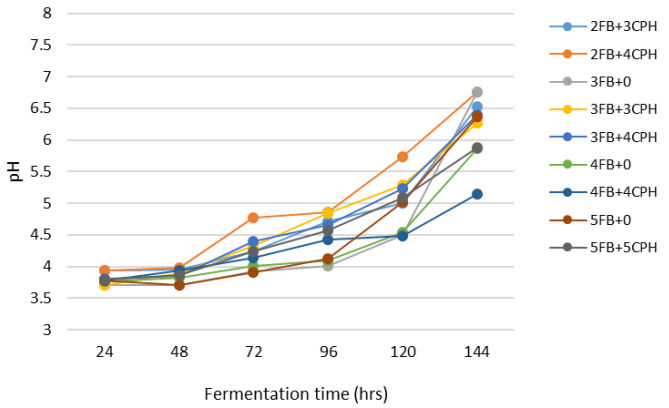

Figure 4. Changes in pulp pH of fermenting cacao beans in the various optimization treatments with and without cacao pod husk (CPH)

The pulp pH at 0 hr ranged from 3.85-4.02 while the pH at 24 hrs of fermentation ranged from 3.70-3.94. It can be noted that lactic acid and acetic acid are produced by lactic acid bacteria. This may connote a sudden drop in pH. The initial pulp pH and the changes in it during the course of fermentation define the succession of microorganisms that will proliferate in the pulp. Organisms come mainly from the hands of workers, knives, unwashed baskets used for the transport of seeds, and dried mucilage left on the walls of boxes from previous fermentations (Schwan and Wheals, 2004). The acidic pH of cacao pulp due to citric acid, although allowing fungal growth, is not favourable for bacterial growth except for lactic acid bacteria (LAB) (Ouattara *et al*., 2016). Yeasts and LAB are the microbial groups that are physiologically better adapted to thrive at the start of

Table 3. Correlation between the mean temperature of fermenting beans and cacao pod husk (CPH) of the various set-ups with CPH used for optimization

Fermentation Time (Hrs)	Temperature (°C)											
	2FB+3CPH		2FB+4CPH		3FB+3CPH		3FB+4CPH		4FB+4CPH		5FB+5CPH	
	Beans	CPH	Beans	CPH	Beans	CPH	Beans	CPH	Beans	CPH	Beans	CPH
24	41.0	41.6	39.6	40.7	40.6	42.0	38.5	39.7	37.8	40.0	38.0	40.7
48	44.0	42.3	43.3	42.7	44.4	44.8	43.7	43.0	43.2	42.2	43.1	42.8
72	47.7	49.0	47.0	47.6	45.3	45.8	48.0	47.0	44.4	45.8	44.6	44.8
96	47.4	51.0	48.6	49.7	46.0	46.1	49.1	49.7	47.4	50.2	45.0	44.6
120	44.8	46.3	45.5	47.0	45.7	44.6	49.5	49.3	46.5	47.8	47.0	46.2
144	43.3	43.7	41.5	42.0	44.0	43.7	46.1	45.4	45.0	45.1	44.2	43.1
r	0.9267		0.9791		0.9133		0.9806		0.9259		0.9488	

r = Pearson's correlation coefficient

fermentation since both groups display a remarkable tolerance to low pH (Axelsson, 2004; Deak, 2006 as mentioned in Lima *et al*., 2011). Additionally, yeast can withstand higher sugar concentrations (Barnett *et al*., 2000 as mentioned in Lima *et al*., 2011). The initial acidity of fresh cacao pulp, approximately pH 3.6, due to citric acid, high sugar content and the low oxygen supply due to the tightly-packed structure of the cocoa bean mass favour yeasts, which outnumber LAB and other organisms during the first day (Roelofsen, 1958; Schwan and Wheals, 2004; Lima *et al*., 2011). The yeast's metabolic activity in the cocoa bean pulp leads to the production of ethanol with an associated increase in temperature (Lima *et al*., 2011) while LAB utilizes citric acid. According to Jespersen (2003), there are some yeasts that are able to metabolize citric acid which contributes to the increase in pH of the pulp. The consumption of citric acid results in a gradual rise in pH and the increase in temperature due to alcoholic fermentation becomes less favourable to yeasts and more favourable to lactic acid bacteria (Roelofsen, 1958). Yeast also has the ability to produce pectinolytic enzymes that reduce pulp viscosity, drain away "sweatings" and increase oxygen pressure (Schwan and Wheals, 2004; Lima *et al*., 2011). With these changes, the yeast population declines and favours the growth of acidoduric LAB. Lactic acid bacteria convert citric acid and residual carbohydrates of the pulp to lactic acid, acetic acid or mannitol (Pereira *et al*., 2012). It can be noted that lactic acid and acetic acid are produced by lactic acid bacteria. This may connote a sudden drop in pH. The production of these acids causes a drop in the pH of the pulp, which can be detected in around 24 to 48 hrs (Nielsen *et al*., 2007). However, the fact that yeasts can assimilate lactic acid, in conjunction with the fermentative ability of LAB to use citric acid, partly explains why the overall effect of acids production may not produce such a pronounced drop in pH during the first two days of fermentation (Camu *et al*., 2007). Another reason for this is the fact that lactic acid and acetic acid have lower acid-dissociation constants than citric acid (Lima *et al*., 2011).

Considering Figure 4, the 72 hrs bean pulp pH in treatments with added CPH has higher pulp pH (4.14 – 4.77) compared with the bean pulp pH of treatments without CPH (3.90-4.01). As fermentation progresses, aeration in the fermenting mass increases with increasing temperature around 37^{o}C or higher. At this stage, acetic acid bacteria (AAB) becomes the dominant microorganism and the population peaks around 88 hrs (Schwan and Wheals, 2004). The low levels of sugars due to yeast and LAB metabolism shifts the AAB metabolism towards oxidation of ethanol produced by yeasts and lactic acid generated by LAB as a carbon source to generate acetic acid and eventually oxidized to carbon dioxide and water (Lima *et al*., 2011; Ouattara *et al*., 2015). Acetic acid produced by AAB is a key metabolite for the cocoa bean fermentation process. Acetic acid diffuses into the beans, and in combination with heat reaching between 45-50^{o}C or more due to the exothermic bioconversion of ethanol into acetic acid, causes the death of the seed embryo, the disruption of the internal cellular structure of the beans, and the end of fermentation (Schwan, 1998; Camu *et al*., 2008).

The pH trend continued to rise until the end of fermentation at 144 hrs. The same trend was observed in the study of Afoakwa *et al*. (2013) where they used various pulp pre-conditioning (storing of cacao pods) before fermentation of 30 kg cacao wet beans. This optimization treatment used pulp pre-conditioning by storing the pods for 5 days. In the said study of Afoakwa *et al*. (2013), the pods which were pre-conditioned by storing for 3 days resulted in a pH increase from 3.98 to 5.04, for pods stored for 7 days gave a pH increase from 4.01 to 5.23 and pods stored for 10 days gave a pH increase from 4.02 to 5.24 at the end of the sixth day of fermentation. This is in contrast to the pods without storing in which pH increased just from 3.88 to 3.96. These pH values at the end of fermentation in the study of Afoakwa *et al*. (2013) are similar to the pH values in this study at 120 hrs of fermentation. At 144 hrs, the pulp pH ranged from 5.14 to 6.76. These gradual increases in pH of the pulp during fermentation were suspected to be due to the reported decline in citric acid concentration (Schwan and Wheals, 2004; Jespersen *et al*., 2005). During fermentation, yeasts and lactic acid bacteria break down the citric acid in the pulp to metabolize the pulp sugars leading to an increase in the pH from 3.5 to 4.2 (Schwan *et al*., 1995; Schwan, 1998; Schwan and Wheals, 2004; Jespersen *et al*., 2005).

In the study by Ardhana and Fleet (2003), the pH of cacao pulp increased from between 3.7 – 3.9 at the start of fermentation to between 4.8 – 4.9 by the end of fermentation. In the study by Schwan (1998) of cacao fermentation in Brazil, the maximum and final pH at the end of fermentation was 5.9. In the review paper by Lima *et al*. (2011), where they compared the pulp conditions among 13 cocoa bean fermentation from various countries, the final pulp pH at the end of fermentation ranged from 4.2 to 5.9. One possible reason for the differences among these fermentations is the geographical source of the beans as shown in the study by Hori *et al*. (2016).

Analysis of variance (ANOVA at $\alpha = 0.05$) showed that there was no significant difference in pulp pH across treatments ($p = 0.9464$). As mentioned earlier, one

parameter considered for optimizing the length of fermentation for this research was the pulp pH. Based on the result of the pH reading for the various optimization treatments (Figure 4) and considering results from different studies, the pH readings at 120 hrs gave the best range of pH that is expected at the end of fermentation. Another reason for recommending a five-day fermentation using the treatments in this research was that most of the beans at 144 hrs were dark brown or black in colour. Also, a putrid smell was observed at 144 hrs. These are considered indicators of over-fermented beans which are unfavourable to the taste and quality of the beans.

4. Conclusion

The parameters used to optimize the pod-husk assisted cacao fermentation treatment were the temperature of beans, the temperature of CPH, and the pH of pulp. All the treatments with cacao pod husk were able to meet the required temperature of 45-50^{o}C from 96 hrs up to 144 hrs of fermentation. Significant differences were determined among the treatments using ANOVA and LSD tests. Among the treatments, the 3 kg wet beans with 4 kg cacao pod husk (3FB+4CPH) gave the best result in terms of all the parameters. One result of the small-scale fermentation optimization process is shortening the fermentation time to 120 hrs as the beans were over-fermented at 144 hrs showing dark brown to black beans with hammy and putrid odours. Also, the growth of moulds commences at 144 hrs which gives deteriorating quality to the beans.

The small-scale fermentation (3FB+4CPH) can be a practical and low-cost treatment that can improve the postharvest processing of small-scale cacao farmers by utilizing the waste product in cacao farms which is the cacao pod husk, giving them the quality of fermented dried beans comparable with beans processed using large scale fermentation. Cacao farmers can be guaranteed value-adding benefits through the use of this treatment, commanding a fair price for their cacao beans in the market in comparison with just selling wet beans or dried beans. Likewise, this small-scale treatment can be a cost-effective treatment that can be used for cacao research studies (e.g., flavour differentiation and development among varied sources of cacao beans, metabolomics of cacao beans, differentiation of cacao sourced from various geographical growing regions). It is recommended to use a thermal probe with a data logger that will give a complete picture of the varying fermentation temperature in the cacao bean mass.

Conflict of interest

The authors declare no conflicts of interest.

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