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Effect of pods storage and fermentation duration on proanthocyanidins content in Malaysian cocoa beans

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Abstract

The objective of this research is to evaluate the effect of pods storage and fermentation duration towards proanthocyanidins content in Malaysian cocoa beans. The pods storage duration was varied from 0 to 6 days with two days interval, while the fermentation duration of cocoa beans was varied from day 0 until day 5. The amount of proanthocyanidins extracted from the cocoa beans was determined by using UV spectrophotometric and high performance liquid chromatography (HPLC) analyses. The results showed that the total proanthocyanidins contents in all samples from different pods storage and fermentation duration are varied for both analyses. From the results, it shows that fermentation duration has more significant effect to the proanthocyanidins content in Malaysian cocoa beans as compared to the effect of pod storage duration.

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1.0 Introduction

Many studies have shown that cocoa powder has more antioxidants than other products (Afoakwa et al., 2008; Krähmer et al., 2015). Polyphenolic compounds, also known as flavonoids are important in cocoa beans due to its potential cardiovascular health benefit, antioxidant protections, and cholesterol controller in the body. The flavonoids contain plenty useful components that function as anti-inflammatory, anti-carcinogenetic properties, and powerful antioxidant which 20 times greater than nutrition C and 50 times higher than vitamin (He et al., 2009; Rahul et al., 2015). Polyphenol in the cocoa beans consists of flavanols, anthocyanins, and proanthocyanidins with high level of flavan-3-ols, represent as monomers of epicatechin and catechin (Mazor Jolić et al., 2011). These compounds give astringent and bitter sensations, and contribute essentially to the green and fruity flavour of cocoa liquors. The many health benefits of cocoa products make it one of the highest sought commodities around the world. According to the statistics given by the Malaysian Cocoa Board on the export and import of the cocoa beans and cocoa product, the demand has been fluctuating (Board, 2016). However, the supply is not available as per demand since the statistics shown that the production of cocoa beans by region is decreasing each year. Other than that, the problem that also arise is the products from Malaysian cocoa beans have not been commercialised and less popular as compared to the products from other countries.

Fermentation becomes a crucial process in order to determine the biochemical changes in the type and concentration of final cocoa flavour (Krähmer et al., 2015). The right choice of fermentation process of the cocoa beans, depends on the countries of origin due to the improvement of the reasonable flavour as well as the flavour precursor (Ho et al., 2014). Malaysian Cocoa Board has endorsed five days fermentation period using shallow box with a single turning on the third day as a standard fermentation preparation for the Malaysian cocoa beans (Sulaiman & Yang, 2015). Malaysian cocoa beans are usually known for its low cocoa flavour, high acidity, and astringent (Reed & Cocoa, 2010). Therefore, it has been reported that this technique is capable to produce Malaysian cocoa beans with low acidity and stronger cocoa flavour (Zaibunnisa, 2002).

In addition, the pods storage has an effect to the quality of Malaysian cocoa flavour, especially on the acidity of the cocoa beans (Afoakwa et al., 2012). It

also helps to decrease the nib acidification and increase in cocoa flavour (Meyer et al., 1989). Furthermore, pod storage had indirectly helped the cocoa farmers to collect their pods until enough beans is obtained for fermentation. (Sulaiman et al., 2016). Therefore, this research was done to investigate in further detail about the effect of pod storage and fermentation time to the proanthocyanidins content in Malaysian cocoa beans that give more impact to the flavour and quality of the products from Malaysian cocoa beans. The finding from this study can be applied to the Malaysian Cocoa Board to increase the quality and productivity of the cocoa beans as well to develop more products from the cocoa beans.

2.0 Methodology

2.1 Material

The ripe and health cocoa pods of mixed clones were provided by the Cocoa Research and Development Centre, Hilir Perak, Malaysia. Standard of catechin and quercetin were also provided by Malaysian Cocoa Board. Methanol, acetic acid, n-hexane and acetonitrile were obtained from the Chemistry Laboratory at the Faculty Chemical Engineering.

2.2 Sample preparation

Malaysian Cocoa Board performed the collection of the cocoa pods, which come from mixed clone. The ripe and health cocoa pods were chosen in order to determine the compound content in the cocoa. The pods storage, fermentation and drying process/method were done according to Sulaiman & Yang (2015). For the effect of pods storage duration, the cocoa pods were kept in a basket for different duration from 0 to 6 days. While for the effect of fermentation time, the cocoa pods were opened, the fresh cocoa beans were extracted and kept in a standard shallow box to undergo fermentation for different time from 0 to 5 days.

2.3 Defatted powder

The dried coca beans were cut and deshelled to obtain nibs. The nibs then grounded to into the fine powder. Then, the fine powder was defatted using n-hexane with ratio of 1:5 (w/v). The powder was suspended in n-hexane, vortexes vigorously for 30 minutes and the solution was discarded afterwards. The remaining defatted powder then was dried before further use.

2.4 Extraction of sample

Prior to analysis, approximately 0.1 g of defatted powder was resuspended in 7 ml of acetone/water/acetic acid (70:29.5:0.5) solution and vortexes for 5 minutes. Lastly, the suspension was centrifuged at 3250 g for 5 minutes and supernatant were collected for analysis.

2.5 Total proanthocyanidins determination

DMAC reagent: Total proanthocyanidins were measured by using 4-dimethylaminocinnamaldehyde (DMAC) assay. The DMAC reagent was prepared by dissolving 2.0% DMAC powder (w/v) in a cold 1:1 mixture of methanol and $6NH_2SO_4$ (v/v) daily. Reagent was kept in dark glass bottle and the freezer (–18ºC) between analysis.

Catechin standards: The catechin stock solution was prepared by dissolving 0.1 g catechin in 10 ml methanol. Then, the stock solution was diluted with methanol for 10, 20, 40, 60, 80, and 100 mg/L catechin preparation.

Spectroscopic analysis: All the experiment was done under minimal light conditions due to light sensitivity. The 3 ml disposable cuvettes were used. Samples were mixed with methanol and DMAC solution was pipetted into the mixture before the mixture was left for 15 minutes at 25 °C for reaction. The readings for all mixtures were taken using UV-Vis spectrophotometer at 640 nm wavelength. The experiment was repeated three times and the mean value was taken. The average total of proanthocyanidins content of sample was reported in milligram catechin equivalents per gram defatted cocoa powders.

2.6 High performance liquid chromatography (HPLC)

The methods of normal HPLC analysis from Segura-Carretero et al. (2014) have been adapted in determination of proanthocyanidins content. The sample that comprises proanthocyanidins compounds injected to the column together with a mobile phase at a certain time. The HPLC has been set the parameter to the appropriate condition where flow rate of 1ml/min and the column was saved at a temperature of 30 °C

with an injection volume of 20 µl. The stationary phase (solvent A) consist of water with 5% acetic acid in determination of proanthocyanidins, while, the mobile phase (solvent B) comprised with methanol and 5% of acetic acid. The gradient of the solvent parameter was achieved using a linear gradient which have been set isocratic/isothermal with 50:50% solvent A and B.

3.0 Results and discussion

3.1 Total proanthocyanidins content

The use of HPLC is most optimum for accurately evaluating the amount of proanthocyanidins present in fermented and unfermented cocoa beans (Segura-Carretero et al., 2014). The standard curve has been generated by using external standard quantitation for HPLC method. Fig. 1 shows the average total proanthocyanidins content for different pod storage and fermentation time. For cocoa beans without fermentation (0 hour), fresh cocoa beans (PS0) mostly produced the highest amount of average total proanthocyanidins, followed by cocoa beans at PS2, PS4 and PS6. The same trend also observed for cocoa beans that have been fermented for 120 hours for all post storage duration. The highest proanthocyanidins obtained was 250±0.5 ppm.

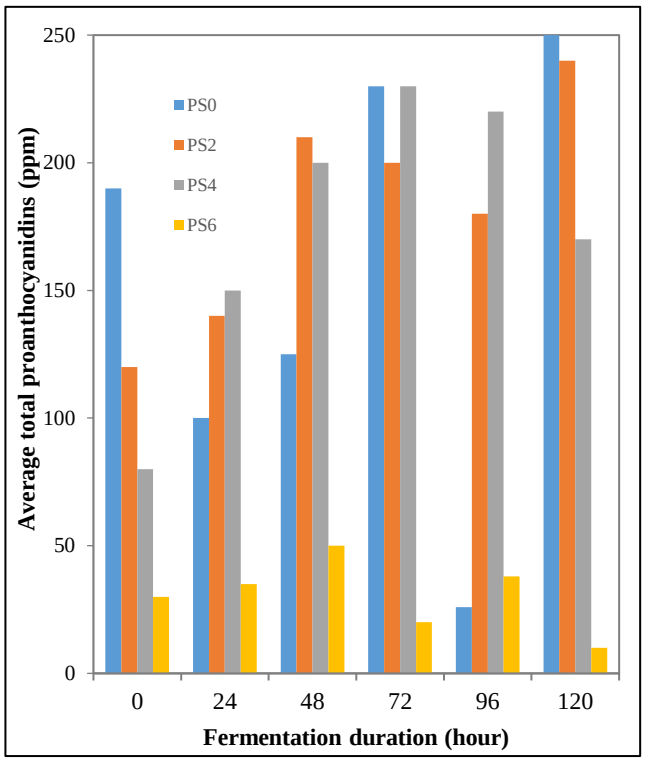
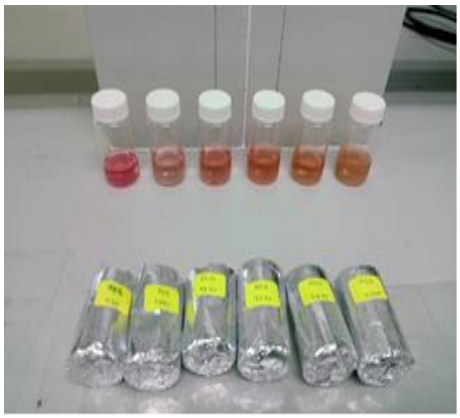
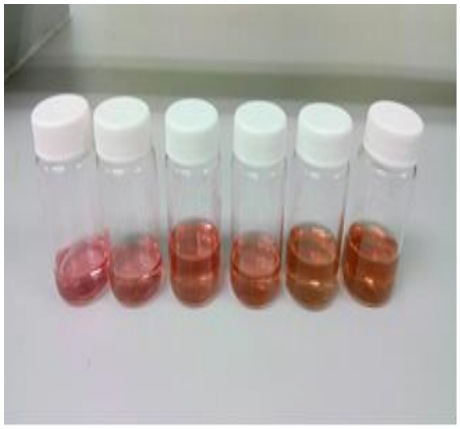


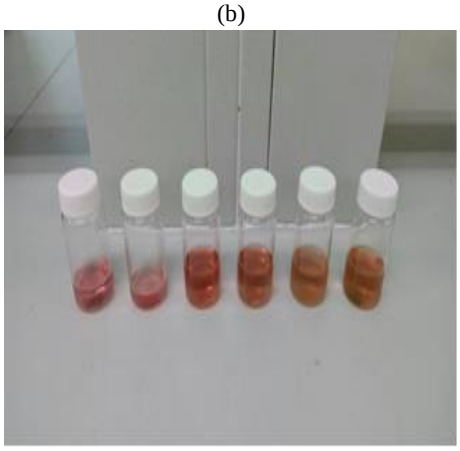
Fig.1: The total proanthocyanidins content (ppm) in all samples from different pods storage and fermentation duration.



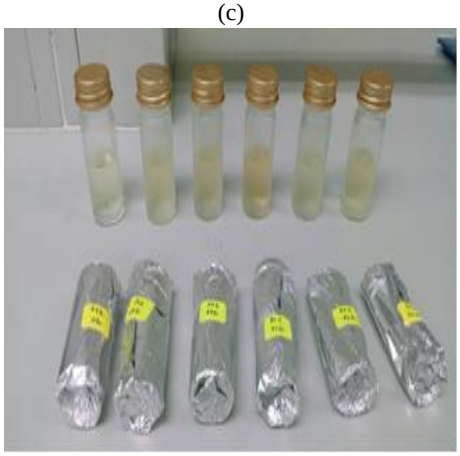
(a)



(b)



(c)



(d)

Fig.2: The colour observation on (a) the PS0 (b) PS2 (c) PS4 and (d) PS 6 with the increment of fermentation duration (0, 24, 48, 72, and 96,120 h).

Other than that, for all fermentation duration, pod storage 6 day (PS6) produces the lowest average total proanthocyanidins content. This result is consistent with is obtained from the longest pods storage duration (PS6) cocoa beans. These results are consistent with (Sulaiman et al., 2016) as their research also PS6 produced the lowest) owes average total proanthocyanidins content. On the other hand, in recent study shows that the total proanthocyanidins decreased as the fermentation time increase (Ioannone et al., 2015). This is because of the polyphenol where it is diminishing essentially due to enzymatic browning and diffusion out of the beans throughout the fermentation and drying process.

However, observation on the colour of the samples (Fig. 2 (a)–(d)), showed that as the pods storage and fermentation duration increased the colour of the sample become light pink and slowly turns to brown colour. This showed that the total proanthocyanidins content in the samples decrease as the duration increase. From this figure, higher total proanthocyanidins should be in pink colour. The colour will turn from pink to the brown colour where the proanthocyanidins decrease in the solvent. Therefore, from this colour observation, it can be stated that unfermented pods without storage should have higher total proanthocyanidins. Meanwhile, PS6 have less content of total proanthocyanidins. However, PS6 have the appropriate result where it has the least proanthocyanidins content in both analyses.

4.0 Conclusions

The total proanthocyanidins contents in all samples from different pods storage and fermentation duration are varied. It can be concluded that pods storage duration does not have significant effect to the proanthocyanidins bioconversion as compared to the effect of fermentation duration. In addition, further study must be carried out in order to determine and characterize more detail about the compound exist in the Malaysian cocoa beans.

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References

A. Krähmer, A. Engel, D. Kadow, N. Ali, P. Umaharan, L. W. Kroh and H. Schulz, (2015). Fast and neat – Determination of biochemical quality parameters in cocoa using near infrared spectroscopy. *Food chemistry* 181. 152–159.

B. Meyer, B. I. Biehl, M. B. Said and R.J. Samarakoddy, (1989). Post-harvest pod storage: A method for pulp preconditioning to impair strong nib acidification during cocoa fermentation in Malaysia. *Journal of the Science of Food and Agriculture* 48(3). 285–304.

E.O. Afoakwa, A. Paterson, M. Fowler and A. Ryan, (2008). Flavour formation and character in cocoa and chocolate: a critical review. *Critical reviews in food science and nutrition 48(9)*. 840–857.

E.O. Afoakwa, J. Quao, A. S. Budu, F.K. Saalia and F.S. Takrama, (2012). Changes in total polyphenols, o-diphenols and anthocyanin concentrations during fermentation of pulp pre-conditioned cocoa (Theobroma cacao) beans. *International Food Research Journal 19(3)*. 1071–1077.

F. Ioannone, C. D. Di Mattia, M. De Gregorio, M. Sergi, M. Serafini, and G. Sacchetti, (2015). Flavanols, proanthocyanidins and antioxidant activity changes during cocoa (Theobroma cacao L.) roasting as affected by temperature and time of processing. *Food chemistry 174*. 256–262.

H. Zaibunnisa Abdul, (2002). Development of fermentation and drying of cocoa beans using a rotary fermenter-drier. 2002. Retrieved from http://psasir.upm.edu.my/8464, WorldCatDissertations database.

J. W. He, T. C. Keatley, M.L. Failla and M.M.Giusti, (2009). Stability of black raspberry anthocyanins in the digestive tract lumen and transport efficiency into gastric and small intestinal tissues in the rat. *Journal of agricultural and food chemistry 57(8).* 3141.

K. L Rahul, N.S. Deeba, M.A. Vaqar, K. Mohammad Imran, K. and M. Hasan, (2015). Dietary polyphenols in prevention and treatment of prostate cancer. *International Journal of Molecular Sciences 16(2)*. 3350–3376.

M. C. Board, (2016). Daily/Monthly Cocoa Prices. Retrieved on January 08,2017 from http://www.koko.gov.my.

S. Khairul Bariah, T. A. Yang and N.Hamizah, (2016). Effects of pods storage and fermentation duration on bioconversion of proanthocyanidins in Malaysian cocoa beans. *Malaysian Cocoa Journal 9(2)*. 78.

S. Khairul Bariah, T. A. Yang, (2015). Colour characteristics of dried cocoa using shallow box fermentation technique. *World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering 9 (12)*, 1213–1217.

S. Mazor Jolić, I. Radojčić Redovniković, K. Marković, D. Ivanec Šipušić and K. Delonga, (2011). Changes of phenolic compounds and antioxidant capacity in cocoa beans processing. *International Journal of Food Science & Technology. 46(9)*. 1793–1800.

S. Reed and C. Cocoa, (2010). Sensory Analysis of Chocolate Liquor: Sophisticated sciences such as sensory and flavour chemistry are needed to understand flavour development. *The Manufacturing confectioner 90(11)*. 43−52.

V.T.T. Ho, J. Zhao and G. Fleet, (2014). Yeasts are essential for cocoa bean fermentation. International Journal of Food Microbiology 174. 72−87.