Effect of Fermentation Duration Using Shallow Box on Ph, Equivalent Percent Fully Brown and Flavour Attributes of Malaysian Cocoa Beans

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Background: Fermentation is an important process in cocoa beans that enabling the occurrence of cocoa flavour precursors. Termination at the correct duration will ensure production of the best quality of dried cocoa beans. In this study, about 1000 freshly harvested cocoa pods from mixed clones were stored for 4 days and fermented using shallow box technique for 4 days with a single turning at 48 h. Three subsamples of 5 kg of fermented beans were taken randomly at duration of 48, 72 and 96 hours of fermentation and sun-dried at one bean thin layer. Upon dried, the beans were assessed for pH, Equivalent Percent Fully Brown (EB) score and sensory analysis. Objective: To evaluate the effect of the fermentation duration using a shallow box on the pH, Equivalent Percent Fully Brown (EB) score and flavour attributes of the resultant dried cocoa beans. Results: The pH value of the resultant dried beans was in between 5.32, 5.09 and 5.29, whereas the EB score increased from 84.02 to 85.72 and 91.44 for fermentation duration of 48, 72 and 96 hours, respectively. Sensory analysis on the dried beans for each of fermentation duration showed that flavour attributes were significantly different at p<0.05 for cocoa, astringent and sour. Conclusion: Based on this study, increasing of the duration will increase the fermentation degree of dried cocoa beans. On the other hand, the cocoa flavour showed the opposite effect. Whereas the effect of fermentation duration on the acidity of cocoa nibs and other flavour attributes was variable.

INTRODUCTION

Fermentation is well-known as an important process in cocoa beans that enabling the occurrence of cocoa flavour precursor development and determine the final colour of dried cocoa beans (Lopez, 1986; Thompson, et. al. 2001). The fermentation process should be carried out properly and terminated at the correct duration to ensure that the dried cocoa beans have high potential of cocoa flavour. Incorrect fermentation duration will not only produce beans with low potential of cocoa flavour but also off-flavour beans (Khairul Bariah, 2013; Biehl 1984).

Usually, the fermentation process can take up to six days depending on the type and the quantity of cocoa beans used. The process started with the activity of yeast at the cocoa pulp layer, followed by the succession of lactic acid bacteria and acetic acid bacteria. Activity of the microbial leads to the increasing of mass temperature, which exceeding 40°C before it gradually decreased. The microbial activity also produced organic acids such as lactic and acetic acid, thus resulted in the acidification of nib. The nib acidification will trigger changes in biochemical composition such as proteins, polyphenols, sugars and cocoa butter (Afoakwa, 2008; Voight et al., 1994).

Currently, Malaysian Cocoa Board recommends five days fermentation using Shallow box with a single turning on the third day. However, this period is considered long for farmers because they have to dry the cocoa beans between 3 to 7 days depending on the weather. In addition, farmers also do cocoa pods storage either to ensure sufficient cocoa beans for fermentation or as a preconditioning to reduce the acidity of the cocoa bean.
All these will delay the return of hard-earned farmers. Therefore, this project is carried out to evaluate the effect of the fermentation duration using a shallow box on the pH, Equivalent Percent Fully Brown (EB) score and flavour attributes of the dried cocoa beans.

**Methodology:**
A total of 1000 ripe and healthy cocoa pods (mixed clone) were harvested at Cocoa Research and Development Centre, Jengka, Pahang, Malaysia. The pods were stored in dry and well aerated conditions under a roof for four days. The pods were sliced opened and beans were extracted manually. Prior to beans loading, a partition was placed in the shallow box (90 X 60 X 31 cm³) to cater 100 kg loading capacity. The box was covered with gunny sack once the beans were loaded and fermentation was carried out for four days with single turning at the 48th hour. Three subsamples of about 5 kg of fermented beans were taken randomly at the end of day-2, day-3 and day-4 of fermentation process. The beans were sun dried at one bean thickness on drying yard until the moisture content reduced to less than 7.5 per cent. During fermentation, mass temperature and pH (pulp) were recorded for every 24 hours.

**Analysis of pH:**
The pH of wet and dried nibs was determined according to Khairul Bariah (2013) with slight modification. The wet and dried cocoa nibs were obtained by grinding the deshelled beans using analytical grinder. Five grams of ground nibs was dissolved in 45 ml of distilled water. The mixtures were filtered and pH was read in triplicate using a pH meter.

**Equivalent Percent Fully Brown score:**
A total of 100 dried fermented beans were taken randomly from 250 gram samples derived by quartering technique. The dried beans were cut lengthwise into halves for maximum surface exposed. Both halves of each surface were inspected for colour under artificial light and divided into fully brown (FB), partly brown (PB), partly purple (PP), fully purple (FP) and slaty. The total of dried beans in each group was counted and score for Equivalent Percent Fully Brown (EB) were determined according to Mamot (1989) by using equation as followed:

\[ EB = \left[ (1 \times FB) + (0.7 \times (PB + PP)) + (0.5 \times FP) + (0.3 \times \text{slaty}) \right] \]

**Sensory Analysis:**
Dried cocoa beans were roasted at 150°C for 30 minute using standard lab oven. Upon roasting cocoa beans were deshelled and liquors were prepared by grinding the nibs in Mortar and pestle mill (Pascal, U.K) until a smooth cocoa paste was obtained. Sensory analysis was carried out by 8 MCB trained panelist at CITC, Nilai. A descriptive analysis with scale of “0” to “10” was used where “0” indicates the absence or minimum intensity and “10” indicates the maximum intensity. Sample was labeled with randomly selected 3-digits numerical code. Ghana cocoa liquor was used as reference sample. The flavour characteristics evaluated were cocoa, bitter, astringent and sour/acidic. Statistical analysis was carried out for one-way Anova and Tukey’s multiple comparison test using Minitab-14 software.

**Statistical analysis:**
ANOVA was conducted using Microsoft Office Excel 2007 to evaluate the impact of fermentation duration on pH, EB scores and sensory attributes of dried cocoa beans.

**Result:**

**Profiles of temperature and pH:**
The profile of mass temperature, pulp mass and wet nibs during fermentation is shown in Figure 1. Temperature of fermenting mass increased rapidly from 31°C to 44.3°C in duration of 24 hours. The temperature was further increased gradually until it attained the highest level, 48.3°C in 72 hours of fermentation duration. After that, the temperature was slightly decreased and suggested as one of the indicator that the fermentation process should be terminated.

The pH profiles of pulp mass and wet nibs were showing a different trend. At the beginning of fermentation, the pH of pulp mass decreased about 12% from 4.47 to 3.95. Thereafter, the pH of pulps mass gradually increased to the final pH of 5.69. On the other hand, the pH of nibs gradually decreased about 6% from 6.43 to 6.04 in 24 hours of fermentation duration. The pH of nibs continued to decrease about 17% until attained the lowest pH, 5.03 in 72 hours of fermentation duration. Then the pH of nibs slightly increased about 6% to 5.36.
Quality and sensory attributes of dried beans:

The quality of dried cocoa beans was assessed based on the pH value, EB score and flavour attributes as shown in Table 1 and Table 2. The pH values of dried beans were in between 5.32 for 48 hours, 5.08 for 72 hours and 5.29 for 96 hours of fermentation duration, respectively. Whereas, the EB scores increased from 84.02 for 48 hours to 85.72 for 72 hours and 91.44 for 96 hours of fermentation duration, respectively.

![Average pH](image)

Fig. 1: The profiles of mass temperature, pH of pulp mass and wet nibs during fermentation process.

<table>
<thead>
<tr>
<th>Fermentation Duration (hours)</th>
<th>pH of dried nibs</th>
<th>EB Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>5.32 ± 0.02</td>
<td>84.02 ± 2.99</td>
</tr>
<tr>
<td>72</td>
<td>5.08 ± 0.02</td>
<td>85.72 ± 0.55</td>
</tr>
<tr>
<td>96</td>
<td>5.29 ± 0.01</td>
<td>91.44 ± 1.64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fermentation Duration (hours)</th>
<th>Cocoa</th>
<th>Bitter</th>
<th>Astringent</th>
<th>Acid/sour</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>72</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>96</td>
<td>4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard (120)</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghana</td>
<td>7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean value followed by different alphabet in the same column are significantly different at p<0.05 (n=8)

Comparison of flavour attributes of dried beans samples with standard fermented beans for 120 hours and Ghana is shown in Table 2. Flavour attributes showed that dried beans fermented for 48 hours have highest score in cocoa compared to other samples and almost close to Ghanaian reference. All the dried beans have bitter score between 4.5, 4.9 and 4.1 for fermentation in duration of 48, 72 and 96 hours, respectively. Whereas, the astringency scores were 4.2, 5.5 and 3.8 for dried beans fermented for 48, 72 and 96 hours, respectively. The bitter and astringency scores in all dried beans were higher compared to Ghanaian reference. All dried beans were found to be moderately sour with score ranged between 2.0, 3.4 and 2.6 for fermentation in duration of 48, 72 and 96 hours, respectively. Overall, all samples in this study were better than beans produced by standard fermentation practice. The beans produce by standard fermentation practice was acidic and very poor in cocoa flavour. Dried beans fermented for 48 hours and 96 hours has very close flavour attributes, however dried beans fermented for 48 hours was the best as it has a better cocoa flavor and the least acidic.

Statistical analysis:

Table 3 shows the ANOVA two factors analysis of pH, EB score and flavour attributes of all dried beans fermented for 48, 72 and 96 hours, respectively. Different fermentation duration give significant effects to the pH, EB score and flavor attributes of all dried beans (P <0.05).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same fermentation duration</td>
<td>2</td>
<td>1.947</td>
<td>0.662</td>
<td>0.537</td>
<td>4.103</td>
</tr>
<tr>
<td>Different fermentation duration</td>
<td>5</td>
<td>3425.811</td>
<td>1165.081</td>
<td>1.730 X 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>3.326</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>2.941</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α=0.05</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>
Discussion:
Cocoa pulps are reported to have pH value in between 3.3 to 4.45 and contain about 14% sugar notably glucose and fructose and 1.5% pectin (Afoakwa, et. al. 2013; Guehi, et. al. 2010). Initially, the pulps are sterile but as soon as cocoa beans are exposed to environment, microbe predominantly yeast will start to colonize. Yeast will utilize the sugars which lead to the production of ethanol, carbon dioxide and organic acids with concomitant of temperature increment (Schwan, 1998). These scenario explained the increasing trend of temperature during fermentation, and rapidly increased of temperature might be due to the fact that yeasts actively metabolize the high concentration of reducing sugar produced by sugar respiration during four day pod storage (Schwan and Wheals, 2004; Meyer, et. al 1989).

At the same time, production of organic acids might be the cause to the drop of pulp’s pH during the first 24 hours of fermentation. After that, the pH value of cocoa pulp started to increase until the value reached its highest level. According to Jespersen (2003), the increase in pH of cocoa pulps is believed to be due to metabolizing of citric acid by some yeasts, draining of organic acid during cocoa sweating and also diffusion of the organic acid into cocoa nibs (Thompson, et. al. 2001; Biehl, et. al. 1985).

Acidification of cocoa nibs together with temperature rising promote the activation of enzymes. During 48 hours of fermentation duration and onwards, temperature of fermenting mass has reached between 45.7 and 46.3°C while pH value of nibs decreased in range of 5.45 to 5.36. These conditions are suggested as optimal for aspartic endopeptidase and carboxypeptidases activities, therefore will be hydrolyzing the vicilin 7S-class globulin to the correct ratio of hydrophilic oligopeptides and free hydrophobic amino acid (Khairul Bariah, 2007, Voigt, et. al. 1994). However, Buyukpamukcu, et. al.( 2001) has revealed that the oligopeptides responsible for cocoa flavour are formed early during fermentation and degraded after day three. This occurrence is reflected with score of cocoa flavour during sensory of cocoa liquor.

Bitterness and astringency of all the dried cocoa beans except fermented for 96 hours is very high compared to Ghana. Bonvehi and Coll (1997) reported that bitterness and astringency derives from polyphenols compounds mainly monomeric flavan-3-ols and polymeric flavan-3-ols. Although all the dried cocoa beans except fermented for 96 hours have high score in bitterness and astringency, the EB score indicated that all the beans are well-fermented. Further investigation on the relation of bitterness and astringency to fermentation degree need to be emphasized as the mechanism is not clearly understood.

Conclusion:
Increment of fermentation duration will increase the fermentation degree of dried cocoa beans. On the other hand, the cocoa flavour showed the opposite effect, the cocoa flavour intensity reduced during the progress of fermentation process. Whereas the effect of fermentation duration on the acidity of cocoa nibs and other flavour attributes was variable.

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