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Changes in Physico-Chemical and Microbiological Properties in Thai Cocoa Bean Fermentation

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Abstract

The purpose of this research is to investigate the quality-related physical, chemical, and microbiological changes in Thai cocoa beans during fermentation in 2 types of wooden containers. The results will compose a book of guidelines for good Thai cocoa fermentation in order to educate Thai farmers. Fresh Thai cocoa beans have a low pH value (5.0 - 5.5) compared to those from other countries in general (6.0 - 7.0). However, fermented temperature is able to reach 40 - 45 °C in 6 days, which is a main criteria for finishing cocoa fermentation. The color of fresh cocoa beans changes from white to brown within 2 days; after that, cocoa beans are mixed from the top to the bottom of the containers. Three groups of microorganism are evaluated with 3 different sampling points in wooden containers. The results reveal that yeast is grown quickly in 2 days on the top of containers, and then acetic acid bacteria and lactic acid bacteria are grown by the utilized yeast's metabolites. These behaviors were found in both of the 2 wooden containers; however, a heap of cocoa beans (200 - 250 kgs) in a wooden box showed better quality of cocoa fermentation than a small volume (40 - 50 kgs) in a wooden tray.

Keywords: Thai cocoa, Fermentation methods, Quality changes, Physico-Chemical, Microbiological

Introduction

Global cocoa demand, based on the present production output of the Ivory Coast, will rise by 30 % [1]. However, climate change is expected to affect global cocoa production, which could result in reduction and in insufficient cocoa supply on an industrial level [2]. To date, countries producing cocoa beans are mainly located in the cocoa belt, 23.5 °N to 23.5 °S around the equator, and the main cocoa production area is in west Africa, which produces more than 1.7 million tons of beans per year. In Thailand, less than 200 thousand tons of cocoa beans are produced each year [3].

Cocoa bean fermentation is one of the few remaining spontaneous microbial processes that occur on the farm through traditional practices, subsequently being processed at other facilities downstream in the food processing chain [4]. The importance of cocoa bean fermentation in chocolate quality determination has been well-characterized for over 100 years, including physico-chemical and microbiological aspects [5]. Spontaneous fermentation is a complex process driven by a large number of fungi and bacteria within particular growing and processing environments [4,6]. The cocoa pods are naturally inoculated with these various fungi and bacteria at the time of harvest [7] and are subsequently affected by these various fungi and bacteria during the fermentation/drying processes. A variety of additional microorganism sources from processing are also found during cocoa fermentation. The dried/fermented beans also come into contact with a variety of inoculating surfaces with their own varied local microorganisms. These contact areas include tool surfaces and various containers where the pods are held for fermentation and drying, the pod surfaces themselves, knives, laborers' hands, banana or plantain leaves (which are used to cover the piles of beans during fermentation), and residual microorganisms from previous fermentation.

Microbial metabolites are essential for chocolate flavor and aroma [8]. The uniqueness of chocolate flavor is driven by the genetic constitution of the cocoa variety, although the fermentation process releases and develops this flavor.

Cocoa production has occurred for 30 years ago in southern Thailand, thanks to the suitable climate conditions. A few studies on cocoa bean fermentation were conducted to adapt cocoa production to Thailand conditions. For example, Aran *et al.* [9] revealed that the changing of the microorganism community during cocoa fermentation in a wood basket was different in different locations in southern Thailand. These studies also pointed out that there were different species of yeast, lactic acid bacteria, and acetic acid bacteria found during the cocoa fermentation process. However, these studies were conducted a long time ago, while the production yield of cocoa pods in Thailand was very low compared to others countries. Thanks to global warming, we now can grow cocoa plants in many provinces in northern Thailand, such as Lampang, Nan, Chiangmai, Chiangrai, and Maehongson. Nowadays, cocoa is a new economic crop in Thailand. However, the quality of cocoa pods which are grown in different parts of Thailand, as well as the dried cocoa beans during the natural cocoa fermentation process, still needs to be systematically evaluated. Therefore, this research aims (1) to understand the physical, chemical, and biological changes during cocoa fermentation, and (2) to establish a standardized cocoa fermentation procedure which is operated by a specialist of a Thai coffee and cocoa company in Lampang province. The results of this study will assist cocoa farmers in obtaining a good quality of dried fermented cocoa beans.

Materials and methods

Raw material preparation

Fresh Thai cocoa pods with at least 70 % ripening were collected from more than 3-year-old cocoa trees. Upon harvesting, collected pods were transported to the lab, which took about 4 to 7 days. Cocoa pod appearance is shown in **Figure 2**; the spoiled pods were discarded, and pods with good appearance were kept for downstream experiments. Good quality pods were cut horizontally with a shape knife manually, and then fresh cocoa beans were accumulated with good sanitation in a plastic box before being transferred to a wooden container (**Figure 3**). For the laboratory scale experiment, a square wooden tray container ($40\times40\times10$ cm^3) with a small hole in each 10 cm consisting of 5 layers was used. The capacity of each shelf was 10 kg of cocoa beans. For the industrial scale experiment, a trapezoid wooden box ($60\times90\times50$ cm^3) was used which could take up 200 - 250 g of fresh cocoa beans from 800 - 1,000 kg of cocoa pods. As a huge amount of fresh cocoa pods was required during our experiment, fresh pods were initially collected separately in 25 kg plastic containers and pooled together for further analyses.

Cocoa fermentation

At the beginning of cocoa fermentation, banana leaf was required to put inside wooden trays or wooden boxes in order to prevent moisture loss. Additional layers for the wooden box were 2 layers of nylon net, placed before adding cocoa beans. Cocoa fermentation was carried out using natural microorganisms. Briefly, 100 g of cocoa beans from the 1^{st} and 4^{th} layers of the wooden tray (for the laboratory scale experiment) and 3 different sampling points in the wooden box (for the industrial scale experiment) were used, as shown in **Figure 1**. The temperature, pH, and microbiological changes during fermentation of these selected samples were monitored and analyzed.

Fermentation condition

Temperature and relative humidity outside of both the wooden tray and the wooden box during cocoa fermentation were measured using a Temperature and Humidity Data-logger (CEM, DT-172). Then, a correlation analysis between the environmental condition results and chemical-microbiological changes during fermentation was performed. To study the fermentation closely, we selected 2 time periods of fermentation; September to October, 2019 (Main Crop), and February to March, 2020 (Mid-Crop). This took 5 - 6 days due to finishing up cocoa fermentation in each period. All experiments were conducted at Mueang District, Lampang Province, Thailand.

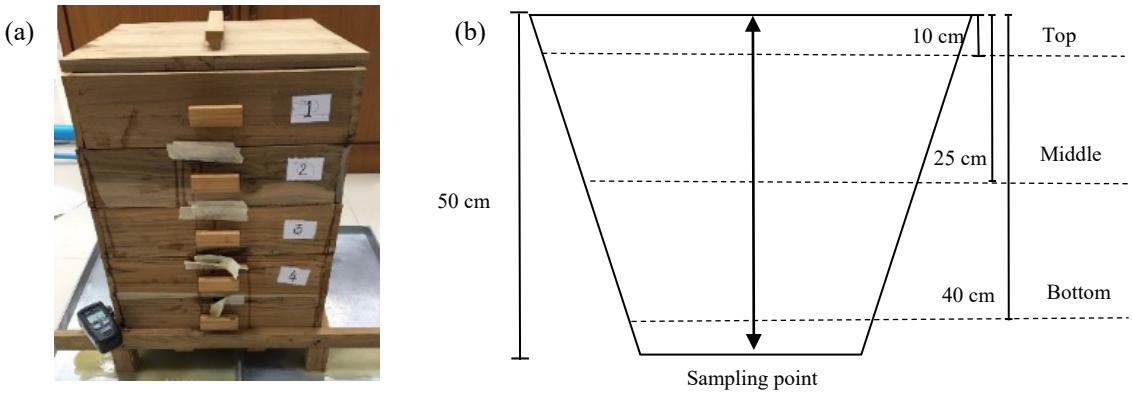


Figure 1 (a) 5 layers of the wooden tray (40×40×10 cm^3) for the laboratory scale and (b) the wooden box (60×90×50 cm^3) for the industrial scale.

Fermentation temperature

The inner temperature of a heap cocoa beans in the wooden tray (the laboratory scale) was measured every 8 h, while that of the wooden box (the industrial scale) was monitored every 24 h, using a thermometer (50S/50D, Fluke, USA 1999).

Acidity of fermented cocoa beans

10 g of cocoa beans were sampled from each spot in the wooden tray and the wooden box, with the same intervals as temperature measurement. Then, 100 mL of distilled water was added, and the mixture of beans and water was blended using a stomacher (Inter science model VAR.SPEED 030213289, Germany) at level 8 for 2 min. The acidity of cocoa beans during fermentation was measured using a pH-meter (Consort C830, CE, Belgium). Each experiment was repeated 3 times.

Enumeration of microorganism

Three groups of native microorganisms are typically found in cocoa fermentation; yeast, lactic acid bacteria, and acetic acid bacteria [9]. Therefore, we analyzed these by sampling 20 g of cocoa beans from each spot of the wooden box with the same interval as chemical property measurement described earlier. An amount of approximately 180 mL of ringer solution was mixed with the cocoa beans and blended using a stomacher (Inter science model VAR.SPEED 030213289, Germany) at level 8 for 2 min. We conducted 3 sampling preparations for microbiological changes during fermentation. For each preparation, 10-fold dilutions with spread plate techniques were used to study the changes of the 3 groups of native microorganisms during cocoa fermentation.

Media and enumeration conditions for yeast

To isolate the natural microorganisms, Potato Dextrose Agar plate (PDA, Merck, Thailand) media supplemented with 100 mg/L of chloramphenicol were prepared and sterilized at 121 °C for 15 min. Then, 0.1 mL of dilution solution was spread on PDA plates and kept at 25 °C for 2 - 5 days. Yeast colonies on PDA plates were counted using the AOAC method number 2002.11.

Media and enumeration conditions for lactic acid bacteria

De Man Rogosa and Sharpe Agar (MRS, Merck, Thailand) media added with 0.2 % sorbic acid, 0.1 % cycloheximide and 01 % cysteine-HCl were sterilized at 121 °C for 15 min. Similarly, 0.1 mL of dilution solution was spread on the MRS plates and kept at 30 °C for 3 - 4 days. Lactic acid bacteria on the MRS plates were counted according to Nielsen *et al*. [10].

Media and enumeration for acetic acid bacteria

Acetic acid bacteria (AAB) media included 0.1 % D-glucose, 1.5 % bacteriological peptone, 0.8% yeast extract, 0.3 % acetic acid, 0.5 % ethanol, and 2.0 % agar (adding 0.1 M NaOH until reach pH 3.5). An amount of 0.1 mL dilution solution was spread on the AAB plates and keep at 25 °C for 3 - 4 days. Acetic acid bacteria on the AAB plates were counted according to Wieme *et al.* [11]

All colonies found on 3 basic media was done by gram stain method [12] in order to see cell morphology under microscopy.

Cut test analysis

A total of 100 dried fermented beans were taken randomly from 250 g samples derived by quartering technique. The dried beans were cut lengthwise into halves for maximum surface exposure. Both halves of each surface were inspected under artificial light and divided into 5 groups (fully brown, partly brown, partly purple, fully purple, and slaty) according to SIRIM [13]. The percentage of the beans was converted into Equivalent Percent Fully Brown (EB) score to compare the degree of fermentation, following the equation below.

EB = 1×% fully brown + (0.7×(% purple-brown)) + (0.5×% fully purple) + (0.3×% slaty)

Statistical analysis

Customized random design was applied for all experiments in this study. Analysis of variance (ANOVA), test of significance, and comparison of means using Duncan test were performed using SPSS version 26 (2019) with a confidence level of 95 %.

Results and discussion

In this research, 70% ripening of fresh cocoa pods was a criteria for raw material selection. Normally, green cocoa pods turn to yellow when they are ripe. However, some of green-yellow cocoa pods were also acceptable, as shown in **Figure 2**.

Good appearance

Fresh cocoa beans in a yellow and a green-yellow pod

Spoilage pod

Figure 2 Cocoa pod appearance during stage of raw material receipt.

Healthy cocoa pods were peeled manually, the middle fibers were discarded, and then cocoa beans were collected for experiments. Fresh cocoa beans were accumulated under good sanitation for in both experimental scales. However, for the industrial level, 25 kg per plastic box were applied in order to achieve a large amount of cocoa beans (200 to 250 kg). These preparation steps are demonstrated in **Figure 3**. Environmental conditions, temperature, pH, and microbiological properties were evaluated every 8 h for Main Crop (the 1st period) in order to understand the change during fermentation. Due to a lack of monitoring data of environmental factors during cocoa fermentation in Thailand, these parameters were evaluated and explored a relationship between the surrounding temperature and the relative

humidity of each sampling time. Change of outer temperature and relative humidity during the 1st period of cocoa fermentation are described in **Figure 4**. There was an inverse relationship between outer temperature and relative humidity. At 2 pm of each day, the outer temperature was highest, while the relative humidity was lowest, and pH of the 1st and 4th tray were decreased at 2 pm of each fermentation day. It was to be seen that the highest outer temperature or the lowest relative humidity of each fermentation day would be a good sampling point for understanding changes in Thai cocoa fermentation, because decreasing pH value meant natural microorganisms could produce more acid that was metabolite product.

Figure 3 Cocoa bean preparation in the industrial scale experiment; (a) Peeling cocoa pods manually, (b) A representation of good quality of fresh cocoa beans, (c) Accumulating fresh cocoa beans with good sanitation in a plastic box before transferring to the wooden box.

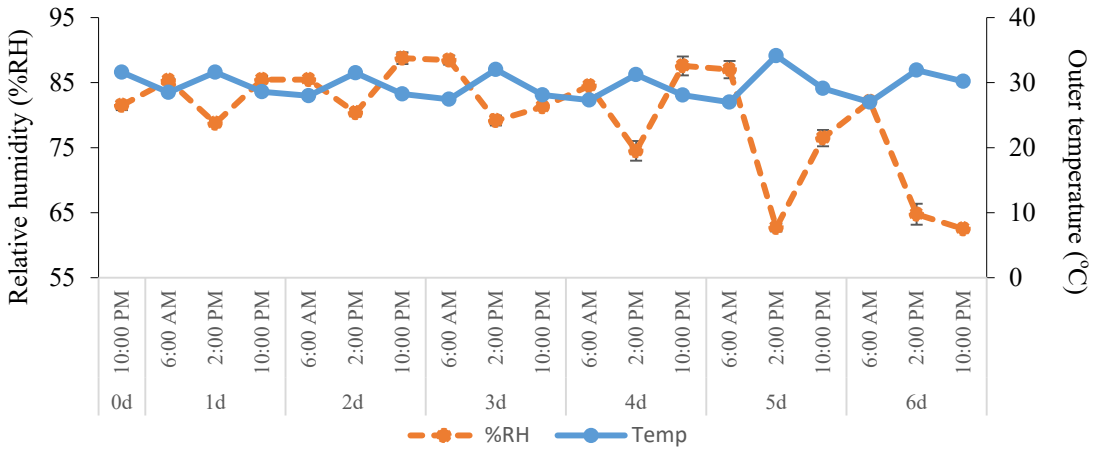


Figure 4 Change of the outer temperature and relative humidity during cocoa fermentation in the wooden tray (the laboratory scale) from September to October 2019.

During 6 days fermentation, inner temperature of each tray was monitored at 6 am, 2 pm, and 10 pm. Different of inner temperature for each day was analyzed (**Figure 1**); also, a relationship between wooden trays and each sampling time was evaluated (**Table 2**). At the beginning of cocoa fermentation, inner temperature increased rapidly and then was stable on the 4th day until the end of cocoa fermentation (**Table 1**). Inner temperature of the wooden tray was not more than 39 °C, which was lower than a suitable inner temperature (40 - 45 °C). This result showed that a small heap of cocoa beans in a wooden tray (40 - 50 kg) was not appropriate for natural microorganisms to grow and obtain a suitable inner temperature.

Table 1 Average inner temperature of the wooden tray on each fermentation day.

Fermentation day	Inner temperature (°C)
0	31.00±0.13^{e}
1	33.18±0.12^{d}
2	35.05±0.24^{c}
3	36.93±0.32^{b}
4	38.49±0.10^{a}
5	38.51±0.15^{a}
6	38.89±0.11^{a}

Remark: Values with different letters in the same column are significantly different at $p < 0.05$.

Relationship of inner temperature of each tray at a different sampling time is shown in **Table 2**. It seems that inner temperature in the 1st wooden tray was not significantly different ($p \geq 0.05$) to that in the 2nd wooden tray. The lowest inner temperature was found in the 4th wooden tray, and sampling times at 2 pm and 10 pm of each tray were not quite significantly different ($p \geq 0.05$). The reason for studying cocoa fermentation in the wooden tray for Main Crop was to confirm a uniformity of cocoa bean color, and the 5th tray was empty because of obtaining a good air flow to induce the growth of natural microorganisms [14]. The position of the cocoa beans was important to the growth of microorganisms, as the 1st and the 2nd wooden tray provided a higher inner temperature, while in the bottom (the 4th wooden tray), there was a lower inner temperature. Therefore, this cocoa fermentation process required a mixing up of cocoa beans in order to induce the growth of microorganisms.

Table 2 Relationship between sampling time and average inner temperature of the wooden tray during 6 days cocoa fermentation.

Tray	Sampling time	Inner temperature (°C)
1	6 am	35.88±0.02^{ab}
	2 pm	37.98±0.01^{a}
	10 pm	37.77±0.01^{a}
2	6 am	36.89±0.03^{ab}
	2 pm	38.07±0.01^{a}
	10 pm	38.76±0.02^{a}
3	6 am	35.61±0.02^{ab}
	2 pm	37.33±0.01^{ab}
	10 pm	37.80±0.02^{a}
4	6 am	34.33±0.01^{b}
	2 pm	36.09±0.02^{ab}
	10 pm	36.60±0.01 ^{ab}

Remark: Values with different letters in the same column are significantly different at $p < 0.05$.

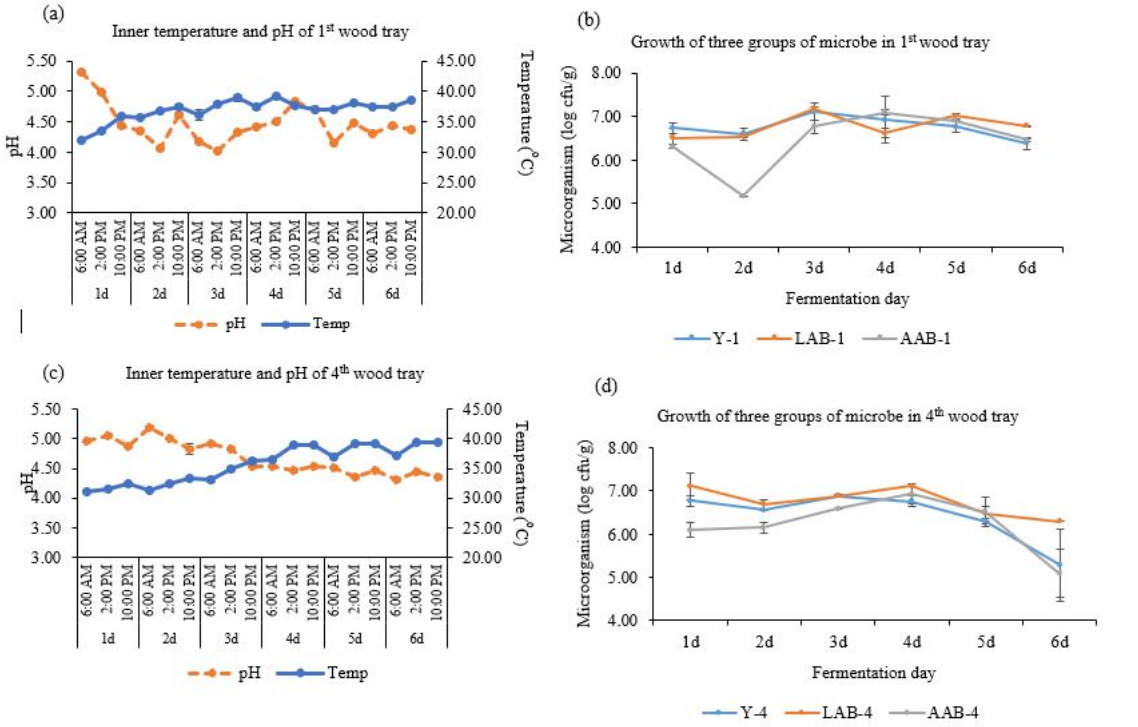


Figure 5 Changes in temperature, pH, and microbiological properties during cocoa fermentation in the 1st and the 4th wooden trays: (a) the inner temperature and pH in the 1st wooden tray, (b) the growth of yeast (Y-1), lactic acid bacteria (LAB-1), and acetic acid bacteria (AAB-1) in the 1st wooden tray, (c) the inner temperature and pH in the 4th wooden tray, (d) the growth of yeast (Y-4), lactic acid bacteria (LAB-4), and acetic acid bacteria (AAB-4) in the 4th wooden tray.

In general, pH and temperature of cocoa beans can be used as indicators for a good quality of cocoa fermentation. The pH value of unfermented fresh cocoa beans would be around 6.6 - 7.0, while the pH of fermented cocoa beans should be 5.5 [15]. However, in our experiment, initial pH value of fresh Thai cocoa beans exhibited at 5.0 - 5.5. Cocoa bean temperature in a fermentation container should be around 40 - 45 °C at the end of the fermentation process. Therefore, we monitored these 2 indicators every 8 h during 6 days fermentation in the wooden tray (laboratory scale). At the beginning of cocoa fermentation in the wooden tray, pH of cocoa beans in the 1st tray decreased more rapidly than in the 4th tray. Also, cocoa bean temperature in the 1st tray increased more quickly than in the 4th tray. This means the growth of yeast increased in the 1st tray (the top side) more than in 4th tray (the bottom side). This behavior was similar to changes in the wooden box, in which yeast had rapid growth on the top of this container. This suggests that a change in cocoa fermentation occurred on the top of the fermentation container and then expanded to the bottom, which is why it was required to mix cocoa beans together every 2 days [10]. For the wooden tray, mixing cocoa beans was not convenient, and it was easy to cross contaminate, so the wooden box would be a proper container because it was easy to mix cocoa beans even when sometimes fermented cocoa beans had a non-uniform color [14]. The growth of yeast and lactic acid bacteria in the 1st tray increased within 3 days of fermentation, while that of acetic acid bacteria was increased after 3 days of fermentation. This observation was in line with a theory of cocoa fermentation mechanism in which temperature is higher at the beginning of fermentation, because yeast and pH values are increased because of yeast and lactic acid bacteria. After 3 days of fermentation, temperature is stable, but pH value still changes because of acetic acid bacteria [10].

On the other hand, the growth of all 3 groups of microorganisms slowly increased on the 4th floor of the wooden tray. The color of cocoa beans in the wooden tray after fermentation was uniform (data not shown). To achieve a good quality of cocoa fermentation by natural microorganisms, a large volume of cocoa beans is required. As per the results, a yield loss of the wooden tray fermentation was 50 % (calculated from a ratio between fermented cocoa beans and unfermented cocoa beans, shown in **Figure 8**), even though the cocoa bean color was uniform. We also studied the temperature, pH, and microbiological changes of Thai cocoa beans during fermentation using normal flora in the wooden box to understand its behavior further.

(a) (b)

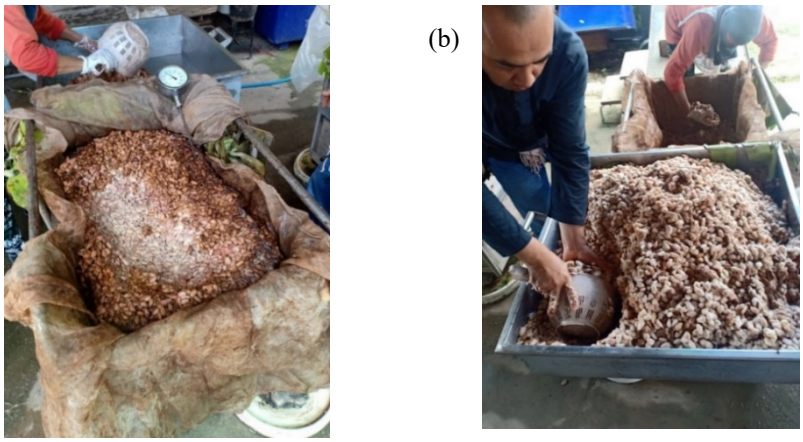


Figure 6 Mixing cocoa beans in the wooden box after incubating them for 2 days. (a) Cocoa bean color change due to a rapid fermentation on the top of the wooden box. (b) A good way for mixing cocoa beans properly in order to relocate cocoa beans from on the top to the bottom.

Fermenting cocoa beans in the wooden box was done in 6 days for the Mid-Crop (February to March, 2020). The outer temperature and the relative humidity were also monitored at every 2 pm, and the results showed that there was no significant difference ($p \geq 0.05$) between both of the 2 periods (**Table 3**). It was confirmed that we are able to perform cocoa fermentation during a whole year. The color of cocoa beans was changed from white to brown at the edge of the top of this container, as shown in **Figure 6(a)**, due to stronger yeast growth [14]. However, this changing did not occur in the bottom of the wooden box. Therefore, mixing by relocating cocoa beans from the top to the bottom in order to add healthy yeast to the bottom was applied after incubating for 2 days. Then, mixing them together every day took place until the inner temperature reached 40 - 45 °C and all of the cocoa beans were turned to a brown color.

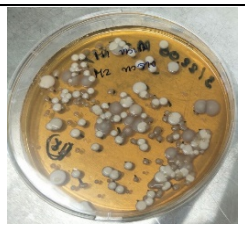
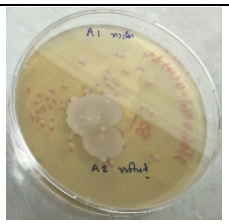
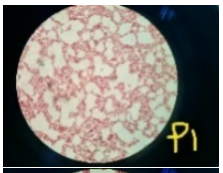
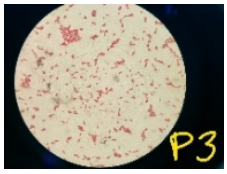
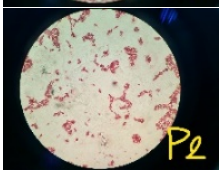
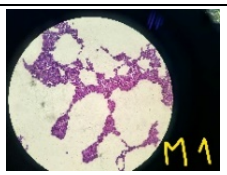
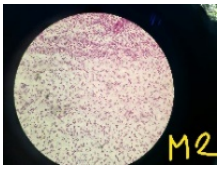
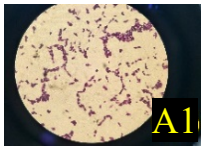
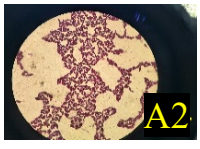
Table 3 Comparison of outer temperature and relative humidity of the Main Crop (September to October, 2019) and the Mid-Crop (February to March, 2020) during 6 days fermentation.

Fermentation day	Outer temperature (°C)		Relative humidity (%)	
	Main Crop	Mid-Crop	Main-Crop	Mid-Crop
Initial	31.05±0.78^{cd}	29.90±0.35^{d}	81.25±1.41^{ab}	80.75±0.35^{ab}
1	31.45±0.21^{cd}	30.10±0.19^{d}	78.85±1.27^{b}	79.45±0.42^{ab}
2	31.85±0.49^{bcd}	34.30±1.32^{abc}	79.20±1.27^{ab}	78.60±0.99^{b}
3	33.15±1.63^{abcd}	36.15±2.00^{a}	80.60±1.34^{ab}	83.90±1.98^{a}
4	31.50±0.40^{cd}	32.50±1.77^{abcd}	73.25±0.14^{c}	70.70±2.26^{c}
5	34.50±0.54^{abc}	35.35±0.24^{ab}	62.55±0.21^{d}	62.50±0.00^{d}
6	32.35±0.64^{bcd}	33.90±0.68^{abc}	64.30±0.68^{d}	63.80±0.28^{d}

Remark: Values with different letters in the same column and row of each parameter are significantly different at $p < 0.05$.

Changes in temperature, pH, and microbiological properties of cocoa bean fermentation in 3 layers of the wooden box are demonstrated in **Figure 7**. pH values of Thai fresh cocoa beans were 5.0 - 5.5, which are lower than the international standard quality of fresh cocoa beans. One reason would be the different geography and soil quality of agricultural land [16]. However, Thai fresh cocoa beans with pH 5.0 could achieve a good quality of dried cocoa beans after fermentation. Inner temperature in the wooden box rose and reached 40 - 45 °C in 6 days fermentation. For the top of the wooden box, temperature was higher than the other layers because of the rapid growth of yeast. After mixing cocoa beans together in 3 days fermentation, temperature quickly increased for all of the layers in the wooden box. As per the results, inner temperature should be an important parameter to be an indicator for Thai cocoa fermentation, as well as good raw material receiving and handling, are key processes at the beginning of this fermentation.

Table 4 Appearance of each colony on plate, under microscopy, and sequencing report of each colony.

Item	PDA media	MRS media	AAB media
Colony on plate			
Colony under microscopy (10x100)	  	 	 

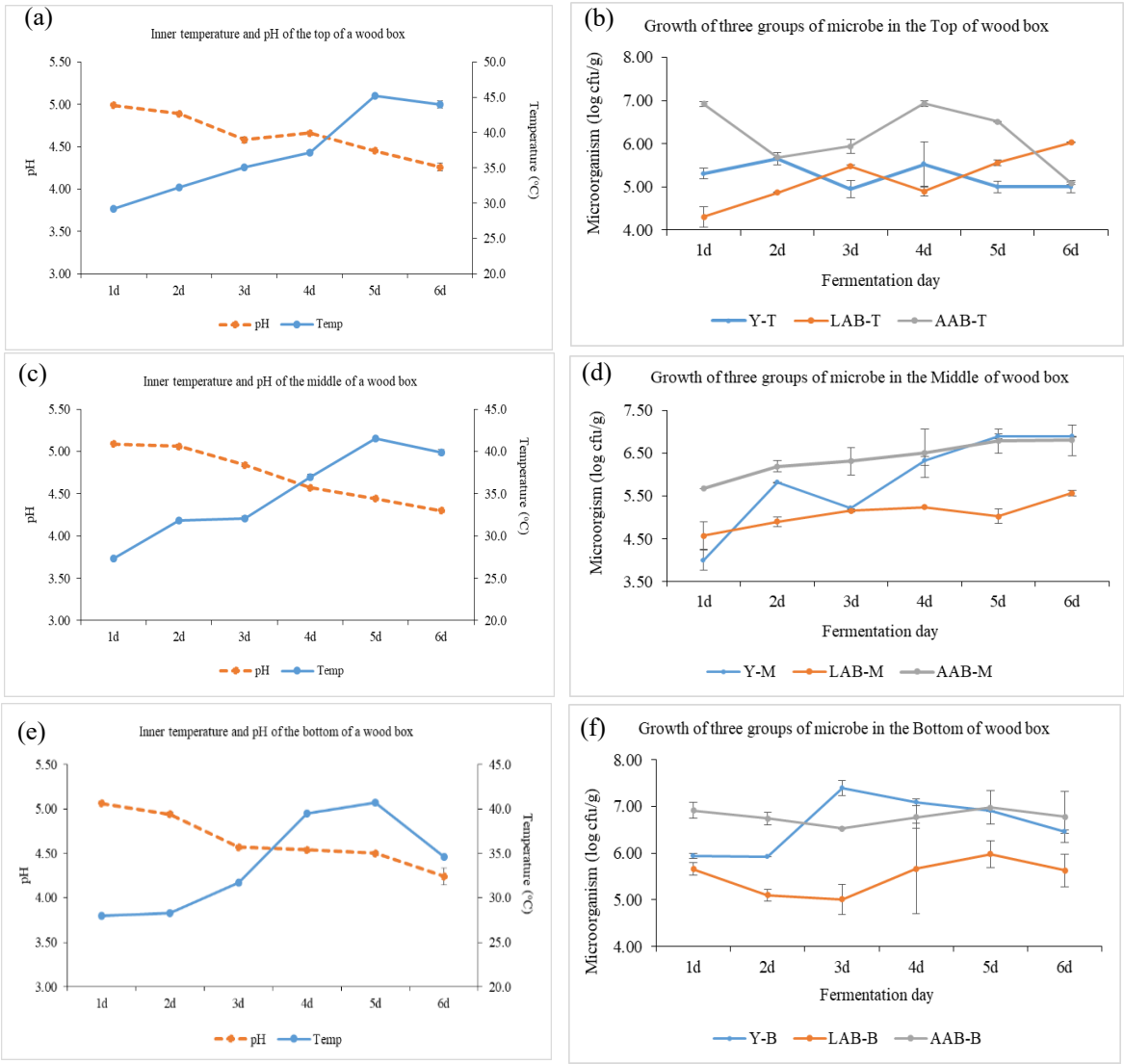
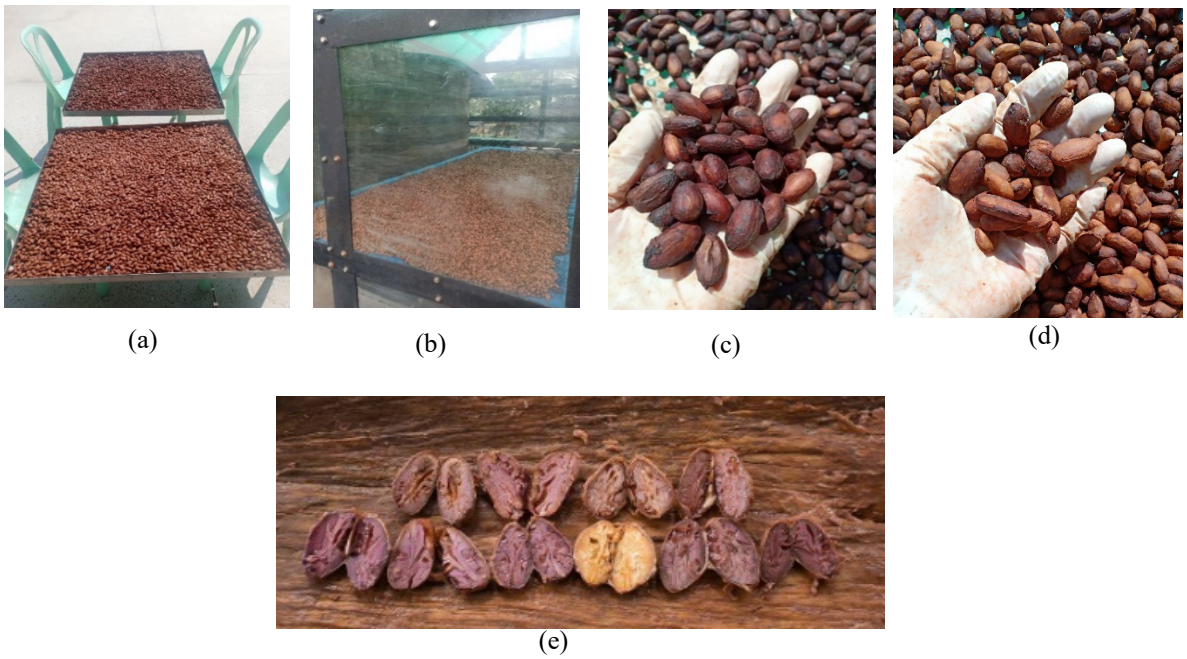


Figure 7 Changes in temperature, pH, and microbiological properties during cocoa fermentation in the top, the middle, and the bottom layers of the wooden box: **(a)** the inner temperature and pH in the top of the wooden box, **(b)** the growth of yeast (Y-T), lactic acid bacteria (LAB-T), and acetic acid bacteria (AAB-T) in the top of the wooden box, **(c)** the inner temperature and pH in the middle of the wooden box, **(d)** the growth of yeast (Y-M), lactic acid bacteria (LAB-M), and acetic acid bacteria (AAB-M) in the middle of the wooden box, **(e)** the inner temperature and pH in the bottom of the wooden box, **(f)** the growth of yeast (Y-B), lactic acid bacteria (LAB-B), and acetic acid bacteria (AAB-B) in the bottom of the wooden box.

Changes in microbiology growth in a different sampling point of the wooden box are also shown in **Figure 7**. In the top layer of the wooden box, yeast was grown very quickly within 2 days. After mixing cocoa beans together, the growth of yeast slowly increased in this layer, while it grew very much faster in the middle and the bottom layers of the wooden box. Lactic acid bacteria were induced by yeast fermentation. Moreover, the amount of acetic acid bacteria increased after yeast fermentation, which was similar in the wooden tray (laboratory scale). The results suggest that a proper mixing of cocoa beans after curling within 2 days is required for completed cocoa fermentation. Interestingly, in the wooden box fermentation, the fresh cocoa beans seemed to contain a high number of acetic acid bacteria at the beginning, which was different from in the wooden tray. Therefore, further investigations of natural acetic acid bacteria behavior in Thai cocoa fermentation needs to be done in the future. Probably, natural microorganisms in Thailand, especially acetic acid bacteria, will differ from those in other countries. Moreover, this could be related to the lower pH of fresh Thai cocoa beans, which never exceeds 5.5. For this research, we did not need to identify those microorganisms, but we would like to monitor changes in the Thai cocoa fermentation process. However, we performed gram strain to confirm the type of colony on a basic media (**Table 4**). Even though there were many types of colony on the plate, they were of the same stain color under microscopy, and cell morphology was not different. However, identification of these microbes with a specific method is required to make this clearer.

After fermentation, cocoa beans were dried using natural sunlight or hot air in a drying room for 4 to 5 days. Three hundred cocoa beans were selected for a cut test, as shown in **Figure 8**. A good quality of dried cocoa bean should have a brown color after cutting at least 70 %. Around 50 % of the dried cocoa beans from the laboratory scale experiment (the 1st and the 2nd tray) showed good quality, whereas 90 % of dried cocoa beans from the wooden box showed good quality. Our results suggest that a suitable size for cocoa fermentation using natural microorganisms should be 200 - 250 kg of fresh cocoa beans in a wooden box as per a commercial scale.



(a) (b) (c) (d)

(e)

Figure 8 Drying cocoa bean after fermentation. (a) Dried cocoa beans using sunlight, (b) Dried cocoa beans in a drying room, (c) Good fermented cocoa beans after drying for 4 - 5 days, (d) Unfermented cocoa beans after drying for 4 - 5 days, (e) Cocoa beans after a cut test.

Conclusions

Traditional cocoa fermentation of a large volume of fresh cocoa beans is still carried out in Thailand. Typically, 3 types of microorganism (yeast, lactic acid bacteria, and acetic acid bacteria) are found during cocoa fermentation. Among these, yeast plays an important role in cocoa fermentation, and their metabolites affect the growth of lactic acid bacteria and acetic acid bacteria. Therefore, curling of a large volume of fresh cocoa beans, carried out in a wooden box for 2 days, is required to induce yeast fermentation. After that, proper mixing of the cocoa beans in the wooden box is required to create a uniform condition for microorganisms to grow in a whole batch. A good indicator of Thai cocoa bean fermentation is an inner temperature of the wooden box, which should rise to 40 - 45 °C at the end of the fermentation process.

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