EFFECT OF POD STORAGE AND FERMENTATION DURATIONS USING SHALLOW BOX ON THE QUALITY OF COCOA (*Theobroma cacao* L.) BEANS

by

KHAIRUL BARIAH SULAIMAN

Thesis submitted in fulfillment of the requirements
for the degree of
Doctor of Philosophy

August 2018

ACKNOWLEDGEMENT

Alhamdulillah, first of all, I would like to express my profound gratitude to Almighty Allah S. W. T. for giving me the capability and strength to complete this study and my shalawat to His righteous messenger, Prophet Muhammad S.A.W

I would like to take this opportunity to express my deepest appreciation and gratitude to my previous supervisor, Associate Professor Dr Tajul A. Yang, for constantly believing in me to finish this study. His invaluable guidance, constructive suggestions and encouraging advice throughout this study will forever leave a feeling of indebtedness that cannot be fully expressed. I am also very grateful to my current supervisor, Assoc. Prof. Dr Fazilah Ariffin for her constructive comments, and willingness to spend her valuable time towards the preparation of this thesis.

My sincere gratitude to the Ministry of Science, Technology and Environment of Malaysia for the financial support on this project through the ScienceFund grant (06-03-13-SF0115). I am also indebted to Malaysian Cocoa Board especially to Dr Sabariah Samsuddin and Dr Alias Awang for scholarship and laboratory facilities, respectively which without their help, the research work would not be possible. My deepest gratitude is also extended to my colleagues and all the staff of CRDC Bagan Datuk Perak especially to Primary Processing Unit, Mr Husin and Mdm Norasah for their continuous assistance to the success of this study. Acknowledgement is also conveyed to all my study-mates (Dr Wahidu, Intan, Hari and Akmal) and the staff of the School of Industrial Technology for their help and cooperation in many ways to complete my graduate study in USM.

Last but not the least, I also wish to express my deepest appreciation to my beloved husband, Cairil Nidzwan, who has always been there and never failed to show

his ardent love, sacrifices, patience, endless prayers and support. My beloved children (Khairunnisa Izzati, Khairil Rashid Alhafiz, Khairil Rafique Alhalim and Khairil Razique Alhazim), father, stepmother, mother-in-law, aunties, brothers and cousins for their understanding and constant support through the duration of my graduate study. Special memory and Alfatihah to my late mother, Hajjah Asiah Kasmat, who encourages me to pursue PhD and taught me the meaning of life.

KHAIRUL BARIAH

August 2018

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ha	Hectare
kg	Kilogram
NaOH	Sodium hydroxide
mg	Miligram
g	Gram
ml	Mililiter
µL	Microliter
HCl	Hydrogen chloride
TPTZ	2,4,6,-Tripyridyl-s-triazine

KESAN TEMPOH PENYIMPANAN BUAH KOKO DAN FERMENTASI MENGGUNAKAN KOTAK CETEK KE ATAS KUALITI BIJI KOKO (*Theobroma cacao* L.)

ABSTRAK

Fermentasi adalah penting untuk pembentukan prekursor warna dan perisa di dalam biji koko, namun dipengaruhi oleh pelbagai faktor termasuk amalan lepastuai. Oleh itu, kesan tempoh penyimpanan buah koko dan penapaian menggunakan kotak cetek pada biji koko Malaysia telah dikaji. Perubahan fizikokimia dalam biji koko basah dan kualiti biji koko kering yang dihasilkan telah dinilai menggunakan rekabentuk rawak lengkap 4 x 6 dengan tempoh penyimpanan buah (0, 2, 4 dan 6 hari) dan fermentasi (0, 24, 48, 72, 96 dan 120 jam) sebagai faktor utama. Pencirian biji koko kering yang dihasilkan dianalisa lebih lanjut kepada profil warna dan komponen meruap secara spektrofotometri, kromatografi cecair prestasi tinggi-pengesan gelombang berubah dan mikroektraksi fasa pepejal-kromatografi gas jisim spektrometri. Hasilnya menunjukkan bahawa penyimpanan buah koko selama empat dan enam hari mempengaruhi kebanyakan perubahan fizikokimia secara signifikan ($p < 0.05$) terutamanya suhu timbunan yang meningkat melebihi 42 °C dalam masa 24 jam fermentasi berbanding buah tanpa penyimpanan atau penyimpanan selama dua hari. Kualiti biji koko kering yang dihasilkan lebih baik dengan biji koko dari penyimpanan buah selama empat dan enam hari masing-masing mencapai skor peratusan setara coklat penuh 76.2% dan 68.9% yang menunjukkan tahap fermentasi baik seawal 24 jam berbanding 48 jam bagi buah tanpa penyimpanan (65.5%) dan penyimpanan selama dua hari (78.6%). Keasidan dalam biji koko kering dipertingkatkan dengan nilaian pH antara 5.09 -

5.53. Selain itu, asid lemak bebas (0.61 hingga 1.22%) di dalam biji koko kering yang dihasilkan adalah lebih rendah daripada 1.75% setara asid oleik iaitu had maksimum yang dibenarkan oleh Codex Alimentarius. Profil sensori bagi likur dari biji koko yang dikeringkan selepas 24 jam fermentasi dari penyimpanan buah selama empat dan enam hari mempunyai rasa koko yang paling kuat, agak pahit dan astringen dengan sedikit masam serta paling hampir dengan profil likur dari biji koko Ghana. Pencirian warna biji koko menemukan dua puncak spektrum penyerapan dan dikenal pasti sebagai asid klorogenik, asid vanila, katekin, asid kafeik, asid ferulik dan asid protokatekik. Manakala sejumlah 281 komponen meruap telah dikenal pasti dari biji koko kering yang dihasilkan dan telah didominasi oleh sebatian ester. Sebagai kesimpulan, penyimpanan buah selama empat dan enam hari sebelum fermentasi selama 24 jam menggunakan kotak cetek boleh digunakan untuk meningkatkan kualiti biji koko kering yang dihasilkan agar setanding dengan kualiti biji koko Ghana.

EFFECT OF POD STORAGE AND FERMENTATION DURATIONS USING SHALLOW BOX ON THE QUALITY OF COCOA (*Theobroma cacoa* L.) BEANS

ABSTRACT

Fermentation is important for the formation of flavour and colour precursor in the cocoa beans which are influenced by various factors including post-harvest practices. Therefore, the effect of pod storage and fermentation duration using a shallow box on the Malaysian cocoa beans was investigated. Physicochemical changes in wet cocoa beans and quality of the resulting dried cocoa beans were evaluated using a 4 x 6 complete randomized design with pod storage (0, 2, 4 and 6 days) and fermentation duration (0, 24, 48, 72, 96 and 120 hours) as the principal factors. The resulting dried cocoa beans were further characterized for profiles of colour and volatile compounds using the spectrophotometer, high-performance liquid chromatography-variable wavelength detector and solid-phase microextraction-gas chromatography-mass spectrometry. The results showed that the pod storage for four and six days were significantly ($p < 0.05$) influenced most of the physicochemical changes especially the mass temperature which had increased exceeding 42 °C within 24 hours of fermentation compared to without storage or pod storage for two days. The quality of dried cocoa beans produced significantly improved with the beans from the pod storage for four and six days had achieved respective scores of equivalent percent of fully brown 76.2% and 68.9% which indicated a well degree of fermentation as early as 24 hours compared to 48 hours from the pods without storage (65.5%) and storage for two (78.6%) days. The acidity in dried cocoa beans was enhanced with the pH between 5.09 - 5.53.

Besides, the free fatty acids (0.61 to 1.22%) was lower than the acceptable limit of 1.75% oleic acid equivalent allowed by Codex Alimentarius. Sensory profiles of liquor from the cocoa beans which dried after 24 hours of fermentation from the pod storage for four and six days had the strongest cocoa flavour, moderately bitter and astringent and slightly low in sourness as well as closest to the profile of liquor from the Ghanaian beans. Colour characterization revealed two spectrum peaks and identified as chlorogenic acid, vanillic acid, catechin, caffeic acid, ferulic acid and protocatechuic acid. While a total of 281 volatile compounds identified from the dried cocoa beans, they were dominated by the ester compounds. As conclusions, the pod storage for four and six days prior fermentation for 24 hours using shallow box can be used to enhance the quality of dried cocoa beans produced and comparable to quality of the Ghanaian cocoa beans.

CHAPTER 1

INTRODUCTION

1.1 General Background

Theobroma cacao L. is commonly known as cocoa and the seeds which are embedded inside the pod are the only source of raw material for the chocolate and cocoa-based industries (Thompson *et al*., 2013; Kim *et al*., 2011). Therefore, cocoa is recognized as one of the ten most active agricultural commodities being traded in the world (InvestorGuide, 2017; Fry, 2011). In Malaysia, cocoa is the fourth commodity which provides job opportunities for more than 31 thousand people including estate workers, smallholders, cocoa processors, manufacturers as well as chocolate entrepreneurs (Abdel Hameed and Arshad, 2014). The industry has contributed RM5.03 billion of the total value of Malaysia's major commodities export earnings in 2015 and has been increased to RM5.74 billion in 2016. The increment is driven by increasing exportation of cocoa-based products such cocoa butter and powder (Harnie, 2017; Anon, 2016).

Currently, Malaysia is the 8th largest cocoa grinder in the world, after the_Netherlands, Cote d'Ivoire, Indonesia, Germany, United States, Brazil, and Ghana (Harnie, 2017). Despite of the increasing demand of dried cocoa beans for grinding industry, the cultivation area and production of cocoa beans are declining. Since 1996, the smallholder sector has become the dominant players in cocoa cultivation with an average area of 94,716 ha as compared to 73,503 ha under estate sector. To date, the Malaysian cultivation area is about 18,122 ha with 95% (17,243 ha) share of smallholders (Anon, 2015). Changing of a dominant player in the cocoa cultivation has affected the production trend of

cocoa seeds. In which the estate usually produce as low as 500 kg cocoa seeds per harvesting day while smallholders only produced as high as 20 kg (Anon, 2015).

After harvesting, cocoa seeds have to undergo a series of processing stages starting from fermentation and drying, subsequently followed by roasting, before a unique chocolate flavour is fully developed. Among these processing stages, fermentation is the most crucial because any imperfections in flavour during this stage is irreversible and will affect the taste in final products. Any effort to improve the flavour in the next stage, especially roasting will only increase the cost of operation and will be very expensive (Hidayatullah *et al.,* 2016; De Vuyst and Weckx, 2016; Beckett, 2015b). Generally, cocoa fermentation practices vary considerably from one producing country to another and even from farm to farm within the same region. Approximately half of the world's cocoa industry is reported to ferment the seeds in boxes while remaining half fermented in heaps, trays or by using other primitive practices such as basket and bucket (Thompson *et al*., 2013).

Regardless of any fermentation practices have been applied, the process should be conducted properly as the high and consistent quality of dried cocoa beans is one of the main factors in determining the price. Normally, dried cocoa beans come in the form of batch to manufacturer and each of the batch will has some inconsistent qualities especially in flavour. The manufacturers have to put an extra budget to reduce the fluctuation of quality by blending or adding with other substances such as vanilla before the dried cocoa beans are further processed. If the cocoa grinders or chocolate manufacturers get the high and consistent quality of dried cocoa beans, their production costs will be lowered. Hence, the budget that has been saved can be used as an incentive to farmers by offering extra premium price from markets of dried fermented cocoa beans (Abdel Hameed *et al*., 2009).

The changes of the players background from estate owners to smallholders in Malaysian cocoa cultivation have not only affected the production trend of cocoa seeds but also the quality of dried cocoa beans. Survey on smallholders from all over the Malaysia has revealed that knowledge level on the importance of fermentation process is still lacking among smallholders. About 59% of the smallholders have been carried out an improper fermentation process by using various fermentation practices (Albert *et al*., 2015). Although the Malaysian Cocoa Board (MCB) has recommended the shallow box fermentation as standard practice since 1999, it is not widely used due to the loading capacity. The smallest shallow box needs at least 25 kg cocoa seeds, but the smallholders usually producing less than required capacity per harvesting day. The smallholders tend to conduct in small batches using primitive practices such as rattan basket, plastic bucket, sack, tray and heap (Hii and Bakri, 2002). Therefore, the cocoa beans which have been produced by smallholders were various in qualities and sold at a discounted price in the world market (Hii *et al*., 2004).

In order to ensure consistency in the quality of the Malaysian dried fermented cocoa beans, the usage of shallow box fermentation is still recommended as it is reported as a well-performed fermentation practice (Papalexandratou *et al*., 2013; Kelvin *et al*., 2013; Zaibunnisa, 2002). Modification on the shallow box such as moveable wall or partition is made to cater for smaller cocoa seeds capacity and at the same time ensure that the depth of fermenting mass still 30 cm. The 30 cm depth of fermenting mass is important as Shamsudin *et al*., (1978) reported that it ensure enough heat generated during fermentation process. The smallholders are also suggested to do pod storage, a practice of storing the cocoa pods for maximum 10 days before fermentation process. The practice is not only helping the farmers to

obtain enough cocoa seeds but also can enhance the flavour (Afoakwa *et al*., 2011a; Nazaruddin *et al*., 2006). However, all the modification and suggestion are failed to give consistent quality and often faced the problem of over fermented seeds. On the other hand, the combination of pod storage and shallow box technique is time consuming because the process requires ten days of cocoa storage before five days of fermentation and takes additional three to seven days to allow the cocoa beans completely dry. In sum, the following problems are the issues discussed in order to improvee the quality of Malaysian dried fermented cocoa beans.

1.2 Problem Statements

i. The flavour of Malaysian cocoa beans is inconsistent and often regards as excessive acidic flavour, weak cocoa flavour and also with certain undesirable flavour such as mouldy and hammy. Thus it has been sold at RM5800 to RM 7150/tonne compared to RM10000/tonne of the world market (Harnie, 2017). Cocoa processors normally blend it with higher quality beans; hence increase their processing cost.

ii. The previous researcher Said *et al*., (1988), has implemented pod storage of ten days prior the fermentation process. However, the implementation of pod storage for ten days prior fermentation is failed to produce consistent good quality beans. This is because the smallholders are not aware of the correct time to terminate the fermentation process. Normally, they just follow the previous five days recommended duration by Malaysian Cocoa Board and faced the over fermentation problem, where the over-fermented cocoa beans are manifested by blackish with mouldy and hammy flavour.

iii. The quality of cocoa beans produce by smallholders is inconsistent due to the duration of the fermentation is standardized to five day regardless the implementation of pod storage or not. The fermentation duration of five days is shortened from six days duration which is adapted from Ghana and implemented without conducting proper optimization. Therefore, the observations for the best duration of pod storage as well as the fermentation process are needed.

1.3 Objective

The main objective of this study was to determine the effects of the duration of pod storage and fermentation process using the shallow box technique on the quality of Malaysian cocoa beans. The objectives are specifically divided as follows;

i. To evaluate the effects of pod storage duration during fermentation using a shallow box on the physicochemical changes of wet cocoa beans.

ii. To assess the effects of pod storage and fermentation duration using a shallow box on the quality of Malaysian dried cocoa beans and compare its sensory profiles to the Ghanaian dried cocoa beans profiles.

iii. To characterize the colour of the dried cocoa beans as affected by pod storage and fermentation using a shallow box.

iv. To characterize the volatile flavour of the dried cocoa beans as affected by pod storage and fermentation using a shallow box.

1.4 Hypothesis

In this study, it is hypothesized that pod storage will reduce the remarkable amount of pulp sugars. The reduction will cause a rapid increase in temperature as well as decreases the formation of acid. At the same time, the usage of the shallow box will provide sufficient heat which helps to enhance the enzymatic reaction and significantly promote faster fermentation. Termination of the fermentation process at the correct time will maximize colour and flavour precursors, hence significantly improve the colour and flavour quality of produced dried cocoa beans.

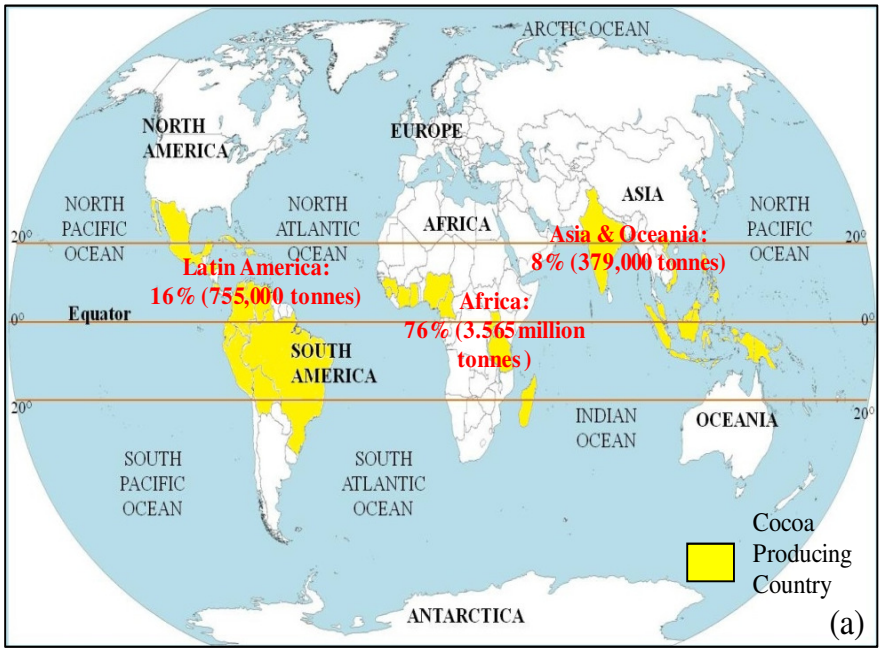
CHAPTER 2

LITERATURE REVIEW

2.1 *Theobroma cacao* L.

Theobroma cacao L.*,* commonly known as cocoa, is a perennial tree that formerly classified in the family of Sterculiaceae; but later reclassified into the Malvaceae family. The tree is widely cultivated in a range between 20° north and 20° south of the equator (Figure 2.1a) with three main growing regions, which are West Africa, South-East Asia and South America (Lopes and Pires, 2014; Colombo *et al*., 2012; Bernaert *et al*., 2012). Côte d'Ivoire is on the top among the 15 cocoa producing countries with estimated 1581 thousand tonnes of cocoa beans have been produced for 2015/16 crop year (Figure 2.1b) and Malaysia has been in the rank of 23 with production of 1.757 thousand tonnes of cocoa beans (Harnie, 2017). The cocoa tree needs general climatic conditions such as yearly rainfall distribution of 1250 - 3000 mm, preferably between 1,500 - 2000 mm. The minimum means of temperature is in between 19 to 21 °C and maximum temperature 30 to 32 °C with no persistent strong winds as it may damage the plants. In addition, the dry season should be not exceeding three consecutive months with not less than 100 mm rain per month. Besides climatic conditions, the cocoa tree also needs suitable soil properties such as more than 100 mm depth of soil as well as good drainage and water holding capabilities to grow well (Ahmad Kamil *et al*., 2013).

The cocoa tree can be propagated by two methods, either via seedling or vegetative methods such as cuttings, grafting, and marcotting. The growth of cocoa seedling which known as hybrid differs from the vegetative propagation, where it has one main stem grows vertically upwards until it reaches a certain height



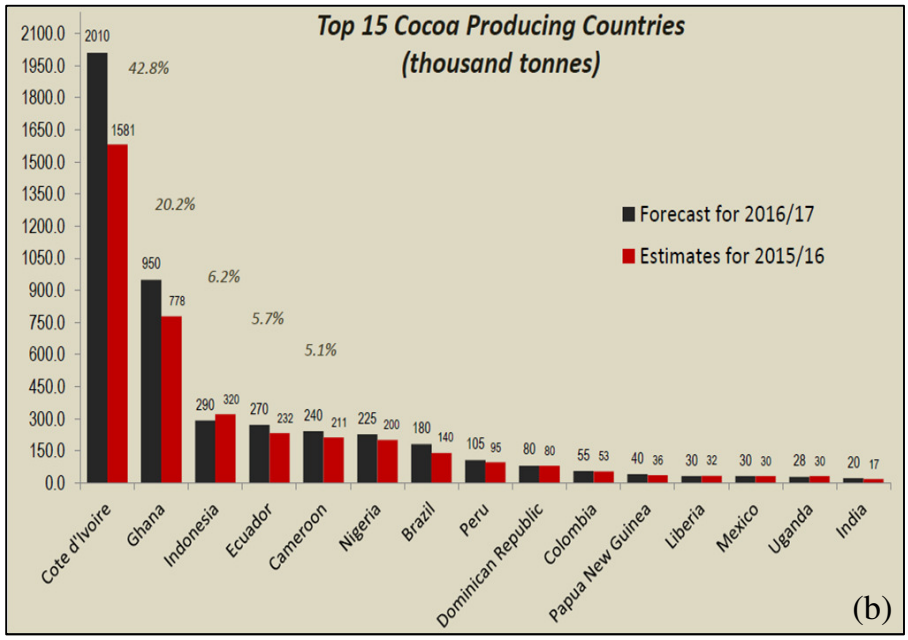


Figure 2.1: World cocoa production. (a) Cocoa cultivation regions and (b) Top 15 cocoa producing countries. (Source: Adapted from cocoanibs.wordpress.com and ICCO, 2017). Denotes the cocoa producing country in the world.

before forming 'jorquette' (Figure 2.2). On top of the 'jorquette' will protrude three to five lateral branching commonly known as fan branches. In contrast, the cocoa tree from vegetative propagation or familiar as clone has only fan branches. On top of the tree morphology, the pod and bean characteristics of the clone are more uniform compared to the hybrid. In addition, certain clones can bear fruit throughout the year and are not depend only on the two peak seasons. Thus, these will allow the farmers to have a stable source of income and due to that, grafting is the highly recommended breeding technique for cocoa in Malaysia (Ahmad Kamil *et al*., 2013; Toxopeus, 2008; Francis *et al*., 2005).

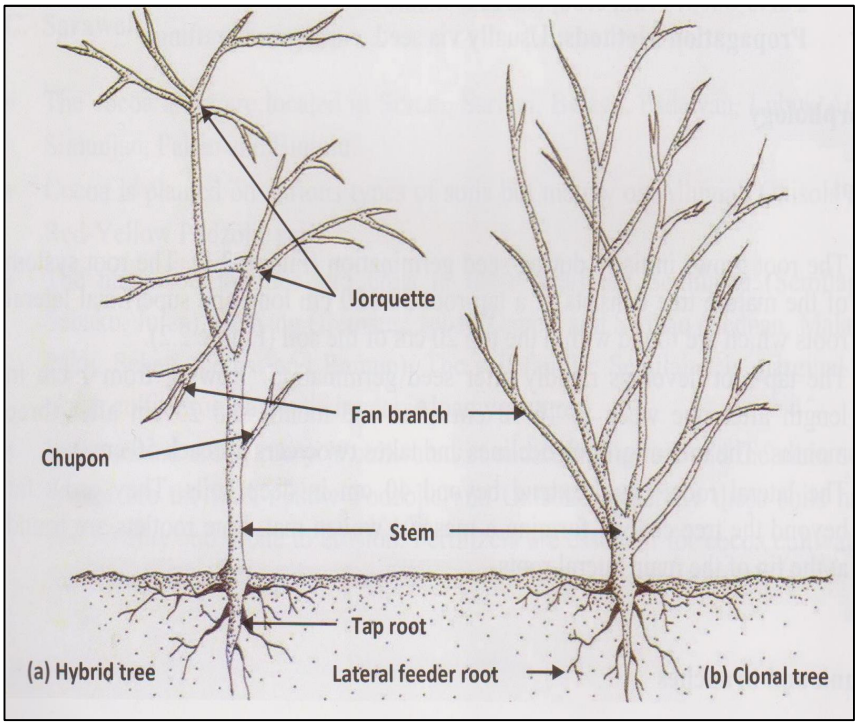


Figure 2.2: Schematic of the cocoa tree. (a) hybrid and (b) clonal (Source: Ahmad Kamil *et al*., 2013).

Depending on the propagation method, the tree will start bearing a drupe fruit after 18 - 30 months being planted in the field and remain productive for several decades. The fruit, also known as cocoa pod, develops in clusters from pollinated flowers which arise directly on the auxiliary bud of main stem as well as branches of the tree (Figure 2.3). The pod will take approximately four to six months to ripen and may differ in terms of size, shape, and colour depending on the variety. The surface colour of pod husk which can generally be either green or red when immature, will turn to yellow or orange (Figure 2.4) upon ripening. During harvesting, the pod should be cut on stalk closely to husk using sharp knives, secateurs or machetes to avoid damage to the flower cushions. Damaging the flower cushions or leaving the pod rotten on the tree will reduce the future yields. Breaking the pod will expose about 20–50 of seeds (refer to the fresh and unfermented seeds) which surrounded by a mucilaginous pulp and embedded to a placenta (Zhang and Motilal, 2016; Ahmad Kamil *et al*., 2013, Thompson *et al*., 2013).

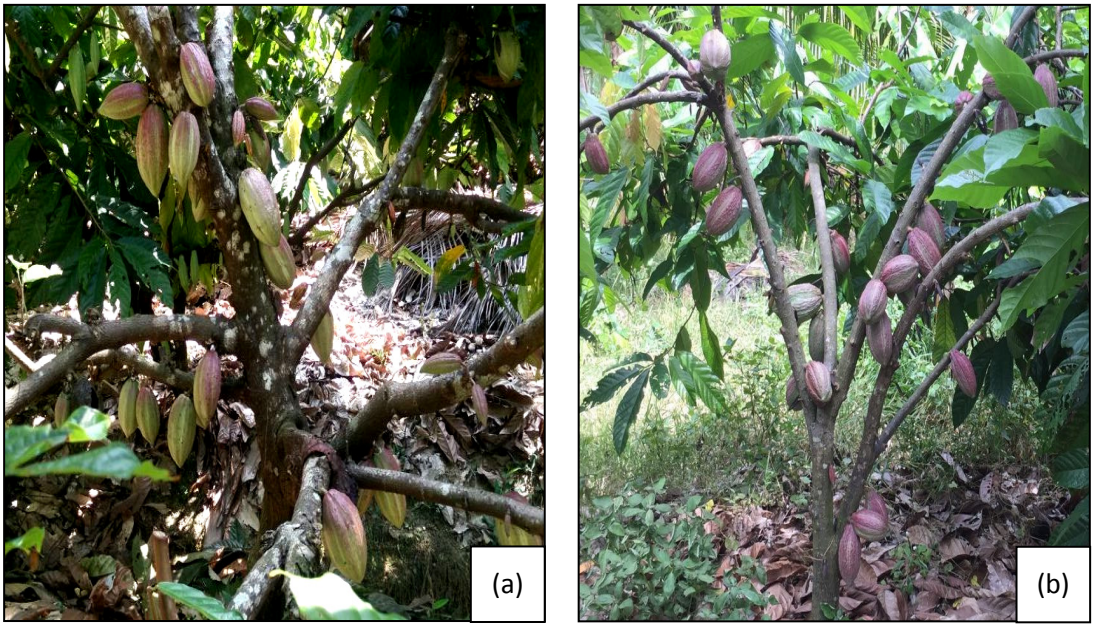


Figure 2.3. Cocoa pods developed in clusters on the main stem and branches of the tree. (Source: Malaysian Cocoa Board gallery (2016), reprinted with permission.)

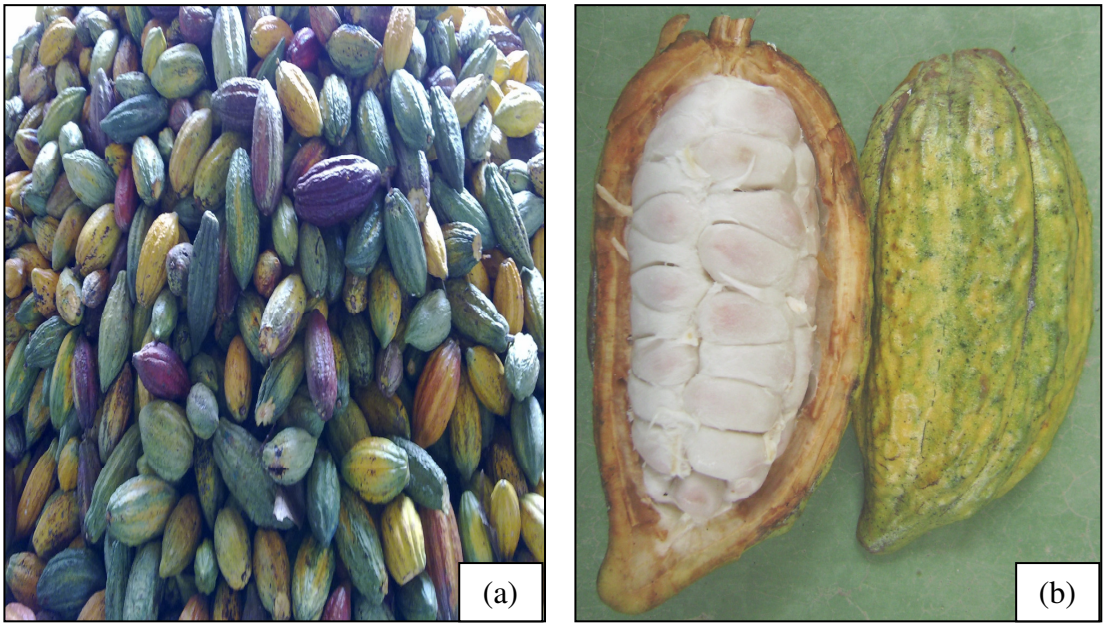


Figure 2.4. Cocoa pods. (a) Variation of colour and size of cocoa pods (b) Cocoa seeds inside the pod husk. (Source: Personal collection).

2.1.1 Cocoa Seed

Cocoa seed is a fresh bean that serves as the primary source of raw material for the chocolate and cocoa-based industries. The seed structure encompassed of three parts that are mucilaginous pulp, seed coat and cotyledon (Figure 2.5). A white and sweet mucilaginous pulp is the outermost part that surrounding the seed. In which, the pulp or external fermentation of cocoa beans takes place and a decisive factor in the outcome of the fermentation step (Nielsen, 2006). Inside of the seed are the cotyledons as the main part, which consists of two convoluted cotyledons and attached together with small germ or embryo. Seed coat, namely as testa is located between cotyledon and pulp, serves as a barrier to control the diffusion of molecules either going inside or outside of the cotyledon by allowing only smaller molecules such ethanol and acetic acid to diffuse into the cotyledons (Lopes and Pires, 2014; Ahmad Kamil *et al*., 2013; Toxopeus, 2008).

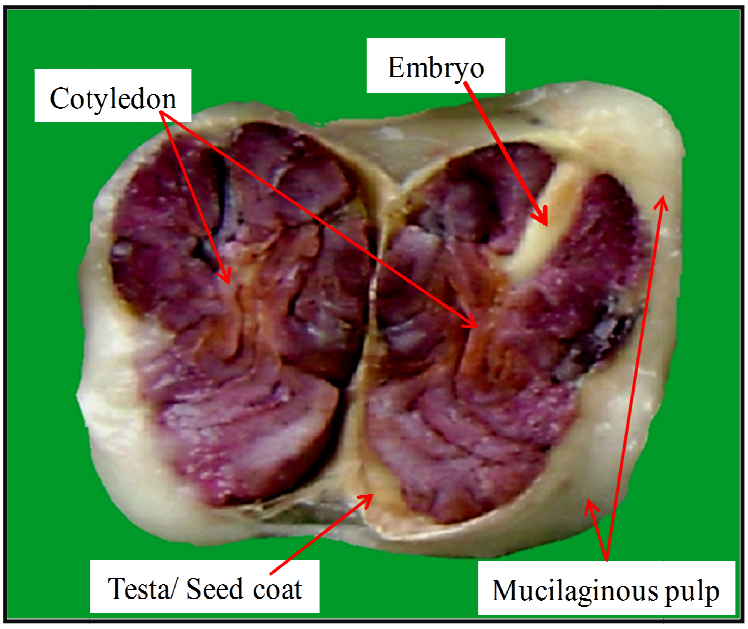


Figure 2.5. Structure of the cocoa seed. (Source: Personal collection)

2.1.2 Classification of Cocoa Seed

Cocoa seed is traditionally classified into three well-known varieties namely Forastero, Criollo, and Trinitario. The classification is based on geographical origins and morphological traits such as colour as well as flavour (Trognitz *et al*., 2013; Motamayor *et al*, 2008; Bartley 2005). Forastero is originated from the Amazon region and caters for more than 95% of the world's cocoa supply (Kongor *et al*., 2016; Saltini *et al*., 2013; Lima *et al*., 2011). The forastero tree variety is more vigorous and resistant to pests and diseases as compared to others (Lopes and Pires, 2014; Rusconi and Conti, 2010). Cross-section of the seeds display that the surface of cotyledon are in deep purple (Figure 2.6a) and upon proper processing will produce strong inherent flavour, incline to be somewhat bitter and usually dark brown in colour (Beckett, 2015a).

On the other hand, Criollo is native to Central and South America as well as the Caribbean Islands and Sri Lanka. It accounts for only 5% of the world's

production (Beckett, 2015a; Jahurul *et al*., 2013). Criollo seeds are white, ivory or very pale purple in colour (Figure 2.6b) and have more aromatic or smoother tastes but milder cocoa flavour. The Criollo seed tends to be bigger and rounder as well as having lower fat content as compared to Forastero (Beckett, 2015a; Jahurul *et al*., 2013). Whereas, Trinitario is reported as the result from hybridization of Forastero and Criollo, which colour characteristic has reflected the variation of Forastero and Criollo (Figure 2.6c) and noted for its fine flavour (Giacometti *et al*., 2015; Toxopeus, 2008). Besides the three well-known groups, there is another group known as Nacional which has been acknowledged for its raisins fruit-flavoured ('Arriba') and only grown in Equador (Giacometti *et al*., 2015; Counet *et al*., 2004). However, recently Motamayor *et al*., (2008) has proposed to classify the cocoa tree into ten major groups based on genetic differentiation using simple sequence repeat (SSR) analysis: Maran˜on, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Puru´s, Nacional and Guiana.

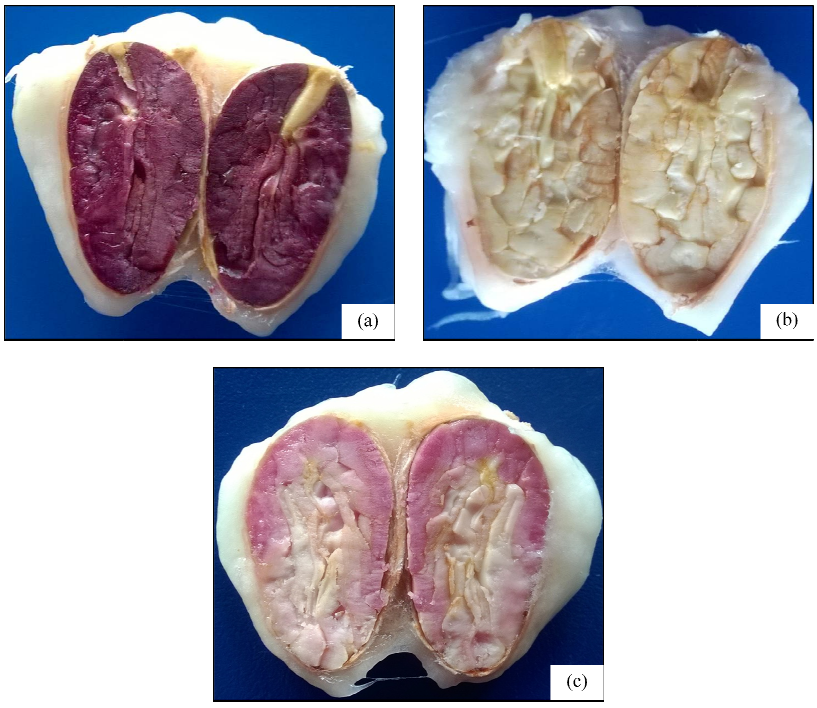


Figure 2.6: Cross-section of cocoa seeds. (a) Forastero; (b) Criollo and (c) Trinitario. (Source: Personal collection)

2.1.3 Composition of Cocoa Seed

In general, the composition of the cocoa seed is shown in Table 2.1. The cocoa pulp is comprised of spongy parenchyma cells which have wall structure that supported by 1 - 2% pectin (Afoakwa *et al*., 2013b). The total sugar content including pentosans is approximately 10 - 15%, with the main sugar of the fresh pulp are glucose (5.4 - 6.6%), fructose (6.3 - 7.4%) as well as small amounts of sucrose (less than 0.3%). The ratio of glucose/fructose to sucrose change with the degree of maturity where the pulp in immature cocoa pods consist of a higher proportion of sucrose, while the pulp in ripe cocoa pods containing mainly fructose and glucose. The pH is relatively low (3.0 - 4.0) mainly due to the content of 0.5 – 3% citric acid (Aprotosoaie *et al*., 2016; Afoakwa, 2010).

Table 2.1: The composition of cocoa seed.

Component	Composition
Pulp	
- Water	80 - 87%
- Sugars (glucose, fructose, sucrose)	10 - 13%
- Pentosans	2 - 3%
- Citric acid	0.5 - 3%
- Salts	8 - 10%
Cotyledon	
- Water	32 - 39%
- Cellulose	2 - 3%
- Starch	4 - 6%
- Pentosans	4 - 6%
- Sucrose	2 - 3%
- Fat	49.9 - 55.2%
- Proteins	8 - 10%
- Theobromine	2 - 3%
- Caffeine	1%
- Acids	1%
- Polyphenols	5 - 6%

The cocoa cotyledons are composed of two types of parenchyma storage cells, known as lipid/protein cells and polyphenolic cells. The polyphenolic cells

which are dark-stained in Figure 2.7 are separated from lipid/protein cells via a grid forming by plasma (Guilherme *et al*., 2016; Martini *et al*., 2008). The lipid/protein cells contain a large number of lipid globules, starch granules as well as protein bodies which are embedded in the cytoplasm. Other organelles such as nuclei and mitochondria are also squeezed together among the vacuoles. The polyphenolic cells are larger cells with a large vacuole and contain almost all of the seed's polyphenolic material as well as alkaloids (Voigt and Lieberei, 2014; Martini *et al*., 2008; de Brito *et al*., 2001).

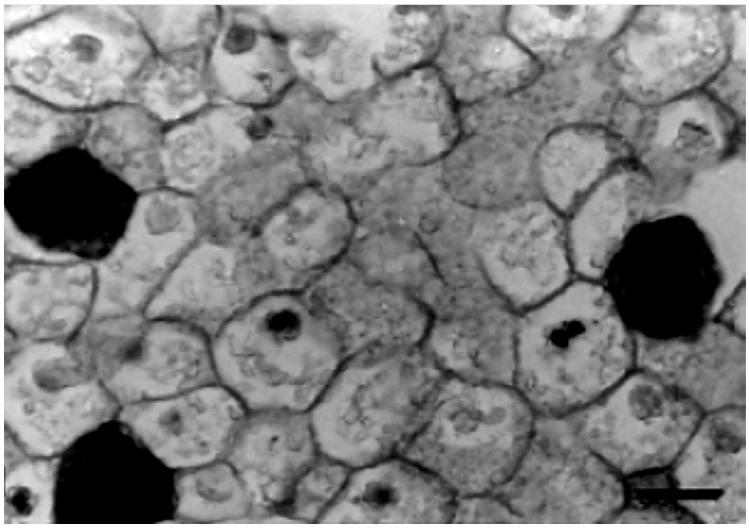


Figure 2.7: Lengthwise cut section of cocoa seed under light microscopy. (Source: de Brito *et al*., 2001). The dark-stained cells are polyphenolic cells.

Although lipid/protein cells are smaller than polyphenolic cells, lipid content constitutes about 49.9% - 55.2%, while protein composes 17.5% - 21.6% of the fresh cotyledons (Afoakwa *et al*., 2013b; Elwers *et al*., 2010; Belitz *et al*., 2009). The protein storage in the cotyledons is classified based on their solubility into four groups albumin, globulin, glutelin and prolamin (Bertazzo *et al*., 2011). The albumin is abundantly present but do not contribute in producing specific cocoa flavour precursor. Only globulin which later known as vicilin (7S)-class globulin, is found to be significantly degraded during fermentation to produce

hydrophilic polypeptide and free hydrophobic amino acids: specific cocoa flavour precursors (Guilherme *et al*., 2016; Voigt and Lieberei, 2014; Marseglia *et al*., 2014; Kratzer *et al*., 2009; Amin *et al*., 2003; Amin *et al*., 2002).

The polyphenolic cells make up about 12 to 20% of dry weight beans, containing with polyphenols and alkaloids including caffeine, theobromine and theophylline (Aprotosoaie *et al*., 2016; Voigt and Lieberei, 2014). The polyphenols are comprised of many classes of compounds including flavonoid. The flavonoid is the predominant class in cocoa and further subdivided into three groups which are proanthocyanidins (58%), flavanols (37%) and anthocyanins (4%). The flavanols can occur both as monomers of epicatechin and catechin or polymerized flavanols or procyanidins (Kongor *et al*., 2016; Bordiga *et al*., 2015; Voigt and Lieberei, 2014; Ackar *et al*., 2013; Hurst *et al*., 2011; Jalil and Amin, 2008). The anthocyanins content is responsible for the fresh cotyledon colour which is being used as an indicator for the degree of fermentation. However, anthocyanins have not been detected in the criollo type beans (Amoa-Awua, 2014; Wollgast and Anklam, 2000; Hansen *et al*., 2000). The remaining content of the cotyledon are 4 - 6% starch, 2 - 3% cellulose and 1% acids (Voigt and Lieberei, 2014; Nielsen, 2006; Goto *et al*., 2002; Bucheli *et al*., 2001).

2.2 Cocoa Processing

Cocoa seeds which are directly dried under the sun are usually lack of chocolate flavour and aroma characteristic. The seeds must go through several stages of processing in order to obtain the desired flavour and colour before being served at the table (Aprotosoaie *et al*., 2016; Aculey *et al*., 2010). The process can be classified into three stages, namely primary, secondary and product development.

The primary stage is carried out by cocoa farmers at farm involving a series of the process after harvesting including fermentation and drying in order to produce fermented and dried cocoa beans (Fowler, 2017; Kongor *et al*., 2016). Some farmers in certain countries such as Ghana and Malaysia are practising pod storage prior fermentation either to ensure enough seeds to ferment or to enhance the quality of the dried cocoa beans.

The dried fermented cocoa beans are subsequently been processed into the semi-finished cocoa products such as cocoa butter, cake, powder as well as liquor at the secondary stage by industry through roasting, winnowing, grinding and pressing. Subsequently after that, the semi-finished product will be further transformed at the product development stage into cosmetics, beverages, confectionery, and chocolate for consumer usage (Thompson *et al*., 2013; Schwan and Wheals, 2004). A flow-chart summarizing the cocoa processing is given in Figure 2.8 (Beckett, 2015b; ICCO, 2014).

2.2.1 Primary Processing

In primary processing, the cocoa seeds will undergo either enzymatic or non-enzymatic transformation to produce flavour precursors in fermented dried cocoa beans. The practices may vary according to the size of the farm and pod yield but the aim is still same, towards high-quality fermented dried cocoa beans (Ahmad Kamil *et al*., 2013; Amanquah, 2013).

2.2.1(a) Pod breaking and seeds preparation

After been harvested, the pods must be broken to prepare the cocoa seeds for fermentation. The preparation can be carried out either at the farm or other places

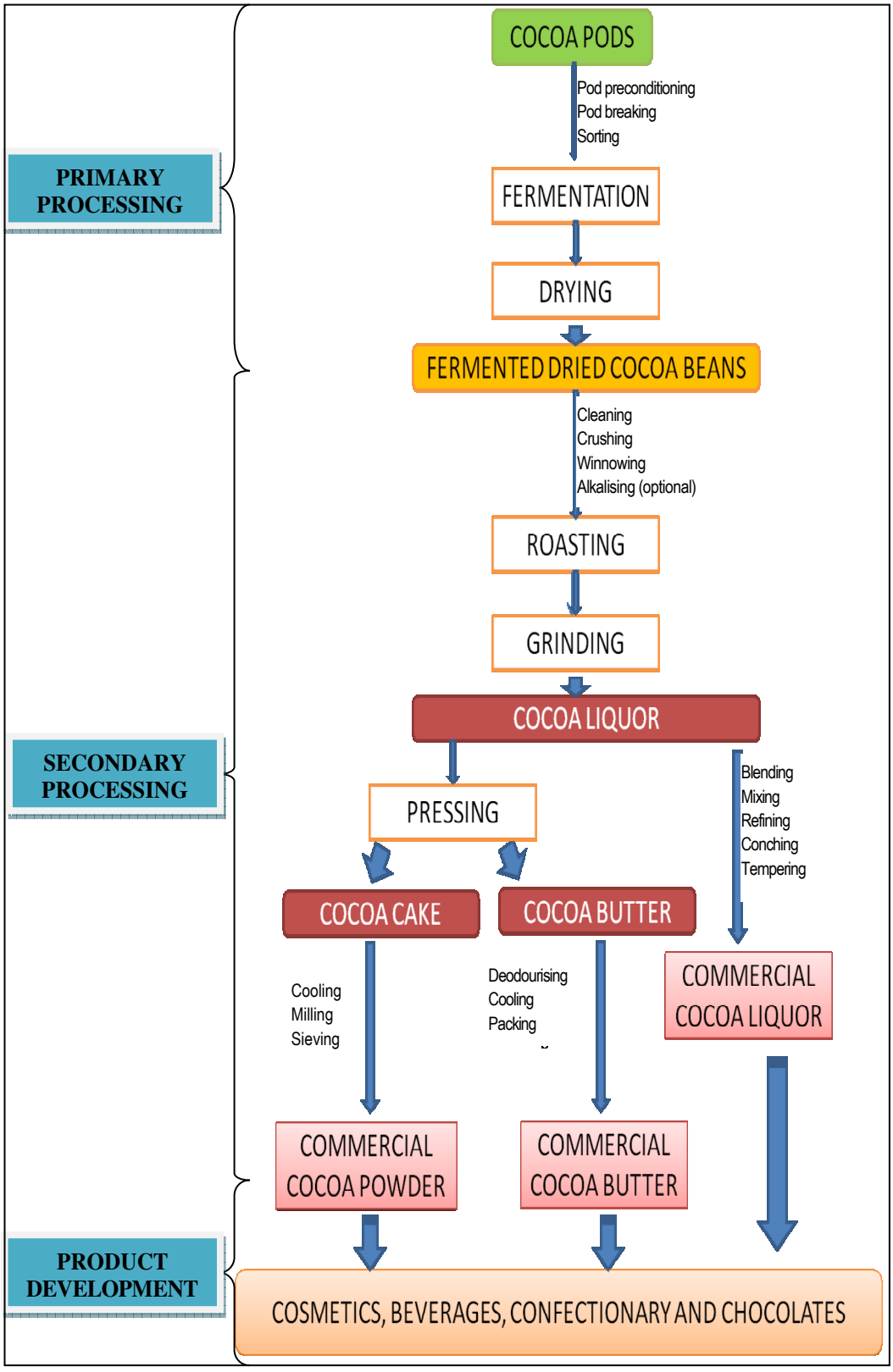


Figure 2.8: Flow-chart summary of the cocoa processing (Adapted from Beckett 2015b; ICCO, 2014).

as long as it is near to the processing centre, where fermentation will take place (Lee *et al*., 2014; Ahmad Kamil *et al*., 2013). The commonest practice for pod breaking is by manually hitting them together. However, the suggested practice is to use a special knife, which has a 'block' that will prevent the seeds from getting cut (Figure 2.9a). Manual pod breaking is considered a time-consuming and labour intensive process. Hence, applying the mechanical cocoa pod breaker or COBRE™ (Figure 2.9b) is most recommended as extracted seeds should be fermented in the same day after breaking.

The fermentation should be carried out as soon as the seeds have been removed and sorted from placenta or husk fragment. Upon completing the sorting, the seeds will be placed into a container and ready to ferment. Yet, if the process is being carried out in the field, only ready to ferment seeds will be taken to the processing centre, while the husks and pods that have been infested by diseases and insects will be left in the field. This practice is not encouraged because it will spread the diseases and insects at the farm. In contrast, centralized pod breaking at other places near the processing centre will help to control disease and insects spread.

2.2.1(b) Fermentation

Fermentation is identified as a crucial process because involving the enzymatic reaction on carbohydrate, protein, fat and polyphenols. As a result, the cocoa-specific flavour precursors such as reducing sugars, peptides, amino acids (Voigt and Lieberei, 2014, Romero-Cortes, *et al*., 2013, Khairul Bariah, 2010) as well as key volatile fractions such as alcohols, esters and fatty acids (Aprotosoaie *et al*., 2016; Rodriguez-Campos *et al*., 2012) are formed at this stage. Besides developing the precursors of cocoa flavour and aroma, fermentation also prevents cocoa seeds from germinate and helps to remove the mucilaginous pulp in

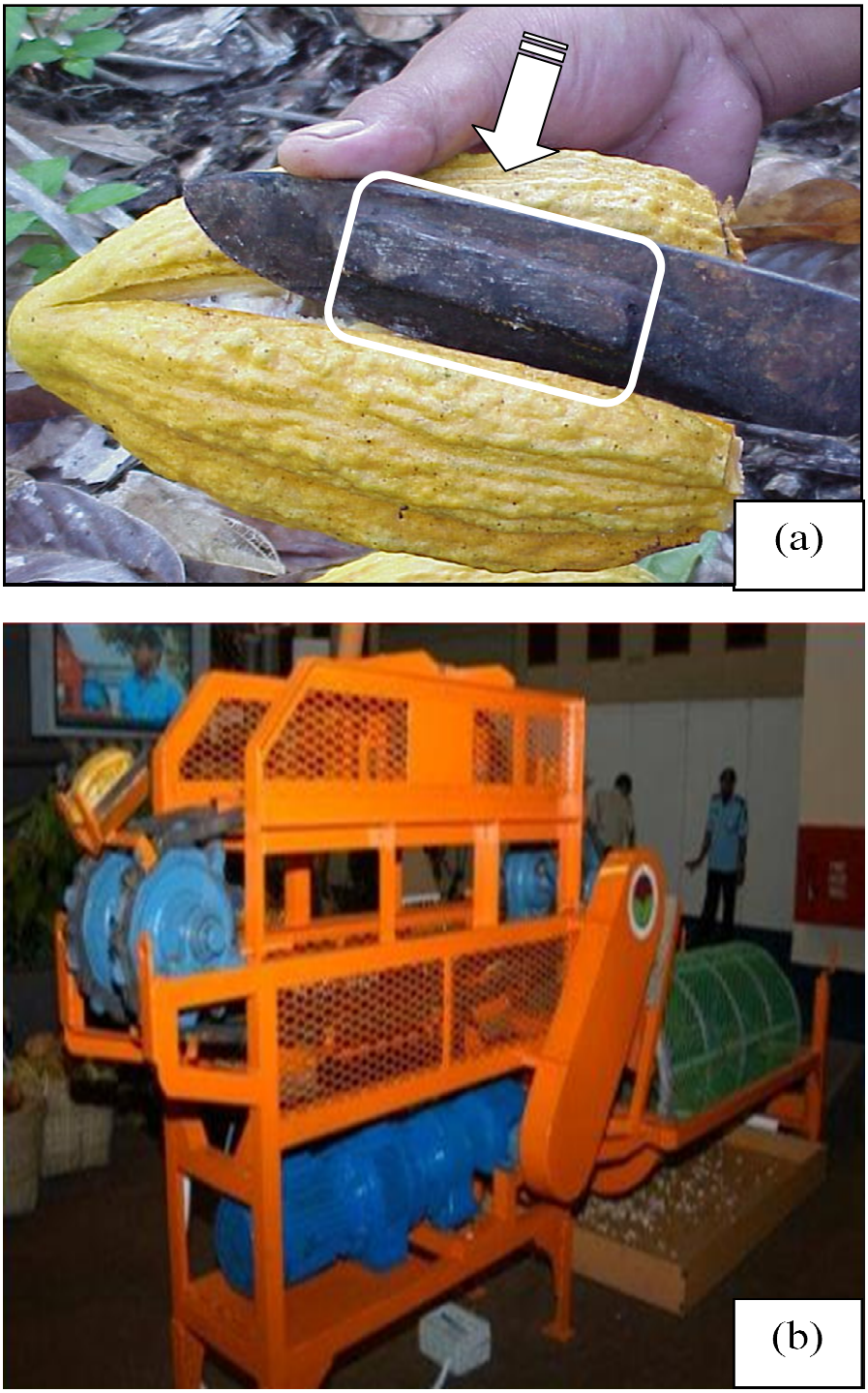


Figure 2.9: Pod breaking practice. (a) Manually using special knife and (b) mechanical using cocoa pod breaker COBRE™. (Source: (a) Personal collection and (b) Malaysian Cocoa Board gallery (2008), reprinted with permission.)

order to facilitate drying. Furthermore, the unfermented cocoa seeds do not produce a good cocoa flavour during roasting (Voigt *et al*., 2016; Schwan and Wheals, 2004).

Fermentation begins as soon as the cocoa seeds are exposed to the environment and microbial is started to grow on the pulp and leads to the production of alcohol and organic acids with concomitant of temperature increment (Schwan *et al*., 2014). Currently, the succession of microbial during fermentation is well documented involving enterobacteria, indigenous yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), bacilli and filamentous fungi (De Vuyst and Weckx, 2016; Illeghems *et al*., 2015; Ho *et al*., 2014). Cocoa fermentation requires a sufficient depth of fermenting mass to ensure enough heat is generated during the process and it depends on which technique has being applied. Besides, fermenting mass should be covered properly to prevent heat from released to the environment and the container must have sufficient perforation for good drainage during sweating. In addition, the fermenting mass should be turned at least once throughout the duration of the fermentation process to allow good aeration and ensure beans are mixed uniformly (Amoa-Awua, 2014; Ahmad Kamil *et al*., 2013).

2.2.1(c) Drying

Drying is a continuation process which carried out as soon as fermentation is completed. Completion of the process is manifested by decreasing temperature of the fermenting mass but there is no exact timer to stop the process. Usually, the process is terminated based on the farmer experience (Saltini *et al*., 2013; Nielsen, 2006). The aims of drying process are to reduce the moisture content of the wet cocoa beans from 40-60% to only 7.5%. This will ensure a good storage condition for dried cocoa beans and prevent the growth of moulds. The low moisture

content can deactivate the endogenous enzymes and prevent the over-fermented or off-flavour beans due to excessive proteolysis reaction. Besides, drying also ensures that colour development in cocoa beans takes place, changing the reddish colour to fully brown. The changes of colour are reported to correlate with reduction of the bitter and astringent taste of dried cocoa beans (Saltini *et al*., 2013; Thompson *et al*., 2013; Zahouli *et al*., 2010).

Normally, the drying process is performed naturally under the sun by spreading the beans on the appropriate surface, preferably on an elevated platform. The process will take about three to seven days depending on the weather as well as the thickness of cocoa beans layer. The cocoa bean layer is limited to ‘one bean thickness' or 5 cm especially on the first day for optimum penetration of sunlight during drying. It there is some mistake in this process, it will result in blackish beans due to the moulds. The cocoa beans need to be turned periodically (every two to three hours) to ensure all the beans are evenly warmed. Upon drying, cocoa beans are physical inspected either sufficiently dried or not by grabbing a handful of the beans and rubbed each other. The cocoa beans are sufficiently dried if crackle sound is produced. Moreover, artificial drying which using oil or solid fuels as a source of power may be resorted in rainy periods (Lee *et al*., 2014; Ahmad Kamil *et al*., 2013; Musa, 2012; Hii *et al*., 2012; Hii *et al*., 2009).

2.2.2 Secondary Processing

At the secondary stage, the fermented dried cocoa beans will be converted into semi-finished product. Traditionally, the fermented dried cocoa beans are processed by the chocolate manufacturer which normally located in a temperate climate country. But nowadays, the cocoa growing countries are preferred to produce

cocoa liquor from their own beans. Regardless where the process is carried out, the principle of cocoa bean processing in this stage has not changed for more than 150 years, where the beans are still cleaned, de-shelled, roasted, and sometimes alkalized. Subsequently, the beans will be ground into cocoa liquor before following two distinctive processing lines. Approximately 65% of the cocoa liquor is reported to be pressed into butter and cake before the cake will finally be pulverized into powder. Whereas, the remaining 35% is processed into commercial cocoa liquor which for directly used by the manufacture of chocolate (Beckett, 2015b; De Zaan, 2009).

2.2.2(a) Beans cleaning

Cleaning step is performed to ensure various types of foreign material which may be left or mixed up during primary processing are removed. The foreign materials; especially plant debris should be removed because it may release unpleasant gasses during roasting which would be spoiled the cocoa aroma. Hard materials such as sand, stones and metals may damage the machinery, especially during grinding. The procedure starts with the fermented dried cocoa beans will be move through a coarse and fine sieve equipped with a vibratory to remove sand and stone while segregating the beans according to similar size within each batch. At the same time, a strong flow of air will act as suction which will draw off the dust, leaves and fibres, whereas iron and other metals will be removed by a magnet. Finally, the cleaned beans will go on to the next stage of processing (Beckett, 2015b; De Zaan, 2009). A few cocoa manufacturers adopt bean blending technique before cleaning procedure to minimize fluctuating characteristics of cocoa flavour.

2.2.2(b) Roasting

Fermented dried cocoa beans are reported to have a bitter, acidic, astringent and musty but rich with flavour and aroma precursors. Roasting will transform the fermentation products such as free amino acids, oligopeptides and reducing sugars via Maillard reactions into full characteristics of chocolate flavours as well as browning of the cocoa seeds (Sacchetti *et al*., 2015; Jinap, 2004). Unlike reactions during fermentation or drying, the Maillard reaction that is occurred during the roasting process is non-enzymatic and driven by thermal treatment as high as 150 °C. Therefore, roasting will virtually sterilize the beans from an excess of bacteria, fungi and moulds involved in fermentation that passed through drying (Beckett, 2015b; Nazaruddin *et al*., 2006). In addition, roasting will further reduce the moisture content from about 7.5% to the ranged between 1.5 - 3% and also volatile acids such as acetic acid by evaporation (Beckett, 2015b).

Nowadays, there are three different kinds of roasting techniques for different purposes or products, namely whole bean, nib and liquor roasting (Ziegleder, 2009). The traditional roasting of the whole bean helps separate the seed coats (shell) from the cotyledons and makes cracking and winnowing much easier. However, the method will result in melting of the cocoa butter and subsequently migrates into the shell. When the shell is removed, up to 0.5% of cocoa butter is estimated to lost. Another disadvantage of whole bean roasting is that there is always a range of different sized beans involved. This will lead to a heterogeneous level of roasting treatment and may result in poorer flavour as the larger beans being undercooked while the smaller beans are being over-roasted (Beckett, 2015b; Owusu *et al*., 2013). Alternatively, some chocolate manufacturers practise nib or cocoa liquor roasting where the beans are de-shelled, crushed and winnowed before roasting. The difference between liquor

roasting and nib roasting is where the nib will be further ground into a paste then liquor after winnowed. The advantages of practising nib or liquor roasting are reported as a more uniform distribution of heat, rapid evaporation of water from the nib and increase in output for the same amount of energy input (Beckett, 2015b; Ziegleder, 2009).

2.2.2(c) Grinding

After roasting, winnowing which is the process to remove shells from the nib takes place. After that, the fermented, dried and roasted cocoa nibs are ground into cocoa liquor. The main objective of grinding is to make the cocoa particles small enough so that as much fat as possible is removed from the cells within the cotyledons. An additional reason is to make the nib readily for chocolate making process without necessary to further mill the nib (Beckett, 2015b). Later, the cocoa liquor will be pressed by hydraulic press into cocoa cake to extract cocoa butter. The cake is then pulverized into fine cocoa powder (Afoakwa, 2014).

2.2.3 Cocoa-Based Product Development

Cocoa is synonymous with chocolate but there is a wide range of product that can be developed from cocoa either by using husk, shell, powder, pulp or butter. In food and beverage, by-products of liquefied pulp such as gin, brandy, vinegar, wine, jam and pectin are developed as an extra income (Cudjoe *et al*., 2009). The cocoa pulp is processed into juice for refreshing drinks and ice cream (Chin, 2016). In cosmetic and personal care products such as toothpaste, mouth rinse, foundation, anti-wrinkle cream, scented perfume, lip balm, lipstick and soap from cocoa-based has been

developed (Azila *et al*., 2016; Akoto *et al*., 2015; Norliza, 2010; Azila and Nur Azilah, 2012; Yap and Aminah, 2011).

2.3 Biochemical Changes During Fermentation

The biochemical reaction of cocoa fermentation can be divided into two stages, namely as pulp and cotyledons fermentation.

2.3.1 Pulp Fermentation

The pulp fermentation is also known as external fermentation which involving activity of microorganisms on the pulp. Before the cocoa seeds are exposed to the environment, the pulp has a relatively low pH (3.0 - 4.0) as well as low oxygen tension that mainly due to the content of 0.5 - 2% citric acid and thickness of the pulp. This condition as well as high sugar content including pectin and saccharides, provide an excellent medium for growth of microorganisms which exist from the environment through either soil, air, dust, banana and plantain leaves as well as gunny used to cover fermenting mass, the utensils and equipment used, the fruit fly, workers hands or husk (De Vuyst and Weckx, 2016; Teng-Sing *et al*., 2016; Hamdouche *et al*., 2015: Crafack *et al*., 2013; Meersman *et al*., 2013). The yeast population flourishes the flora on the pulp for about first 24 to 48 hours of fermentation. During this phase, a typical alcoholic fermentation occurs with the yeasts metabolizing pulp sugars and citric acid to ethanol, carbon dioxide, glycerol, acetic acid, succinic acid, and heat. The resulting heat is enough to cause the increasing of mass temperature from an ambient temperature between 25 - 30 °C to 35 - 40 °C within 48 hours (De Vuyst and Weckx, 2016; Ho *et al*., 2014; Papalexandratou *et al*., 2013).

At the same time, the pulp cell is broken down by the action of pectolytic enzymes which produced by certain yeasts result in liquefying of pulp. The liquefied pulp will drain as other called as “sweating” and carried the flakes of the pulp away. The drainage reduces pulp thickness and forming spaces that in turn allows some air to percolate through the mass thus make the conditions become aerobic (De Vuyst and Weckx, 2016; Crafack *et al*., 2013; Daniel *et al*., 2009; Schwan and Wheals, 2004; Ardhana and Fleet 2003). The new pulp conditions make the environment become more convenient for lactic acid bacteria (LAB) to grow and become as coexistence with yeast during the process in between 24 to 72 hours of fermentation. The LAB use citric acid as a co-substrate and convert it into lactic acid during heterolactate fermentation. Conversion of citric acid into lactic acid resultant slightly increases in pH of the pulp as well as changing the composition of the fermenting mass. Consequently, this condition influences the microbial succession and in which favour the acetic acid bacteria (AAB) to growth. The AAB which produce acetic acid by oxidation of ethanol, are dominantly growth between 48 to 112 hours of fermentation (De Vuyst and Weckx, 2016; Ho *et al*., 2014; Papalexandratou *et al*., 2013).

As the fermentation has progressed, the concentration of acetic acid becomes higher especially after turning of the fermenting mass, where the aeration getting better and encourages the exothermic oxidation of ethanol to acetic acids. Hence, liberates more heat as well as rising of mass temperature up to 45 - 50 °C (De Vuyst and Weckx, 2016; Moens *et al*., 2014). The rising of temperature beyond 45 °C is unfavourable not only to the acetic acid bacteria hence resulting in a decline of all microorganisms except for spore-forming aerophilic bacteria types or bacilli. Starting from that moment onward, the bacilli comprise over 80% of microflora and dominate the mass environment. At this phase of fermentation, the pulp layer is

totally depleted hence the fermenting mass becomes more aerobic and together with water formation as well as good aeration promotes the temperature decrease. Decreased of temperature is suggested as an indicator for fermentation to be ended (Lima *et al*., 2015; Schwan and Wheals, 2004). The biochemical changes occur during pulp fermentation is summarized in Figure 2.10.

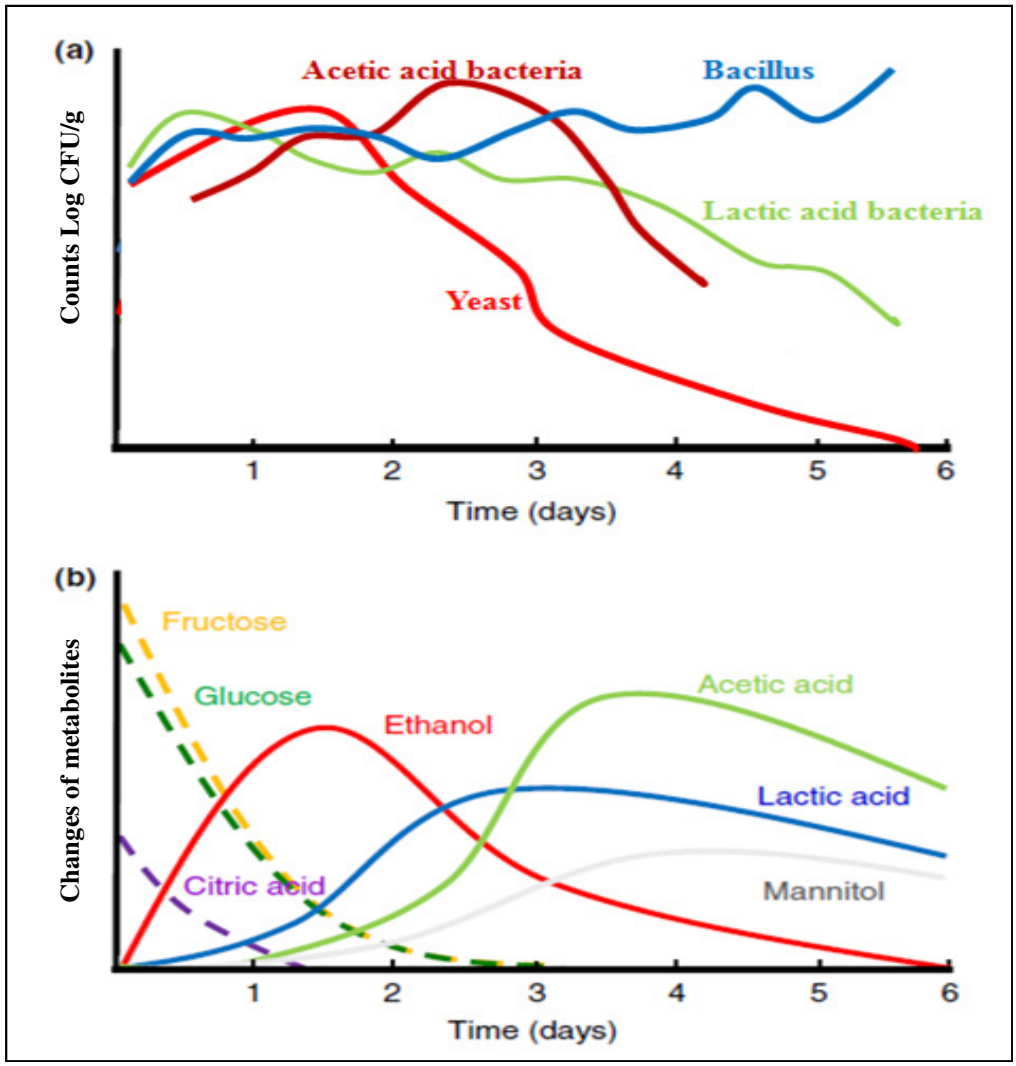


Figure 2.10: Trend of microbial and metabolites changes during the pulp fermentation. (a) Microbial succession (Source: Kouame *et al*., 2015) and (b) metabolite changes (Source: De Vuyst and Weckx, 2016).

2.3.2 Cotyledon Fermentation

Enzymatic and other biochemical reactions occur inside the cocoa beans during fermentation are known as cotyledon or internal fermentation (Lima *et al*., 2011). It starts with the diffusion of ethanol into the micropyle, changes the cellular structural causing loss of the of testa selective permeability. Consequently, this will allow the diffusion of acetic acid into the cotyledons, kill the embryo and will cause the death situation of beans (Anderson *et al*., 2006; de Brito *et al*., 2001). As the cocoa beans death, the membrane barrier has lost its function and allowed the cellular components such as seed enzymes (e.g. invertase, endoprotease, glycosidase and polyphenol oxidase) and substrates (e.g. anthocyanins, flavanols, phenols and storage proteins) are free to mix. The accumulation of acetic acid as well as other organic acids in the cotyledon will cause nib acidification, which in response to heat will be activated the cocoa enzymes. Hence, these will induce the onset of flavours precursors formation during fermentation (Brillouet and Hue, 2017; Kumari *et al*., 2016; Sousa *et al*., 2016; Kadow *et al*., 2015; Lima *et al*. 2011).

As previously mention, cocoa is a rich source of polyphenols which encompassed about 4% of anthocyanins, 37% of flavanols and 58% of proanthocyanidins. Anthocyanins are water-soluble pigment which consists of four compounds namely, cyanidin arabinoside, cyanidin galactoside, cyanidin rutinoside as well as cyanidin pentoside and are responsible for the colour of cacao seed. The pigment has the ability to convert orange-red to blue-violet colour in food and beverage products (Aprotosoaie *et al*., 2016; Bordiga *et al*., 2015; Voigt and Lieberei, 2014; Wallace and Giusti, 2011; Cakirer *et al*., 2010). Although in intact condition, the pigment has not given any marked taste or aroma, it is proven that there is an inverse relationship between flavour development and the retained

purple colour after cocoa fermentation (Kongor *et al*., 2016). Whereas, the proanthocyanidins which known as condensed tannins are dimers, trimers or up to hexamers of flavanols or catechins and are reported to be correlated with flavour quality of cocoa (Brillouet and Hue, 2017; Jolic *et al*., 2011; Counet *et al*., 2004).

During fermentation, hydrolyzation of the anthocyanins by glycosidase releasing sugar either galactoside, arabinoside, rutinoside or pentoside from cyanidin is occurred and results in the bleaching of the pigment of cotyledon. In line with the increase of oxygen, the oxidative phase becomes prominent and has subsequently resulted in the free cyanidin undergo enzymatic oxidation to form oligomers with epicatechin and catechin. Besides, it has been previously described that proanthocyanidin can elongate up to 18mers to form insoluble high molecule-weight compound or tannin. Hence, this can significantly reduce the astringent and bitter taste in the final product of cocoa (Brillouet and Hue, 2017; Aprotosoaie *et al*., 2016; De Taeye *et al*., 2016).

Apart from that, there is another enzymatic reaction that occurs in this phase which is proteolysis of cocoa storage proteins by two endogenous proteases. Cocoa seeds contain two storage proteins, namely vicilin-class (7S) globulin (VCG) and albumin. The albumin with relative molecular mass of 21 000 daltons is the predominant storage protein that account about 52% of total cocoa seed proteins and cannot be degraded during fermentation (Kochhar *et al*., 2000). While the vicilin-class (7S) globulin with the total 43% of total cocoa seed protein consists of three polypeptide subunits with relative molecular masses of 47 000, 31 000 and 15 000 daltons. These polypeptides are reported to be selectively hydrolyzed by the combined action of aspartic endoprotease and carboxypeptidase into hydrophilic and hydrophobic peptides as well as amino acids. The resulting peptides and amino

acids are served as cocoa flavour precursors will subsequently undergo Maillard reaction during roasting (Marseglia *et al*., 2014; Voigt and Lieberei, 2014). Studies also have showed that moderate acidification of cotyledons during cocoa fermentation will only degrade the polypeptide subunits of 46 000 and 31 000 daltons and will produce a high cocoa-specific aroma precursors in raw cocoa batches (Kratzer *et al*., 2009).

The activities of the two endogenous proteases are controlled by environmental factors such as temperature and pH during the fermentation process. The *T. cacao* aspartic endoprotease is documented to contain at least two polypeptides which apparently serve as an important protease during fermentation (Guilloteau *et al*., 2005). The activity of protease is optimal at pH of 3.0 and within temperature range between 42 to 47 °C. The protease is efficiently cleaved the VCG between hydrophobic amino acid to produce low molecular peptides with hydrophobic amino acid at the ends (Guilloteau *et al*., 2005; Voigt *et al*., 1994). On the other hand, carboxypeptidase which exhibits optimal activity at pH 5.8, is considered as the key enzyme for formation of cocoa flavour precursors due to its role to split hydrophobic amino acid from the carboxy-terminus of peptides which has been produced by T. cacao aspartic endoprotease (Laloi *et al*., 2007; Bytof *et al*., 1995). Previous studies have showed that these enzymes will only hydrolyse the 46 000 and 31 000 daltons polypeptide subunits of vicilin to the correct ratio of hydrophilic oligopeptides and free hydrophobic amino acids when been provided with optimum pH in the range of 5.5 to 5.0. Since the activities of these two enzymes are pH dependent, slow acidification of seed is necessary to ensure the formation of certain hydrophilic oligopeptides and free hydrophobic amino acids (Voigt and Lieberei, 2014; Guilloteau *et al*., 2005). In addition, fermentation

duration is a vital factor in influencing the peptide patterns of dried cocoa beans as been reflected by finding of Marseglia *et al*., (2014) and Buyukpamukcu *et al*., (2001).

2.4 Factors Affecting Fermentation

There are many factors which can influence the fermentation process such as the microbial communities (De Vuyst and Weckx, 2016; Ho *et al*., 2014; Papalexandratou *et al*., 2013), technique (Hatmi, 2015; Ganeswari *et al*., 2015; Lima *et al*., 2011, Guehi *et al*., 2010a), duration (Asante, 2015; Guehi *et al*., 2010a), pulp preconditioning either through pod storage, beans spreading or beans pressing (Afoakwa 2014a; Afoakwa *et al*., 2012; Nazaruddin *et al*., 2006), maturity level of pod (De Bertorelli *et al*., 2009) and cocoa genotype (Ramos *et al*., 2014; Trognitz *et al*., 2013; Kadow *et al*., 2013). These factors will lead to significant differences in the quality of cocoa (Aprotosoaie *et al*., 2016).

2.4.1 Microbial Communities

The microbial communities will affect the cocoa beans quality if unwanted microbial interferes. According to the recent finding, it is concluded that successful cocoa fermentation requires indigenous yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) succession. However, the activities of these microbial are depending on their total colony and diversity. Besides, the varying physicochemical parameter such as temperature, pH, oxygen tension and sugar or substrate content of the fermenting mass will influence the total and diversity of colony (De Vuyst and Weckx, 2016). Papalexandratou *et al*., (2013) has suggested that prevailing species *Hanseniaspora opuntiae, Saccharomyces cerevisiae,*

Lactobacillus fermentum, and *Acetobacter pasteurianus* during fermentation are responsible for successful fermentation and manage to produce good chocolates from fermented dry cocoa beans.

2.4.2 Technique of Fermentation

There are various cocoa fermentation techniques which have been practiced all over the world especially among the producing country. However, regardless of any technique, the basic of fermentation is still involved a heaping of fresh seeds to allow a proliferation of microorganisms and generation of heat (Ahmad Kamil *et al*., 2013). The fermentation techniques can be classified into heaps, baskets, trays, drying platforms as well as boxes. In general, the most common practices among the majority of farmers in West African countries such Ghana and Ivory Coast is the heaps fermentation. The technique is the simplest and does not require any container. Judging by the quality of product, it obviously produces good quality cocoa (Fowler and Coutel, 2017; Amoa-Awua, 2014; Aneani and Takrama, 2006).

The basket fermentation technique is mainly practised in Nigeria, Amazon region, Philippines, Vietnam and some parts of Ghana. Fermentation in trays is also being conducted in Ghana, while technique of drying platform is mostly practised in Ecuador as well as parts of Central America where the Criollo cocoa is usually grown (Ozturk and Young, 2017; Saltini *et al*., 2013; Thompson *et al*., 2013). There is a similarity between the fermentation technique using tray and drying platform, where cocoa beans are spread over both containers to a certain thickness. In addition, fermentation using the platform is reported to have a low fermentation rate and it can be applied adequately to Criollo beans. As mention previously Criollo

beans require only two or three days fermentation as compared to the Forastero cultivar (Giacometti *et al*., 2015; Saltini *et al*., 2013).

Although there are various techniques, fermentation using boxes are the most frequently used (Ozturk and Young, 2017; Amoa-Awua, 2014; Saltini *et al*., 2013; Thompson *et al*., 2013; Aneani and Takrama, 2006). The fermentation using the boxes are widely used in most of the producing countries including Brazil, Indonesia, Malaysia and Trinidad with estimation, approximately half of cocoa are fermented in boxes (Thompson *et al*., 2013; Lima *et al*., 2011). Although the fermentation technique is a similar using box, the practice is varied among those prevailing country, where Malaysia is practising fermentation technique known as the shallow box. In contrast to Guehi *et al*., (2010b) finding, it has revealed that heap fermentation can produce a better quality of the dried cocoa beans as compared to fermentation using the wooden and plastic box, Papalexandratou *et al*., (2013) has revealed that cocoa beans from spontaneous fermentation using shallow box managed to produce good chocolates. Their study also has found that the fermentation technique which is carried out according to Malaysian outline using high-quality raw material and clean equipment only allows growth of the right microorganisms and ensure the success of the fermentation.

2.4.3 Shallow Box

The shallow box is designed by Shamsuddin and co-workers in 1978 to improve aeration in fermenting mass (Figure 2.11). Size of the box can be different but the height of the box is limited to 32 cm, in order to ensure that the depth is always not more or less than 30 cm. This is important because previous research has showed that the depth of 30 cm has allowed sufficient heat generation during

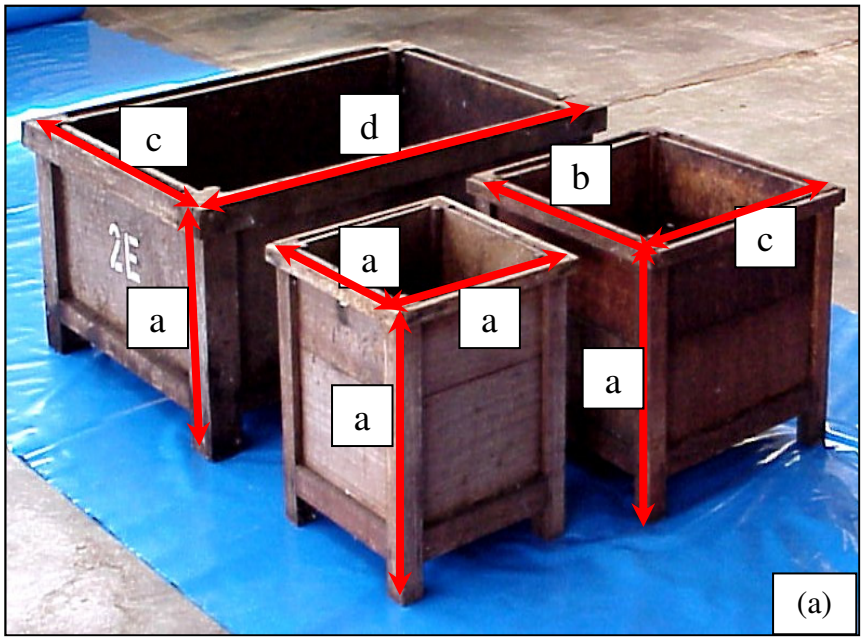


Figure 2.11: Shallow box used for cocoa fermentation. (a) A single unit of the shallow box may be of various sizes, but must be of 32 cm in height (a = 32 cm; b = 45 cm; c = 60 cm; d = 90 cm) and (b) Cascade type of shallow box. (Source: Personal collection).

fermentation and prevented the growth of visible mycelium mould which will damage the quality of cocoa beans (Mamot, 1987; Shamsuddin *et al*., 1978). The shallow box can be built in separate unit but need to be arranged in three cascade boxes (Figure 2.11b). The boxes are placed on the tier rack, where they will be stacked on top of each other to facilitate the handling as well as for saving space. In addition, the arrangement of the wood making up the side and bottom of the box is spaced to form slits which facilitate proper aeration and drainage (Figure 2.12).

Figure 2.12: Slit (white line) on the sides and bottom of the shallow box. (Source: Personal collection).

Fermentation commence by placing the cocoa seeds into the second level of shallow box which arrange in cascade type (figure 2.11b). In the event of harvesting is conducted on the following day, the newest cocoa seeds may be placed on the highest box without disturbing the ongoing fermentation in the second box. The cocoa beans are mixed to ensure uniformity with a single turning carried out on the third day. As a guideline, the wet cocoa beans should be mixed by turning when it seem relatively dry, separated from each other, have a very thin pulp layer as well

as 30% of them have red spots (Figure 2.13). The turning process is performed by transferring the cocoa beans from the upper box to the lower box (Figure 2.14).

2.4.4 Pod Storage

Pod storage is a practice which delaying the breaking of cocoa pods for few days after harvest. Previously, the practice is carried out unintentionally by farmers to gather enough pods to be broken in a single day (Quao, 2010). Nowadays, pod storage is a popular practice among farmers, especially in Malaysia, Vietnam, Trinidad, and Ghana. The pod storage is reported to help fermentation process especially at the beginning stage and results in a faster fermentation. During pod storage, the pulps lose moisture which allows better aeration in beans mass during fermentation. In addition, pod storage reduce the sugar content especially sucrose and subsequently lowering the formation of ethanol as well as acetic acid. The quality of dried cocoa beans is improved, with a higher percentage of brown beans, lower count of purple beans and reducing the acidity. It is also reported that chocolate made from beans which undergo pod storage tends to enhance the final taste of chocolate (Amoa-Awua, 2014; Afoakwa *et al*., 2014b; Saltini *et al*., 2013).

In Malaysia, pod storage not only faster the fermentation process but reported to overcome excessively acidic flavour as well as a weak chocolate flavour in Malaysian dried cocoa beans (Nazaruddin *et al*., 2006). The excessive acidic in Malaysian cocoa beans is due to high residual acetic and lactic acids content which closely links to a large amount of pulp in Malaysian seed compared to Ghanaian seed (Rahmat and Fisal, 2016). The pod storage helps in reducing acidity problem by partially removing or reducing the pulp content prior fermentation and increase the cocoa flavour. The pod storage also reported to reduce over 40% to 50% of water and

Figure 2.13: The cocoa beans appearance before mix. (Source: Personal collection).

Figure 2.14: Mixing of fermented cocoa beans. (Source: Personal collection).

dry matter per seed amount by water evaporation and respiration of sugars (Amoa-Awua, 2014; Afoakwa *et al*., 2013c). Additionally, in Malaysia, the pod storage is performed by storing the cocoa pods upon their harvest in a rattan basket. The pods are left aerobic in a dry airy condition for about seven up to 21 days (Nazaruddin *et al*., 2006). However, all the research which have been carried out on pod storage by previous researchers have used cocoa hybrid (Afoakwa *et al*., 2013c; Amanquah, 2013; Nazaruddin *et al*., 2006). Therefore, new research should be conducted because nowadays clones are the recommended planting material in Malaysia. Moreover, besides reducing the acidity of the dried cocoa bean, pod storage is preferable because it can help farmers to ensure the collection of cocoa seeds is sufficient for fermentation. However, pod storage, especially for damage pods, is not suggested for unduly long periods due to increase the likelihood of mould growth (Amoa-Awua, 2014; Quao, 2010).

2.4.5 Duration of Fermentation

The duration of fermentation is important for flavour development, but difficult to know exactly when to terminate the process since there is no clear definition of complete fermentation (Amoa-Awua, 2014; Nielsen, 2006). However, the duration is known to be affected by pH and temperature during the fermentation. In which, subsequently will also affect the enzymatic processes such as aspartic endoprotease, carboxypeptidase, aminopeptidases, invertase, glycosidases and polyphenol oxidases. These enzymes are pH and temperature dependent and required certain conditions for activating their active site optimally. For instance, a significant reduction of flavour precursors can be detected when the pH becomes too acidic too soon; because some of these enzymes will be inactivated (Aprotosoaie *et al*., 2016;

Saltini *et al*., 2013; Voigt and Lieberei, 2014; Camu *et al*., 2008). Whereas, other studies have reported that fermentation duration is the key factor in controlling the synthesis of aroma compounds such as methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and dimethyl disulphide (Rodriguez-Campos *et al*., 2012; Counet *et al*., 2002).

The fermentation process will be terminated by drying process because when the cocoa beans are dry, they will be separated from each other, blotted and reddish in colour (Figure 2.15). Besides, the temperature of fermenting mass start to decrease as well as the smell of acetic acid/vinegar diminished. The lengthwise cut of wet fully fermented cocoa bean will be revealed by the surface colour of cotyledon (Figure 2.16). The colour is slightly faded or bleached and surrounded by thin layer of brown (Lee *et al*., 2014; Ahmad Kamil *et al*., 2013).

2.5 Quality of Dried Cocoa Beans

The quality of cocoa beans is determined by various pre- and post-harvesting factors such as varieties, soil, climate, harvesting, fermentation, drying and storage (Kongor *et al*., 2016; Niemenak *et al*., 2014; Hii *et al*., 2004). The production of the good quality of cocoa beans is important as it will be sought after by chocolate and cocoa-based product manufacturers to ensure the quality of their final product (CAOBISCO/ECA/FCC, 2015; Amoa-Awua, 2014). In general, the quality of dried cocoa beans is assessed based on their physical, chemical and flavour characteristics through a randomized sampling from a total number of bags in a particular batch of cocoa beans. The samples of cocoa beans will be collected from the selected bags by thrusting a probe at three different positions of each selected bags (Figure 2.17). The collected cocoa beans shall be thoroughly mixed in

Figure 2.15: Appearance of cocoa beans when fermentation should be ended. (Source: Personal collection).

Figure 2.16: Lengthwise cut of wet fully fermented cocoa beans. (Source: Personal collection).