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COLOUR AND ANTIOXIDANT PROPERTIES OF COCOA BEANS FROM PODS STORAGE AND FERMENTATION USING SHALLOW BOX

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Abstract

The study aims to evaluate the effect of duration during pods storage and fermentation using a shallow box technique on the colour and antioxidant properties of the cocoa beans. The fermentation experiment was conducted at the Cocoa Research and Development Centre Bagan Datuk, using 150 kg of fresh cocoa seeds from four pods storage duration (0, 2, 4 and 6 days). A total of 15 kg of samples were taken out randomly for the duration of 0, 24, 48, 72, 96 and 120 hours of fermentation and subjected to sun drying. The dried samples were analyzed by the score of equivalent percent fully brown (EB), Browning index (BI) and antioxidant properties by Ferric Reducing Power (FRAP) Test. The results demonstrated that EB scores increased significantly (P <0.05) from 37%, 34%, 47% and 42% at 0 hours for PS0, PS2, PS4 and PS6, respectively to 87%, 93%, 95% and 94% after 120 hours of fermentation. Similarly, significant

(P<0.05) increased of the BI to more than 1.00 after 48 hours for PS4 and PS6, whereas at 72 hours of fermentation for PS0 and PS2, respectively. On the other hand, the antioxidant properties fluctuated with respect to the different duration of fermentation.

Keywords

Fermentation, Duration, Cocoa, Colour, Antioxidant

1. Introduction

Everyone, especially kids love to eat chocolate, but not everyone aware that the primary raw material of the chocolate is the seeds from a tree of a tree of *Theobroma cacao* L. The seeds generally contain about 20 to 60 and are laid inside the fruits known as a pod. The pods developed from the pollinated flowers on both the trunk and branches of the tree. The seeds contain two cotyledons which are in the form of spherical or flat. The colour of cotyledons is ranging from deep purple to pink as well as white and surrounded by white mucilage pulp (Lopes & Luis Pires, 2014; Ahmad Kamil *et al.,* 2013; Cakirer, Ziegler & Guiltinan, 2010). The pulp has a typical aromatic and sweet to eat, but the cotyledons are almost inedible due to their unpleasant taste which is excessively bitter and astringent. The seeds need to be processed by fermentation, followed drying and roasting in order to obtain the full spectrum of chocolate flavour (De Vuyst & Weckx, 2016; Voigt & Lieberei, 2014).

The variations of colour and unpleasant taste of the cocoa seeds are reported to be associated with the fact that cocoa is rich in polyphenols compound (Aprotosoaie, Luca, & Miron, 2016; Cakirer *et al.,* 2010). The polyphenols are stored in the polyphenolic cells, which encompassed about 12% to 20% dried weight of the defatted cotyledons. A previous study revealed that the unfermented cocoa bean containing 120 to 180 g/kg of polyphenolic compounds. The polyphenols compositions in cocoa seeds are 4% of anthocyanins, 37% of flavanols and 58% of proanthocyanidins (Kongor *et al.,* 2016; Bordiga *et al.,* 2015; Voigt & Lieberei, 2014). The anthocyanins are a pigment compound that responsible for the purple colour of cacao seed. It has the ability to convert from the colour of orange-red to blue-violet in food and beverage products (Wallace & Giusti, 2011; Cakirer *et al.,* 2010). The flavanols and the proanthocyanidins, which are polymers for flavanols are reported to be correlated with the flavour quality of cocoa. Whereby, the higher content of the flavanols such as (+)-catechin and (-)-epicatechin is responsible for bitterness. Whereas, the taste of astringency is stronger with

increased of a soluble content of the proanthocyanidins (Brillouet & Hue, 2017; Counet, Ouwerx, Rosoux & Collin, 2004).

Unfortunately, the polyphenols content is affected by fermentation, drying and roasting process. During fermentation, the polyphenols are reported either being hydrolyzed by a glycosidase or oxidized by polyphenol oxidase (Kongor *et al.*, 2016). The anthocyanins are hydrolyzed and producing a component of anthocyanidins and sugar, resulting in the colour of cotyledon gradually faded or bleached. Later, the drying process will further transform the cotyledon colour towards slaty, fully purple, partly purple-brown, and fully brown (Brillouet & Hue, 2017; Bordiga *et al.,* 2015). It is also suggested that the free anthocyanidins are oxidized to quinones and subsequently complexed with proteolysis products of proteins to form insoluble high-molecular-weight of tannins. In which, the bitter and astringent taste of cocoa beans will reduce (Brillouet & Hue, 2017; Counet *et al.,* 2004). Therefore, this study was to evaluate the effect of different pods storage and fermentation duration using a shallow box on colour and antioxidant properties of the Malaysian cocoa beans.

2. Material and Method

2.1 Cocoa pods

Healthy cocoa pods (mix clone) were harvested at the same ripen stage from Cocoa Research and Development Centre (CRDC), Bagan Datuk, Perak, Malaysia. The pods were stored under dry and well-aerated conditions of the rooftop as in figure 1 (a) and described by Khairul Bariah *et al.,* (2016) at predetermined times of 0, 2, 4 and 6 days. Prior to fermentation, the pods were opened, extracted and sorted manually to ensure only fresh cocoa seeds were used.

2.2. Fermentation

Four fermentations in the shallow box (90 X 60 X 32 cm^3) with a capacity of 150 kg were carried out as in Figure 1 (b). Fermenting mass was covered with clean gunny sacks and turned by transferring from one box to another at 72 hours. A total of 15 kg of wet cocoa beans was taken randomly out from the top, middle and the bottom layer of mass at 0, 24, 48, 72, 96 and 120 hours of fermentation, respectively.

2.3 Drying

The respective wet cocoa beans were sun-dried under the transparent roof by spreading on the drying platform at one layer thickness. The beans were turned using stainless steel rake at

every three hours during daylight to ensure uniformity and covered during night time with gunny to avoid dewdrop. The practices were repeated until the moisture content of the cocoa beans reduced to about 7.5 percent. Upon dried, sampling using quartering tools was performed until each quarter contain about 250 grams. The samples were placed in a vacuum sealed container, labeled and stored accordingly until further analysis.

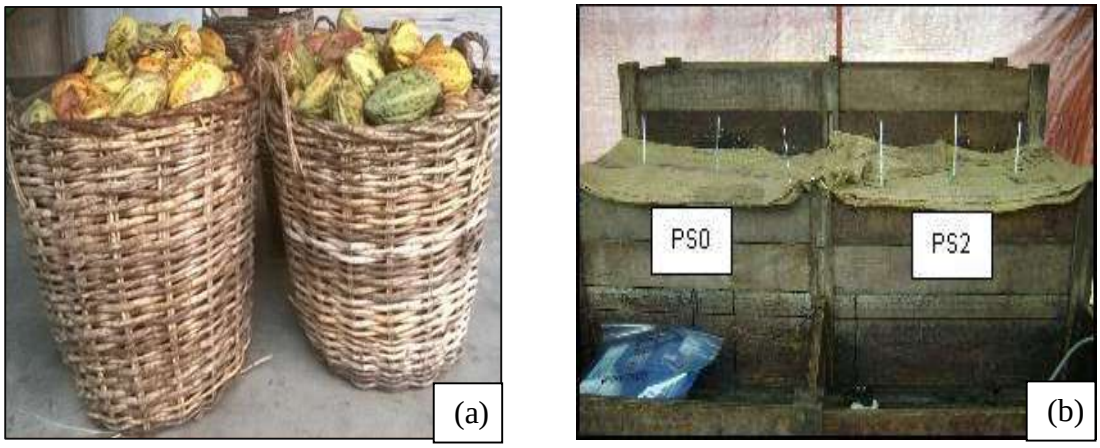

Figure 1: *Pods storage in the basket (a). Fermentation was carried out in the shallow box (b)*

2.4 Equivalent Percent Fully Brown Score

Equivalent Percent Fully Brown (EB) score was determined according to Khairul Bariah, (2014). A total of 100 dried fermented beans randomly taken out from the respective quarter prepared in section 2.3. The dried fermented beans were cut lengthwise into halves for a maximum surface of exposure and inspected for surface colour under artificial light. The beans were divided into fully brown (FB), partly brown (PB), partly purple (PP), fully purple (FP) and slaty as displayed in Figure 2 and calculated by the following equation:

$$EB = \left[\left(1\times\%FB\right)+\left(0.7\times\left(\%PB+\%PP\right)\right)+\left(0.5\times\left(\%FP\right)\right)+\left(0.3\times\left(\%slaty\right)\right)\right] \quad (1)$$

Figure 2: *The surface colour of cocoa bean*

2.5 Browning Index

Browning Index (BI) previously known as fermentation index (FI) was determined as described in Khairul Bariah, (2014) with slight modification. The shells of dried cocoa beans cut from previous section 2.4 were removed to obtain nibs and grinded into powder using an analytical grinder. About 0.4 g of the powders were homogenized in 40 ml methanol: HCl (97:3) solution and incubated at 4^{o}C overnight. The homogenate was filtered and read in triplicate at the wavelength of 460 and 530 nm, respectively. The absorbance ratio of 460 to 530 was calculated as BI.

2.6 Ferric Reducing Antioxidant Power Test

The test was performed according to Albertini *et al.,* (2015) with slight modification. The remaining 0.4 g powder from previous section 2.5 was defatted according to Niemenak *et al.,* (2006). The powder was homogenized in the 5-fold volume of cold n-hexane for 30 min before centrifuged (5000 rpm, 10 min, and 4°C). The sediment collected and the defatting procedure was repeated for three times before dried.

Approximately 0.1 g of the defatted dried powder was resuspended in a solution mixture (7 mL) of acetone/water/acetic acid (70:29.5:0.5), vortexed (5 min) prior to the centrifuge (3250 g, 5 min) and the supernatant collected. A total of 100 μL of supernatant was mixed with ultrapure water (v/v). The mixture was added to 1.5 mL of the Ferric reducing antioxidant power reagent [TPTZ (0.3% w/v) containing of 0.04M HCl; ferric chloride aqueous (0.3% w/v) and 0.3 M acetate buffer (pH 3.6) in a ratio of 1:1:10]. The mixture was incubated for 4 min at room temperature before an absorbance was read at 593 nm using a UV-Vis Spectrophotometer. At the same time, a standard curve was prepared using Trolox (10 - 50 mg/mL). The Ferric reducing activity was determined by comparing the absorbance readings of samples against the standard curve. The experiment repeated in triplicate and results were expressed as milligrams of Trolox equivalents per gram of defatted cocoa (mg TE/g DC).

2.7 Statistic analysis

All data obtained were subject to analysis of variance (ANOVA) and means compared for significant differences ($P < 0.05$) by Tukey Method using Minitab version 16.1.0.

3. Result and Discussion

The Equivalent Percent Fully Brown (EB) scores for all dried cocoa beans at the different duration of pods storage and fermentation are presented in Figure 3. Initially, the EB scores for cocoa beans which stored for 0 (PS0), 2 (PS2), 4 (PS4) as well as 6 (PS6) days and dried at 0 hours of fermentation were 37%, 34%, 47% and 42%, respectively. The EB scores were increased as the duration of pods storage and fermentation extended. The study revealed that all the EB score of the cocoa beans increased rapidly especially for PS4 as well as PS6 to achieve more than 80%. After 72 hours of fermentation, the EB scores for dried cocoa beans were followed in decreased order of PS4 > PS6 > PS2 > PS0. Afterwards, the scores reached a plateau with the EB scores at 120 hours of fermentation were in between 87%, 93%, 95% and 94% of cocoa beans from PS0, PS2, PS4, and PS6, respectively.

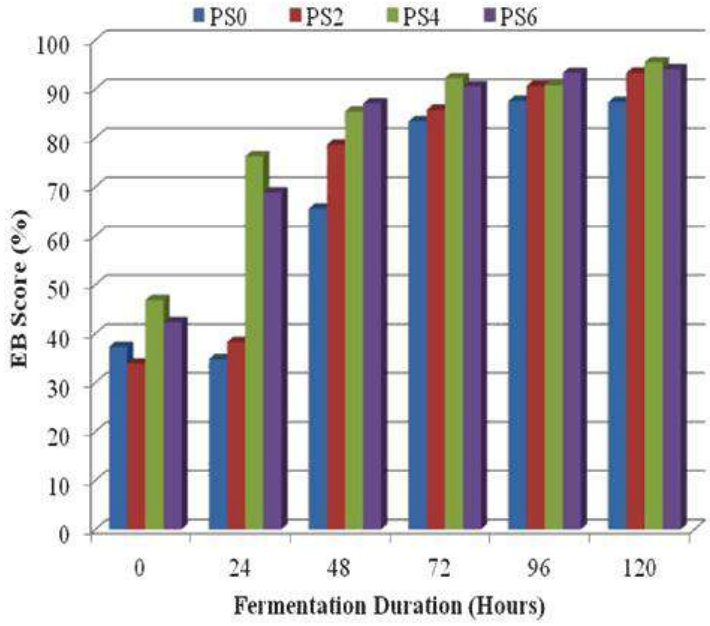

Figure 3: *The EB scores of all the dried cocoa beans*

The BI values for all of the dried beans from PS0, PS2, PS4, and PS6 at the different duration of fermentation were presented in Figure 3. The BI values for dried cocoa beans at 0 hours of fermentation for PS0, PS2, PS4, and PS6 were in between 0.47, 0.40, 0.47, and 0.41, respectively. Similar to EB scores, the BI values increased as the duration of pods storage and fermentation was extended. In which the BI values for PS4 and PS6 achieved more than 1.00 after dried at 48 hours, whereas at 72 hours of fermentation for PS0 and PS2, respectively. A previous study suggested that the cocoa beans considered as under-fermented if the value of BI

was below 1.000, while fully fermented if the BI value in between 1.000 to 1.599 and over-fermented for the BI more than 1.600. In addition, the BI value more than 1.400 was suggested that the cocoa beans were approaching the over-fermented stage, hence the process should be terminated (Khairul Bariah, 2014). Therefore, the study revealed that the BI value for the cocoa beans from PS4 as well PS6 were fully fermented as early as 48 hours of fermentation, while PS0 and PS2 at 72 hours of fermentation. The BI value of PS0 was more than 1.600 and suggested as over-fermented. However, this study revealed that after achieving more than 1.400 for PS6, PS4 and PS2, the BI values were exhibits decreased and increased trend. Whereas, the BI value for PS0 were decreased after achieved the BI value 1.600. The BI value exhibited the increased and decreased trend may be due to a transition of the colour compound.

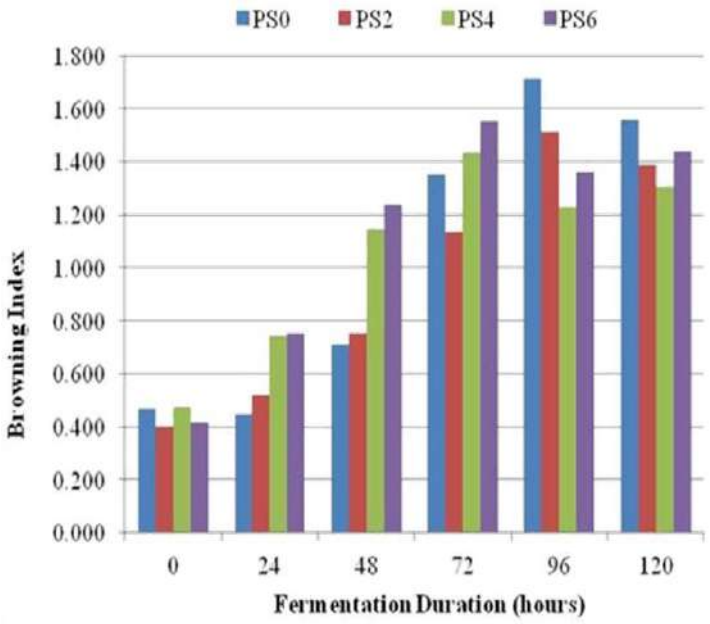

Figure 4: *The BI values of all the dried cocoa beans*

The ferric reducing activity in all the dried beans from PS0, PS2, PS4, and PS6 at the different duration of fermentation was presented in Figure 4. The ferric reducing activity in all the dried cocoa beans from batch PS0, PS2, PS4, and PS6 fluctuated. The highest ferric reducing activity (33.94 ± 0.05 mg TE/g DC) was in dried cocoa beans from PS0 which is fermented for 0 hours of fermentation duration. In dried fermented cocoa beans, the ferric reducing activities were almost at the same except for PS4 at 48 hours of fermentation, PS0 at 96 hours of fermentation and the PS2 at 120 hours of fermentation.

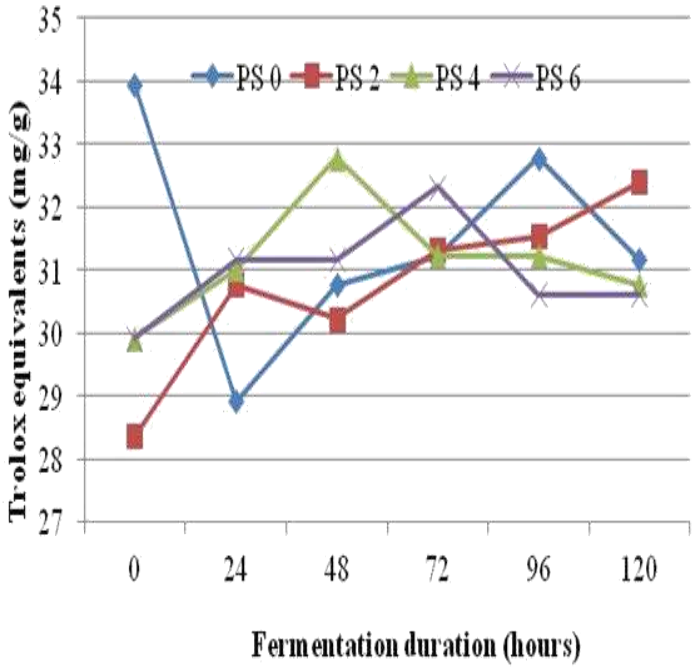

Figure 5: *The ferric reducing activity of all the dried cocoa beans*

The effect of the pod storage and fermentation duration on the EB scores, BI values, and ferric reducing activity were evaluated by General Linear Model Analysis of Variance (GLM ANOVA) and presented in Table 1. The interaction between duration of pod storage and fermentation suggested that either duration of pods storage or fermentation was significantly affecting the EB scores [$F(15, 120) = 17.65$, $p < 0.001$], the BI values [$F(15, 48) = 3.20$, $p = 0.001$] and the ferric reducing activity [$F(15, 48) = 7.29$, $p < 0.001$]. Multiple comparisons by Turkey Method exhibited that regarding pods storage, the EB scores of cocoa beans which dried from 0, 24 and 48 hours of fermentation duration were significantly different. Whereas, the unfermented dried cocoa beans from PS0 has significantly higher the ferric reducing activities and suggested had stronger reducing power compared to others.

Table 1: *The effect of the pod storage and fermentation duration on the EB scores, BI values, and ferric reducing activity*

Pod Storage (days)	Fermentation Duration (hours)	EB scores (%)	BI values	FRAP (mg TE/g DC)
0	0	37.3^{i}	0.465^{d}	33.94^{a}

	24	34.8^{i}	0.446^{d}	28.91^{de}
	48	65.5^{g}	0.709^{cd}	30.77^{bcde}
	72	83.4^{cde}	1.352^{ab}	31.23^{bcd}
	96	87.5^{abcd}	1.713^{a}	32.78^{ab}
	120	87.3^{abcd}	1.556^{ab}	31.15^{bcd}
2	0	33.9^{i}	0.398^{d}	28.37^{e}
	24	38.3^{hi}	0.519^{d}	30.77^{bcde}
	48	78.6^{de}	0.753^{cd}	30.23^{bcde}
	72	85.7^{bcde}	1.133^{bc}	31.31^{abcd}
	96	90.6^{abc}	1.509^{ab}	31.54^{abcd}
	120	93.3^{ab}	1.389^{ab}	32.39^{abc}
4	0	46.8^{h}	0.479^{d}	29.92^{cde}
	24	76.2^{ef}	0.743^{cd}	31.00^{bcde}
	48	85.4^{bcde}	1.147^{bc}	32.78^{ab}
	72	92.2^{abc}	1.434^{ab}	31.23^{bcd}
	96	90.8^{abc}	1.226^{ab}	31.23^{bcd}
	120	95.4^{a}	1.305^{ab}	30.77^{bcde}
6	0	42.3^{hi}	0.414^{d}	29.92^{cde}
	24	68.9^{fg}	0.749^{cd}	31.15^{bcd}
	48	87.1^{abcd}	1.237^{ab}	31.15^{bcd}
	72	90.5^{abc}	1.550^{ab}	31.32^{abcd}
	96	93.3^{ab}	1.363^{ab}	30.61^{bcde}
	120	94.0^{ab}	1.439^{ab}	30.61^{bcde}
F (PS*FD)		17.65	3.20	7.29
P (PS*FD)		<0.001	0.001	<0.001

Means ± sd (n=3) that do not share the same letter were significantly different. All pairwise comparisons were among levels of duration of pods storage and Fermentation at Tukey 95% simultaneous confidence intervals

4. Conclusion

This study revealed that the colour indicators, either the EB scores or BI values were significantly increased and decreased in between of the fermentation duration. Similarly, the ferric reducing activities in all the dried cocoa beans fluctuated at the different fermentation duration. All of the findings suggested that the colour and antioxidant properties of the cocoa beans were significantly affected by both factors ie the duration of pods storage and fermentation. This study was limited to evaluate the effect of post-harvest processing to the changes of the colour indicator and antioxidant properties of the cocoa beans. The reaction

mechanism of the colour changes during the post-harvest processing of cocoa beans is suggested for future research.

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REFERENCES

Ahmad Kamil, M.J., Alias, A., Azmi, C.A., Boney, M., Harnie, H., Haya, R., Khairul Bariah, S., Lee, C.H., Meriam, M.Y., Mohd Yusoff, A.S., Mohd Zamri, A.G., Mohamed Helmi, S., Navies, M., Nuraziawati, M.Y., Nurfadzilah, M., Rozita, O., Saripah, B. & Shari Fuddin, S. (2013). Cocoa Planting Manual, Sustainable Cocoa. Sabah, Malaysia: Malaysian Cocoa Board.

Albertini, B., Schoubben, A., Guarnaccia, D., Pinelli, F., Vecchia, M.D., Ricci, M., Di Renzo, G. C., and Blasi, P. (2015). Effect of Fermentation and Drying on Cocoa Polyphenols. J. Agric. Food Chem, 63 (45): 9948-9953.

Aprotosoaie, A.C., Luca, S.V. & Miron, A. (2016). Flavor Chemistry Of Cocoa And Cocoa Products-An Overview. Comprehensive Reviews in Food Science and Food Safety 15: 73-91.

Brillouet, J.M. & Hue, C. (2017). Fate of proanthocyanidins and anthocyanins along fermentation of cocoa seeds (*Theobroma cacao* L.). Journal of Applied Botany and Food Quality 90: 141 – 146.

Bordiga, M., Locatelli, M., Travaglia, F., Coïsson, J. D., Mazza, G., & Arlorio, M. (2015). Evaluation of the effect of processing on cocoa polyphenols: Antiradical activity, anthocyanins and procyanidins profiling from raw beans to chocolate. International Journal of Food Science and Technology. https://doi.org/10.1111/ijfs.12760

Cakirer, M.S., Ziegler, G.R. & Guiltinan, M.J. (2010). Seed color as an indicator of flavanol content in Theobroma cacao L. Chocolate, fast foods and sweeteners: consumption and health. Nova Science Pub. Inc, New York, 257-270.

Counet, C., Ouwerx, C., Rosoux, D. & Collin, S. (2004.) Relationship between Procyanidin and Flavor Contents of Cocoa Liquors from Different Origins. J. Agric. Food Chem. 52: 6243-6249.

De Vuyst, L. & Weckx, S. (2016). The cocoa bean fermentation process: from ecosystem analysis to starter culture development. Journal of Applied Microbiology, 121: 5-17.

Khairul Bariah, S., Yang, T. A. & Nur Hamizah, S. (2016). Effect of pods storage and fermentation duration on bioconversion of proanthocyanidins in Malaysian cocoa beans. Malaysian Cocoa Journal 9(2): 102-109.

Khairul Bariah, S. (2014). Impact of Fermentation Duration on the Quality of Malaysian Cocoa Beans Using Shallow Box. KKU Research Journal 19:74-80.

Kongor, J.E., Hinneha, M., deWalle, D.V., Afoakwa, E.O., Boeckx, P. & Dewettinck, K. (2016). Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile - A review. Food Research International 82: 44–52.

Lopes, U.V. & Luis Pires, J. (2014). Botany and Production of Cocoa. In Schwan, S.F & Fleet, G.H (Eds), Cocoa and Coffee Fermentation (pp. 43-70). Boca Raton: CRC Press.

Niemenak, N., Rohsius, C., Elwers, S., Ndoumoua, D.O. and Lieberei, R. (2006). Comparative study of different cocoa (*Theobroma cacao* L.) clones in terms of their phenolics and anthocyanins contents. Journal of Food Composition and Analysis 19:612–619.

Voigt, J. & Lieberei, R. (2014). Biochemistry of Cocoa Fermentation. In Schwan, S.F & Fleet, G.H (Eds), Cocoa and Coffee Fermentation (pp. 194-226). Boca Raton: CRC Press.

Wallace, T.C. & Giusti, M.M. (2011). Selective Removal of the Violet Color Produced by Anthocyanins in Procyanidin-Rich Unfermented Cocoa Extracts. Journal of Food Science 76(7):1010-1017.