Effect of clonal variety and fermentation duration on cocoa flavor

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ABSTRACT The quality of cocoa is determined by its unique and complex flavors which are contributed by several factors. This study aimed to investigate the cocoa flavor of three Malaysian cocoa clones, MCBC5, 4, and 2 fermented at different durations. Fresh cocoa beans were fermented for six days, and were sampled every 24 hours for drying. The quality of fermentation was determined by cut test and fermentation index (FI) of the dried beans. The cut test results showed that MCBC5 took the longest to be fully fermented (6 days) whereas MCBC4 and 2 took only five days. However, FI analysis, presented earlier completed fermentation. A portion of dried beans were roasted for 25 min at 127°C and ground to cocoa liquors for sensory evaluation by the Malaysian Cocoa Board's trained panelists with Ghanaian cocoa liquor as the standard. The most intense cocoa flavor was MCBC2 fermented for five days (4.11 ± 0.83). The strong cocoa flavor in MCBC2 could be due to its low bitterness (2.86 ± 0.68) and astringency (3.56 ± 0.66) level and moderate level of acidity (1.86 ± 0.76) as high levels of bitterness, astringency, and acidity are known to mask the cocoa flavor, thus produce poor cocoa flavor. In conclusion, MCBC2 fermented for five days had the best cocoa flavor when compared with the MCBC5 and MCBC2.

KEYWORDS: *Theobroma cacao*; cocoa flavor; cut test; fermentation index; Malaysian cocoa clones
Received 21 April 2021 **Revised** 16 June 2021 **Accepted** 26 June 2021 **Online** 30 June 2021

Original Article

INTRODUCTION

Cocoa beans are mainly used for making chocolate. The most crucial parameter of the cocoa quality is the flavor characteristic (Kongor *et. al*., 2016). Cocoa flavor precursors in the beans were produced during fermentation (Aprotosoaie et. al., 2015) and were further expressed into cocoa flavor after roasting. Fermentation process should be conducted for the right time period in order to produce good cocoa flavor. Under-fermented and over-fermented beans will produce beans with off-flavor and low cocoa intensity (Aprotosoaie *et. al*., 2015). In Malaysia, mixture of cocoa beans from different clones are usually fermented for five days in fermentation box (Sulaiman *et. al*., 2014). However, a pulp-preconditioning technique prior to the fermentation can increase the percentage of well-fermented beans in shorter time (Afoakwa *et. al*., 2013). In regard to this, a study by Sulaiman (2014) suggested that good quality of Malaysian cocoa beans can be attained as early as three days of fermentation. Flavor of cocoa is also affected by the genotype of the cocoa tree (Kongor *et. al*., 2016). Different cocoa clones have different amount of certain components such as theobromine, thus producing different intensity of cocoa flavor (Suzannah, 2006; Othman *et. al*., 2008). Despite this, studies on cocoa flavor intensity of Malaysian cocoa clones at different fermentation stage are still lacking. Therefore, this study aimed to determine the effect of Malaysian cocoa clones and fermentation duration on the cocoa flavor and other sensory profiles.

METHODOLOGY

Cacao beans collection and extraction

Selection of cocoa clones was carried out based on their intensity of cocoa flavor recommended by the Malaysian Cocoa Board (MCB) which are MCBC5 (high), MCBC4 (medium), and MCBC2

(low), respectively. Cocoa pods were harvested from Cocoa Research and Development Centre (CRDC) Tawau Sabah and Sg. Sumun, Perak, Malaysia.

Fermentation and drying

Fresh cocoa beans extracted for each clone were divided into equal amount of 7 samples for day 0 to 6 and was put into jute bags. The bags were put on three layers with a glass thermometer inside. To sustain the heat during fermentation, fresh cocoa beans of PBC123 were used to fill up the box. Temperature of cocoa bean mass was recorded and a jute bag was taken from each clone at every 24 hours. The beans were fermented in a shallow wooden box with 1ft (W) x 1ft (H) x 1ft (L) for two replicates. The fermented cocoa beans were oven-dried at 45^{0}C for 8 hours daily and was continued throughout the night at 35^{0}C until the moisture content reduced to 7-8%. Dried cocoa beans were labelled and stored in sealed container in -80^{0}C freezer.

pH measurement

Acidity of beans was determined from its pH level according to Sulaiman (2014) with a slight modification. De-shelled dried beans (5g) were grinded using conventional dry blender and was mixed with 50 ml of hot distilled water. The mixture was stirred and filtered. The filtered mixture was let cool and the pH level were measured by using calibrated pH meter.

Cut test

Approximately 100 dried beans were randomly taken from each sample and were cut lengthwise into half. The surface of the cut beans was observed and was divided into five groups based on the colour (fully brown, partly purple, partly brown, fully purple, and slaty). Using the MS 293:2005, the percentage of fully brown beans were calculated and signified the level of fermentation, as a quality indicator of cocoa beans. The samples with more than 60% of fully brown beans are considered high quality of fermented beans. Samples with 45 – 60% of fully brown beans are medium quality and less than 45% of fully brown beans are low quality of beans.

Fermentation Index (FI)

Fermentation Index were determined according to Sulaiman (2005). De-shelled dried cocoa nibs were grinded in conventional grinder into coarse powder and was mixed with 50 ml of methanol:HCl (97:3) solution and was put for incubation overnight at 4^{o}C. Then, it was filtered and was made up to 50 ml. The absorbance of the solution was measured at 460 and 530 nm. Absorbance value of 1.000 and below is considered under-fermented, those with absorbance value of 1.000-1.599 are deemed as completely fermented and cocoa beans with absorbance value of 1.600 and above is considered as over fermented.

Cocoa liquor preparation

Cocoa nibs were roasted in an oven for 25 min at 127^{o}C (Farah *et. al*., 2012) and were ground into cocoa liquor using mortar and pestle. The cocoa liquors were evaluated by 9 trained panellists at Cocoa Innovation and Technology Centre (CITC), Nilai. The samples were scored with scale ranging from 0-10 with 0 as the lowest intensity and 10 as the highest intensity. Ghana cocoa liquor was used as the standard. The panellists scored the cocoa liquors based on four flavor attributes which were cocoa, bitterness, astringency and sour/acidic.

RESULTS AND DISCUSSION

Figure 1 showed the temperature profiles during the fermentation process. The temperature of cocoa fermenting mass started to increase from the start of fermentation until they reached its peak

at 45^{0}C ± 2.34 on the third day. Then the temperature started to decrease until the end of fermentation where they reached 39^{0}C. There was increase of surrounding temperature from day 0 to day 2 but decreased slightly onwards due to rain.

The acidity level of the beans was determined and recorded as shown in Figure 2. All clones showed the same trend throughout the fermentation process. Unfermented beans have low acidity ranged from 6.28 to 6.53. After being fermented for 24 hours, their level of acidity increased slightly except for MCBC5 that decreased from 6.28 to 6.34. Nevertheless, all clones' acidity started to increase until they reached and maintained the lowest pH on the third and fourth day. Beans fermented for five days showed decreasing acidity until the end of fermentation where they reached almost the same level as the unfermented beans ranged from 5.87 to 6.50. Again, MCBC5 had the highest acidity level on day 6 compared with the other two clones.

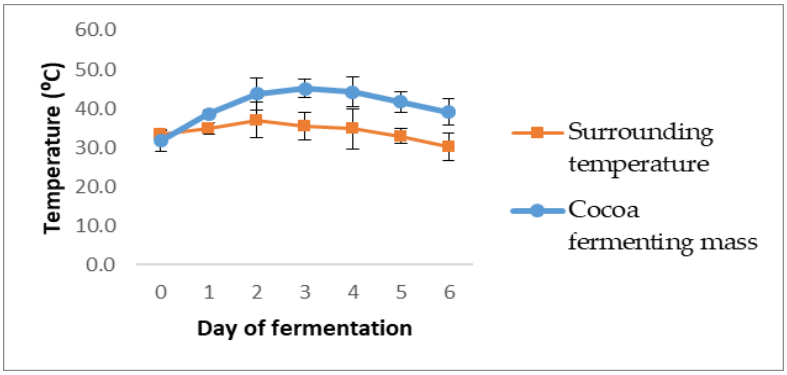

Figure 1. Temperature profiles of fermenting cocoa mass during fermentation process. Error bars indicate standard deviation

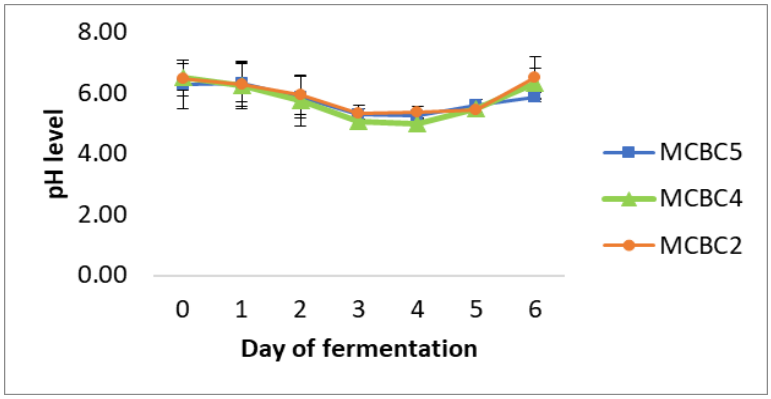

Figure 2. pH level of the bean cotyledons of three cocoa clones at different fermentation duration. Error bars indicate standard deviation

Most commonly used technique by the industries for fermentation analysis is the cut test (Table 1). The percentage of FBB for all clones increased steadily from day 0 to day 2. MCBC5 and 2 showed quite similar start (14 to 16%) compared with MCBC4 (5%). Interestingly, beans fermented for 4 days have higher percentage of FBB from the ones fermented for 3 and 2 days (50-53%) altogether even though there was a slight dip for the beans fermented for 3 days. From then, the percentage increased but in different speed for the three clones. During the last two days of fermentation, MCBC5 has the steadiest and lowest FBB percentage of 58% and 65%, in orderly. MCBC4 has higher percentage of FBB after being fermented for 5 days (69%) while MCBC2 has the highest percentage of it (76%). MCBC4 and 2 had earlier fully fermented of beans which were on the 5th day compared to MCBC5 that reached the fully fermented level on the last day of fermentation.

From the FI results (Table 1), all three clones were fully fermented at least after three days of fermentation. This is also in line with a study by Sulaiman (2014) which showed well-fermented beans through FI on day 3. As fermentation process progresses, the biochemical reaction inside the bean cotyledon will produce higher concentration of anthocyanidins formed from anthocyanins (Kongor, 2013). The oxidation of products will then result in the browning of the cocoa beans especially after the Maillard reaction during roasting. Thus, the fermentation level of the beans was quantified by the ratio of the brown to purple pigments of the bean cotyledons. Logically, an increasing and unvarying pattern of FI value should be expected from the three clones throughout the fermentation process. Yet, they exhibited a fluctuated trend from the rise and fall of the FI value shown except MCBC2. MCBC2 had a steady increment of FI value from the unfermented beans (0.485 ± 0.13) until the over-fermented beans (1.731 ± 0.32).

Table 1. Cocoa beans fermentation level analysis from Cut Test and Fermentation Index (FI)

Clones	Day of fermentation	FBB (%)	FI
MCBC5	0	16	0.620 ± 0.27
	1	27	0.601 ± 0.24
	2	41	0.886 ± 0.16
	3	35	1.101 ± 0.21
	4	50	1.133 ± 0.14
	5	58	1.305 ± 0.22
	6	65	1.271 ± 0.12
MCBC4	0	5	0.358 ± 0.01
	1	32	0.564 ± 0.16
	2	42	0.788 ± 0.19
	3	39	1.257 ± 0.26
	4	53	1.404 ± 0.19
	5	69	1.284 ± 0.22
	6	75	1.154 ± 0.178
MCBC2	0	14	0.485 ± 0.13
	1	28	0.539 ± 0.17
	2	51	1.051 ± 0.30
	3	40	1.107 ± 0.39
	4	52	1.308 ± 0.20
	5	76	1.415 ± 0.13
	6	77	1.731 ± 0.32

Table 2 shows the average sensory evaluation scores. MCBC2 has the highest intensity cocoa flavor (4.11 ± 0.83) instead of MCBC5 (3.64 ± 0.05) after fermented for five days. All clones showed a drop in score after being fermented for six days. This could be due to the excessive oxidation resulting in higher bitterness and astringency. MCBC2 fermented for five days also has the least score for bitterness (2.86 ± 0.68) and astringency (3.56 ± 0.66) when compared with the other two clones. Contrast to the other attributes, unfermented MCBC4 has the lowest acidity (0.67 ± 0.80) which was even lower than the standard (1.39 ± 0.87). Beans from the three clones fermented for 3 and 4 days gave higher sourness when compared with other duration which was as also exhibited by the pH level of non-roasted dried beans (Figure 2). Nevertheless, the sourness for the three clones decrease on the sixth day of fermentation since the reduction of volume, water and sugar from fermentation will give lesser acidic beans (Afoakwa *et. al*., 2008).

As opposed to the first criteria of the cocoa clone selection, the MCBC5 recommended as one of the Malaysian cocoa clone with good cocoa flavor turned out to give moderate score. This may be

due to the high bitterness, astringency and acidic taste that masked the cocoa flavor, causing the detection of cocoa flavor to be difficult (Guehi et. al., 2010). This can be seen from the high intensity of cocoa flavor evaluated from MCBC2 fermented for day 5 which may be due to the low bitterness and astringency enabling the cocoa flavor to be detected easily.

Table 2. Cocoa liquor sensory evaluations. *used as standard. C5 = MCBC5; C4 = MCBC4; C2 = MCBC2. D0 = unfermented; D1–D6 = Day 1 to 6, respectively.

Sample	Cocoa	Bitter	Astringent	Acidic/ Sourness
***Ghana**	4.89 ± 1.22	3.50 ± 1.24	3.78 ± 1.22	1.39 ± 0.87
C5D0	2.14 ± 1.41	5.78 ± 1.48	6.11 ± 1.49	0.76 ± 0.64
C5D1	2.03 ± 2.05	5.25 ± 1.95	5.50 ± 1.87	0.83 ± 0.79
C5D2	3.19 ± 1.90	4.92 ± 2.05	5.19 ± 2.22	1.53 ± 0.87
C5D3	3.28 ± 1.26	4.03 ± 1.22	4.86 ± 1.46	2.92 ± 0.69
C5D4	3.33 ± 0.92	4.14 ± 1.34	4.25 ± 0.94	2.75 ± 0.96
C5D5	3.64 ± 0.05	3.92 ± 0.99	4.36 ± 1.04	2.19 ± 0.81
C5D6	3.22 ± 1.00	3.61 ± 0.90	4.44 ± 0.97	1.58 ± 0.90
C4D0	0.78 ± 0.71	5.75 ± 1.56	5.92 ± 1.24	0.67 ± 0.80
C4D1	1.97 ± 1.56	5.89 ± 1.84	6.06 ± 1.72	1.06 ± 1.06
C4D2	2.31 ± 1.23	4.78 ± 1.23	5.03 ± 1.12	2.08 ± 1.18
C4D3	3.19 ± 1.30	4.14 ± 1.77	4.61 ± 1.46	2.39 ± 0.95
C4D4	2.64 ± 0.80	3.72 ± 1.17	4.72 ± 1.10	3.31 ± 0.79
C4D5	3.25 ± 1.40	4.00 ± 1.18	4.44 ± 1.33	2.03 ± 0.88
C4D6	3.08 ± 0.88	3.97 ± 1.17	4.44 ± 1.10	1.64 ± 0.90
C2D0	1.31 ± 1.15	5.75 ± 1.54	6.14 ± 1.56	0.89 ± 0.80
C2D1	1.31 ± 1.10	5.78 ± 1.52	5.89 ± 1.32	0.72 ± 0.77
C2D2	2.64 ± 1.21	4.81 ± 1.55	4.89 ± 1.17	0.97 ± 0.72
C2D3	3.47 ± 1.94	3.75 ± 2.00	4.47 ± 1.76	2.50 ± 0.97
C2D4	3.94 ± 2.02	3.92 ± 1.98	4.67 ± 1.86	2.14 ± 0.89
C2D5	4.11 ± 0.83	2.86 ± 0.68	3.56 ± 0.66	1.86 ± 0.76
C2D6	3.11 ± 1.23	4.08 ± 1.20	4.33 ± 1.04	1.58 ± 0.97

CONCLUSION

In conclusion, clonal variety affects the intensity of cocoa flavor through the different duration of fermentation process. MCBC2 fermented for 5 days was scored higher cumulatively by the panelists for its cocoa flavor intensity. Cocoa beans fermented over five days lowered the cocoa flavor intensity thus decreasing the quality.

ACKNOWLEDGEMENTS

This work was funded by the UMSGreat grant GUG0145-1/2017. The authors acknowledge the assistance of CRDC Hilir Perak and CRDC Madai in conducting the cocoa beans fermentation and providing the samples.

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