Rapid And Non-Destructive Evaluation On Cacao Pigments, Flavonoids And Nitrogen Contents During Pod Development And Maturity Using A Fluorescence Sensor

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Abstract. The detection of pigments in cacao pods together with colourless flavonoids serves as a useful indicator for pod maturity using a fast and non-destructive multiparametric fluorescence sensor. In this study, the contents of anthocyanin, flavonol, chlorophyll and nitrogen balance were determined monthly (1-5 months) after flower fertilization as pod developed and matured using a fluorescence-based portable sensor on cacao pods from five different clones of DESA1, KKM22, KKM25, MCBC1 and PBC221. There were significant differences (P≤0.05) observed between the interaction of five different cacao clones and pod development periods in flavonol, chlorophyll and nitrogen balance contents. As pods developed, anthocyanin and flavonol accumulated while the content of chlorophyll decreased only when pod matured with nitrogen balance showed a decreasing trend in cacao pods. Among these clones, as expected, natural red appearance in cacao pods of DESA1 showed significantly highest index of anthocyanin (0.637), following by KKM22 (0.255). In addition, there was no significant difference observed in KKM25, MCBC1 and PBC221 for anthocyanin content. During pod development, MCBC1 showed the least content in flavonol (P≤0.05) and the chlorophyll contents in KKM22 and MCBC1 were lower compared to other clones. As a conclusion, non-destructive fluorescence-based indices can be used to measure the pigments and flavonoids in cacao which can provide valuable non-destructive indicators for cacao pod maturity across different cacao cultivars.

Keywords: non-destructive; fluorescence-based indices; fluorescence sensor; cacao pod; maturity

Introduction

Cacao (Theobroma cacao L.), also called cocoa, is a perennial crop from the Malvaceae family (alternately, Sterculiaceae) which produces pods continuously to sustain the large market demand for chocolate. Good quality of cacao beans accounts for high price rate is much depends on pod maturity during harvesting. There are several factors account for the poor quality of cacao produced by some farmers in Malaysia, including poor fermentation and wrong harvesting time of pods. Pods which are harvested immature or over-ripe may influence the quality of the beans during fermentation. Beans are...
extracted from the harvested pods prior to fermentation, drying and roasting and these processes contribute significantly to the development of cacao flavor [1-3, 4-6]. Thus, harvesting pod at the right timing with optimum maturity stages is important because it influences the subsequent process to develop good flavor of beans.

For farmers, pod maturity with good bean quality is usually related to pod size and appearance changes of color during harvesting. However, pods with different cultivars perform distinct characteristics in terms of pod color, shape and size. Sometimes, it is hard to identify the maturity stages of pods due to its similar color changes during pod development and maturity. So, the detection of pigments, flavonoids and nitrogen content in cacao pods is expected to serve as a combined indicator to determine pod maturity indices among different cacao cultivars.

Cacao possesses compounds such as pigments and flavonoids, which includes anthocyanin and flavonol in pods. There are many factors can affect the biosynthesis of these compounds in the pod during development such as genetic differences, soil characteristics, environmental changes and cultural practices. The composition of these compounds may vary significantly depending on the cultivars [7]. Thus, precision agriculture by applying the optical method of fluorescence sensor has become a great tool to assess the variability of cacao pods at different maturity stages from young until harvesting. This technology allows rapid and non-destructive measurements of cacao pods and hence enables the monitoring of the pigments and flavonoid contents throughout cacao ripening [8].

Materials and Methods

Plant material
Five cultivars of cacao (DESA1, KKM22, KKM25, MCBC1 and PBC221) were collected from the field plot (N 03°53.752' E 100°52.061') in the Research and Development Centre Hilir Perak. Cacao pods at one-month until five-month old after flower fertilization were harvested and brought to laboratory for further analysis. Pods were cleaned with distilled water before measurements were taken.

Fluorescence equipment
Cacao pods at different maturity stages were measured using the Multiplex 3® (Force-A, Orsay, France) sensor. The sensor consisted of a fluorimeter with six light-emitting diode sources in the UV-A (370 nm), and the blue (470 nm), green (516 nm) and red (635 nm) spectral regions. The excitation light of the light-emitting diodes was pulsed sequentially at 240 Hz with 45 µs per flash and synchronised with the photodiode detectors to record the fluorescence signals. According to Ben Gozlen et al. [9], the combinations of fluorescence signals in the red at 680-690 nm (RF) and far-red at 730-780 nm (FRF) were acquired with the different excitation bands which provide the following indices of anthocyanin (ANTH), flavonol (FLAV), chlorophyll (CHL) and nitrogen balance index (NBI):

\[
\text{ANTH} = \log \left( \frac{\text{FRF}_{\text{red}}}{\text{FRF}_{\text{green}}} \right) \quad (1)
\]
\[
\text{FLAV} = \log \left( \frac{\text{FRF}_{\text{red}}}{\text{FRF}_{\text{UV}}} \right) \quad (2)
\]
\[
\text{CHL} = \frac{\text{FRF}_{\text{red}}}{\text{RF}_{\text{red}}} \quad (3)
\]
\[
\text{NBI} = \frac{\text{FRF}_{\text{UV}}}{\text{RF}_{\text{green}}} \quad (4)
\]

Measurements were taken by keeping in contact the cacao pods with a 4 cm diameter window of the sensor at a distance of 0.1 m from the sources and detectors.

Statistical analysis
A 5x5 factorial experiment was conducted (five cacao cultivars and five maturity stages) and data was analyzed using Statistical Analysis System (SAS Institute, 2002). Multiple mean comparisons were
analyzed by using Least Significant Difference (LSD). Results were further computed in graphs to study the trend of each parameter during pod development and were displayed as means ± standard error using Microsoft Excel (Microsoft Corporation, 2003).

Results and Discussion

Changes of fluorescence indices on cacao cultivars during pod development and maturity

There were significant differences (P≤0.05) observed between the interaction of five different cacao clones and pod development periods in flavonol, chlorophyll and nitrogen balance contents (table 1). For the anthocyanin, according to the definition expressed in Equation 1, the anthocyanin index increased proportionally with the anthocyanin content. From the study, DESA1 was expected to have significantly higher anthocyanin compared to other cultivars due to its natural red pod color. Therefore, the optimal localization of anthocyanin in the epidermis of DESA1 enables them to efficiently filter part of the green excitation light travelling towards the chlorophyll molecules in the pod layers [10-11]. Furthermore, the anthocyanin content increased by 50.4% as the pod matured at five months after fertilization compared to young and growing pod at one month after fertilization (table 1). This indicated that with increasing anthocyanin concentration, the green light was attenuated much more than the red excitation light. Similar trend was also observed for flavonol where the content increased as the pod matured.

Table 1. Changes of fluorescence indices in cacao pods of five cultivars harvested at five maturity stages.

| Factor | Fluorescence indices |
|--------|----------------------|
|        | Anthocyanin | Flavonol | Chlorophyll | Nitrogen balance |
| Clones (C) |           |
| DESA1   | 0.637a*  | 0.844a  | 1.214a  | 1.141a  |
| KKM25   | 0.100c   | 0.893a  | 1.249a  | 0.387c  |
| KKM22   | 0.255b   | 0.856a  | 1.050b  | 0.504c  |
| MCBC1   | 0.140c   | 0.276c  | 1.155ab | 1.071a  |
| PBC221  | 0.100c   | 0.612b  | 1.266a  | 0.744b  |
| Months after fertilization (M) |           |
| 1       | 0.232b   | 0.329c  | 1.221b  | 1.299a  |
| 2       | 0.252b   | 0.347c  | 1.247b  | 1.205a  |
| 3       | 0.222b   | 0.607b  | 1.312ab | 0.812b  |
| 4       | 0.177b   | 1.053a  | 1.413a  | 0.366c  |
| 5       | 0.349a   | 1.145a  | 0.739c  | 0.164d  |
| Interactions | C x M | n.s. | ** | * |

*Means followed by the same letter in the same column separately are not significantly different by DMRT at P>0.05.

n.s., *, ** Non-significant different at P>0.05 or significant difference at P≤0.05 or P≤0.01, respectively.
index increases when the chlorophyll concentration increases. In this study, chlorophyll and nitrogen contents in cacao pods decreased as pod maturity progressed (Table 1).

![Anthocyanin Index](image1)

![Flavonol Index](image2)

**Figure 1.** Non-destructive indices of anthocyanin (A) and flavonol (B) of five cacao cultivars during pod development.

Both of the non-destructive indices of anthocyanin and flavonol showed sudden increased at five-month old of cacao pod, where the pods are matured and ripened, except cultivar of DESA1 (figure 1). The accumulation of flavonoids, including anthocyanin and flavonol as pod matured, can be explained by the multiple functional roles played by flavonoids in operating as antioxidant compounds to overcome the light induce oxidative stress [13]. In addition, the increased in flavonoids of anthocyanin and flavonol might be primarily due to the loss of chlorophyll but also due to changes in optical properties [14].
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