

Quantification of Tannins in Wet Fermented Cocoa Beans by UV - Vis Spectrophotometric Analysis at 500nm to 800nm

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Abstract—The UV - Vis spectrophotometric analysis was conducted to quantify the tannins content in the *Theobroma cacao* L. Colour change of extracted cocoa samples also have relationship to the total polyphenol content in the extracted cocoa samples.

Keywords— *Cocoa beans, fermentation duration, cocoa pod storage, tannins, total polyphenol content, Theobroma cacao L.*

I. INTRODUCTION

Cocoa (*Theobroma cacao* L.) plant are commonly planted in the West Africa, South America and some other tropical regions around the world (Ardhana & Fleet, 2003) such as Malaysia. In West Africa, cocoa is broadly planted in many countries, where Cote d'Ivoire being the first and Ghana being the second largest producers of cocoa beans in the world (Ghana Cocobod, 2015). Cocoa beans are the major economic part of the cocoa fruit where its role in the manufacture of chocolate are main ingredients.

Tannins exist as polyphenolic secondary metabolites of upper plants and also can be either galloyl esters. Tannins' derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores, or they can be condensed tannins that can possess different interflavanyl coupling and substitution patterns which are oligomeric and polymeric proanthocyanidins. (Khanbabaee & Ree, 2001).

Cocoa beans are rich in polyphenols in particular catechin and proanthocyanidins. The polyphenols in cocoa beans are stored in the

pigment cells of the cotyledons. Those pigments also called as polyphenol-storage cells depending on the amount of anthocyanins. There are three groups of polyphenols that can be distinguished which are catechins or flavan-3-ols (ca. 37%), anthocyanins (ca. 4%) and proanthocyanidins (ca. 58%). The main catechin is (-)-epicatechin with up to 35% of polyphenol content. According to Kim & Keeney (1984), the (-)-epicatechin contents ranged from 34.65 to 43.27 mg/g of defatted sample. In smaller amounts, (+)-catechin as well as traces of (+)-gallo catechin and (-)-epigallocatechin have been found. Meanwhile, the total amount of soluble polyphenols in dried fat-free mass of fresh cocoa beans is 15 to 20% which equals to approximately 6% in air dried cocoa beans, containing 54% fat and 6% water. In fermented beans is approximately 5% of polyphenol content (10% and more are considered as a bad fermentation). These value are valid for Forastero beans, Criollo cocoa beans have approximately 2/3 of the amount of polyphenols, anthocyanins have not been found (Lange & Fincke, 1970). In cocoa liquor, total polyphenols content was also reported ranged from 45 to 52 mg/g, in cocoa beans 34 to 60 mg/g, and 20 to 62 mg/g in cocoa powder. For (-)-epicatechin contents, the avarage are 3.81 mg/g in cocoa powder, 2.53 mg/g in cocoa liquor and 4.61 mg/g in cocoa beans (Nazaruddin et al., 2001)

Recent studies are focusing more on enhancing the cocoa flavour and beneficial effects of polyphenols toward human health. Their antioxidant properties may be responsible for many of their pharmacological effects including as an anti-carcinogenic, anti-inflammatory, anti-microbial, anti-mutagenic, and chemo protective effects as well as analgesic activities (Hii et al., 2009).

However, research on relationship between fermentation degree,

flavour (astringency and bitterness) and colour changes of cocoa beans are still new.

Therefore, this study was conducted to quantify the tannins content and the relationship of the total polyphenol content with the change of the extracted cocoa samples colour.

II. METHODOLOGY

A. Materials

Cocoa bean samples at different Pod Storage (PS) which is PS0, PS2, PS4 and PS 6 that have different fermentation hours for each pod storage (0 hour, 24 hours, 48 hours, 72 hours, 96 hours and 120 hours). This samples was obtained from Malaysian Cocoa Board, Hilir Perak. N-hexane, methanol, Hydrochloric Acid (HCl), catechin.

B. Sample preparation

Grinded cocoa bean (0.5g) samples added with 25mL of n-hexane was shaken for two hours. The mixture then is filtered by filter paper no.4 and rinsed with n-hexane. The defatted cocoa powder form leaved to dry at room temperature. The samples were stored in deep freezer to keep it from oxidized.

C. Extraction procedure

The defatted cocoa bean (0.1g) samples were homogenized with 10mL of methanol: HCl(97:3) solution in wrapped glass bottle and incubated overnight at 4°C. The mixture then is filtered by filter paper no.4 and be analyzed by UV- Vis Spectrophotometer.

D. Spectrophotometric analysis

About 1.5ml from the filtrate was subjected to visible spectrum reading by UV- Vis Spectrophotometer between 500 nm to 800 nm wavelengths. The increment took for this absorbance reading is 10 intervals. This experiment was repeated three times. The standard used for this study is catechin with dilution of 10, 20, 40, 60, 80 and 100 mg/L in methanol.

III. RESULTS AND DISCUSSION

A. Spectrophotometric analysis

From this UV-VIS spectrophotometric analysis shown in Figure 1, the tannins in the extracted samples express their highest intensity at 520nm, therefore this wavelength was chosen for quantification. The trends then decreasing gradually for each fermentation duration of the cocoa pod storage.

Figure 2 shows the close up view of the absorbance trends for each fermentation duration at 520nm wavelength. At the pod storage PS0, highest peak for is at fermentation duration 0hr with 0.275% abs; while the lowest peak is at fermentation duration 24hr.

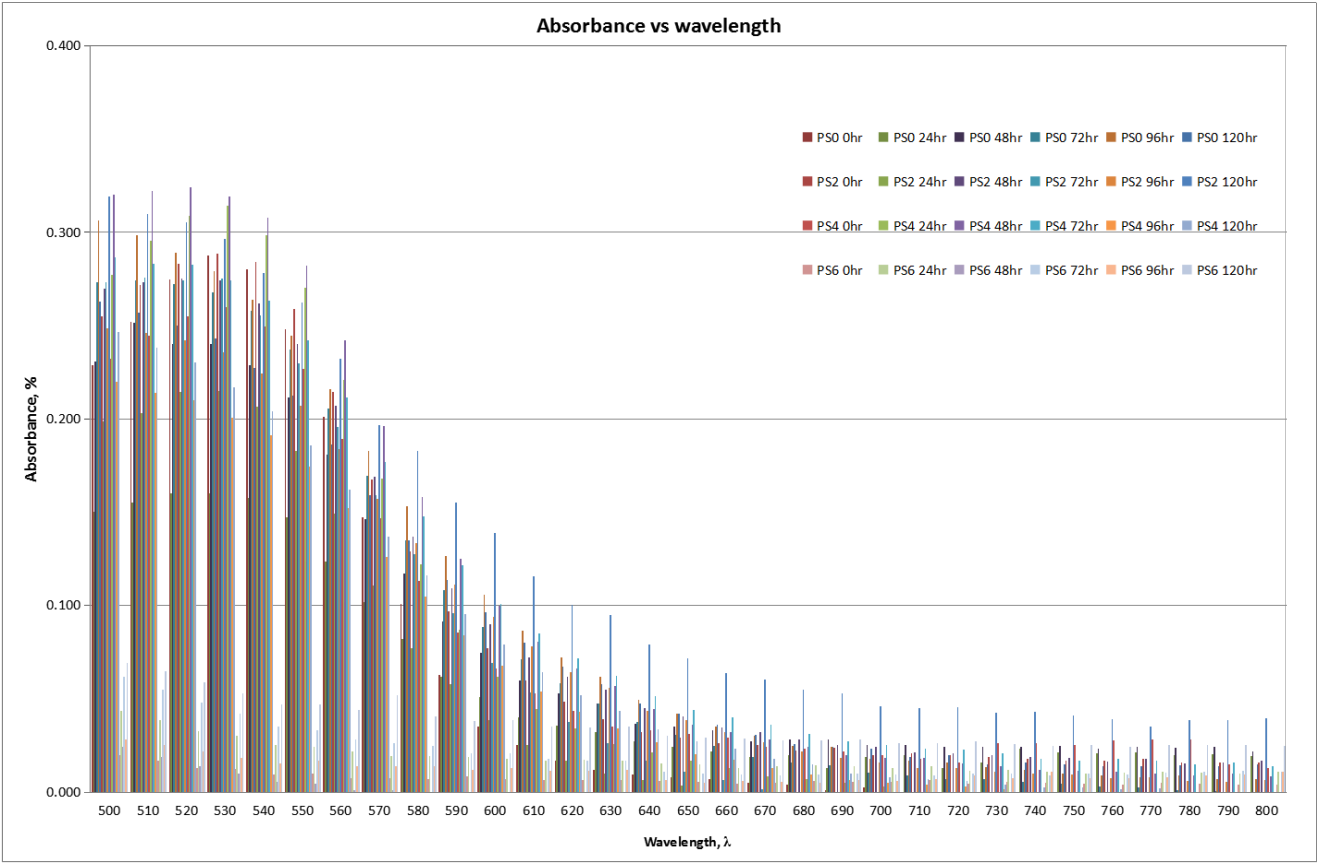


Figure. 1: Absorbance trends for each fermentation duration of pod storage from wavelength of 500nm to 800nm.

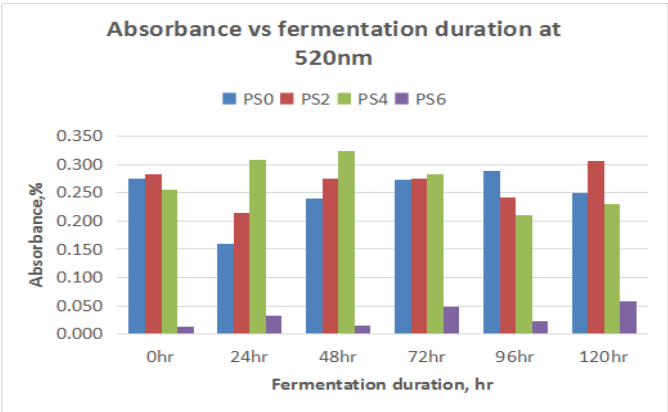


Figure. 2: Absorbance trends close up view for each fermentation duration at 520nm

B. The effect of total polyphenol content to colour change of extracted cocoa samples

The colour intensity of extracted cocoa samples also could show the concentration of the polyphenol content in the samples.

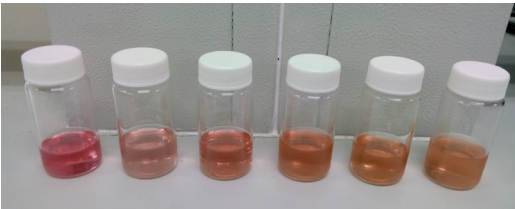


Figure. 3: Color differential of PS 0 from the extracted samples (0hr, 24hr, 48hr, 72hr, 96hr, 120hr respectively from left)

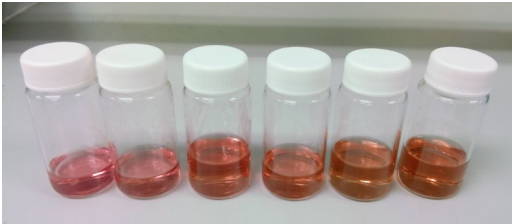


Figure. 4: Color differential of PS 2 from the extracted samples (0hr, 24hr, 48hr, 72hr, 96hr, 120hr respectively from left)

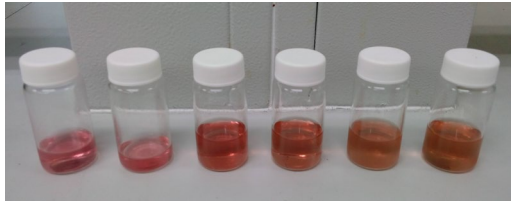


Figure. 5: Color differential of PS 4 from the extracted samples (0hr, 24hr, 48hr, 72hr, 96hr, 120hr respectively from left)



Figure. 6: Color differential of PS 6 from the extracted samples (0hr, 24hr, 48hr, 72hr, 96hr, 120hr respectively from left)

IV. CONCLUSION

The study was conducted to quantify the tannins content and the relationship of the total polyphenol content with the change of the extracted cocoa samples colour.

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