EFFECT OF DIFFERENT POD STORAGE AND FERMENTATION DURATION USING SHALLOW BOX ON AROMA COMPOUND IN THE DRIED COCOA BEANS

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ABSTRACT – *Cocoa fermentation is a process influenced by various factors including duration. This study investigated the effect of different durations of pod storage and fermentation using a shallow box on the volatile compound of Malaysian dried cocoa beans. Four batches of shallow box fermentation were carried out at the cocoa research and development center, Bagan Datuk with a loading capacity of 150 kg fresh cocoa beans. Wet cocoa beans (15 kg) were randomly taken out at 0, 24, 48, 72, 96, and 120 hours of fermentation and subsequently sun-dried until the moisture content was reduced to 7.5%. The volatile compounds for each cocoa powder prepared from samples were extracted by solid phase microextraction using 65 µm polydimethylsiloxane-divinylbenzene coating fiber and analyzed in a Gas Chromatography system equipped with a mass spectrometer detector. A total of 293 volatile compounds were identified and grouped into more than ten chemical groups. Esters predominated throughout the fermentation, followed by hydrocarbons. While, the number of compounds for acids, aldehydes, pyrazines and others such as alkaloids, respectively, increased in line with the fermentation durations. However, the effect of pod storage durations on the profile of volatile compounds is still unclear and needs further study.*

Key words: Cocoa, shallow box fermentation, aroma, pod storage, duration

INTRODUCTION

Fermentation is a critical process that enables the formation of the flavour precursor in the dried cocoa beans. The flavour of chocolate will be fully developed during drying and roasting, corresponding to the quality of precursors produced during fermentation (Barišić *et al.,* 2019; Kongor *et al.,* 2016). Generally, the flavour is divided into volatile and non-volatile compounds. The non-volatile compounds which are prominently known as a taste will be detected by receptors on the tongue and other parts of the mouth during a sensory test. Whilst the volatile compounds are the aromas which will be detected by smell receptors on the nose (Menis-Henrique, 2020).

The effect of fermentation and drying on non-volatile compounds in dried cocoa beans have been extensively studied (Niikoi Kotey *et al.,* 2022; Herrera-Rocha *et al.,* 2021; Delgado-Ospina *et al.,* 2020; Abhay *et al.,* 2016; Kumari *et al*., 2016). During fermentation, sugar in the cocoa pulps is utilised by a succession of three groups of micro-organisms, namely yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB), which results in the production of mannitol, ethanol, glycerol, also organic acids such as acetic acid, lactic acid, succinic acid and gluconic acid (Herrera-Rocha *et al.,* 2021; De Vuyst and Weckx, 2016). Whereas, the storage proteins in cotyledon are mostly the vicilin-class (7S) globulin that is selectively degraded by aspartic endoprotease and carboxypeptidase activity to amino acids and oligopeptides (Scalone *et al.,* 2019). Simultaneously, the polysaccharides are converted into glucose and fructose by invertase. The polyphenols are either hydrolyzed by a glycosidase or oxidised by polyphenol oxidase (Gil *et al.,* 2021). All of these fermentation metabolites, known as flavour precursors, will subsequently, react with each other during drying and roasting to develop the typical flavour and colour of chocolate (Santander Muñoz *et al*., 2020).

On the other hand, researchers are actively investigating the effects of fermentation and drying on volatile compounds (Moreira *et al.,* 2018; Aprotosoaie *et al.,* 2016; Rodriguez-Campos *et al*., 2012). To date, alcohols, carboxylic acids, aldehydes, ketones, esters, pyrazines, thiazoles, oxazoles, pyrrole derivatives, pyridines, and furans have been found as part of 600 various volatile compounds in cocoa (Kongor *et al.,* 2016; Voigt and Lieberei, 2014). According to previous research, several volatile compounds can be produced during the fermentation and drying process. However, it is still scarcely known which essential compounds are primarily produced from the fermentation process and is responsible for good or undesirable flavour. Identification of the essential compounds during fermentation is critical for discriminating the degree of fermentation and can be considered as an indicator for the quality of cocoa beans. In Malaysia, studies regarding the effect of duration during pod storage as well as fermentation on volatile compounds in Malaysian cocoa beans are hardly found. Therefore, this study has investigated the effect of pod storage and fermentation duration on the volatile flavour of Malaysian dried cocoa beans.

MATERIALS AND METHODS

Cocoa Pods

The experiment was arranged in a 4 x 6 factorial design with 24 treatments formed as combinations of two factors ie duration of pod storage (0, 2, 4 and 6 days) and duration of fermentation (0, 1, 2, 3, 4 and 5 days). Healthy cocoa (2500 pods per batch) which is considered ripe according to Ahmad Kamil *et al.*, (2013) was obtained from Cocoa Research and Development Centre (CRDC), Bagan Datuk, Perak, Malaysia. The pods were stored in the basket which had been labeled (pods without storage (0), pod storage for two, four and six days) and placed under a roof with dry and well-aerated conditions. Prior to fermentation, the pods were split opened and fresh cocoa beans were extracted manually.

Cocoa Fermentation

Four batches of fermentation were carried out concurrently, using four shallow boxes measuring 60 cm x 90 cm x 32 cm which were arranged in cascade. The fresh cocoa beans were placed in the box, covered with a clean gunny sack for five days to ferment. At 72 hours, the cocoa beans were mixed by transferring them from one box to another. Samples (15 kg) were scooped out diagonally from the top, middle and the bottom layer of each treatment mass according to Kelvin *et al.*, (2013) at a predetermined duration 0, 24, 48, 72, 96 and 120 hours of fermentation for drying. Every time the sampling procedure was carried out, the wall of the fermentation box was adjusted accordingly to maintain the depth of the fermenting mass and reduce the potential affect on biochemical changes in the beans.

Cocoa Drying

The drying was carried out by sun drying on the platform under the transparent roof and at ambient temperature. Each of the respective samples was uniformly spread out in the single layer of the beans and exposed to daylight for approximately 9 hours from 8.00 am to 5.00 pm. The cocoa beans were jumbled every three hours using a stainless-steel rake to ensure the beans were uniformly dried. At night, the platforms were covered with gunny to avoid dewdrops. The practices were repeated until the moisture content which was measured by using a Protimeter (Grainmaster, USA) reduced to about 7.5%.

Cocoa Beans

Upon drying, the resulting 24 samples ranging from 4.6 kg to 6.1 kg were sub-sampling by using quartering tools as described in detail in Malaysian Standard MS230:2007 (Anon, 2007) until each quarter reduced to about 250 grams.

Sample Preparation

Each of the dried and unroasted cocoa beans were manually peeled to obtain nibs and then ground to a fine powder using the analytical grinder (IKA model A11, Germany). The powders were subsequently kept in a tight container until solid phase microextraction (SPME) was carried out.

Isolation of Volatile Compounds

The volatile compounds from all the samples were extracted by solid phase micro extraction (SPME) procedure using 65 µm Polydimethylsiloxane-divinylbenzene (PDMS-DVB) coating fibre (Supelco, USA). Before extraction, the fibre was conditioned according to the supplier's instructions. A total of 0.64 g from each powder samples was placed into headspace vial with aluminium crimp cap (tef/si 20 mm). The PDMS-DVB coating fibre was then inserted through the septum and conditioned for 30 min at temperature of 142 ºC in paraffin oil bath (Thermoscientific, USA) to allow the absorption of the analytes. After absorption, the PDMS-DVB coating fibre was introduced into the capillary column at injector port of the Agilent 7890A Gas Chromatograph (USA) equipped with 5975C inert MSD triple axis detector in the scan mode for 30 min. The oven was initially held at 50 °C for 2 min, heated to 200 °C at 10 °C min^{-1}, 280 °C at 15 °C min^{-1}, then held for 2 min. The inlet temperature of the GC was set at 250 °C for sample desorption from SPME fibre.

Data Analysis

The area of peaks and retention time obtained were analysed using software which intergrated in the equipment. The volatile compounds were identified by comparison of their mass spectra to the reference mass spectral database of the National Institute of Standards and Technology library (NIST 14.lib) using MassHunter software (Version B.05.00). The identities of the volatile compounds were selected when they had a similarity percentage greater than 80%.

RESULTS AND DISCUSSIONS

Cocoa Volatile Compounds

Overall, a total of 853 chromatographic peaks were detected from all the dried cocoa bean samples, which are presented in Table 1. There was no apparent pattern in the number of chromatographic peaks regardless of the durations of the pod storage or fermentation. Based on comparisons of their mass spectra to the reference mass spectral database, 293 volatile compounds were identified for all 24 treatments and classified in the group of acids, alcohol, aldehydes, esters, hydrocarbons, ketones, pyrazines and others such as alkaloid, acetal, furan as well as phenols (Figure 1). Esters were found in a majority of the dried cocoa beans samples with a total of 90 compounds. The esters

are functional groups that are reported to be correlated with a fruity or flowery aroma. The compounds are formed by the condensation reaction between an alcohol and a carboxylic acid. Aprotosoaie *et al.*, 2016 reported that esters in dried fermented cocoa especially acetates are derived from amino acids. Whereas, yeast activities contributed to fatty acid esters production (De Vuyst and Weckx, 2016). Compounds namely ethyl palmitate, ethyl myristate, methyl palmitate, phenethyl acetate, methyl 2-methylpentanoate and 2, 3-dihydroxypropyl elaidate were found to exist in most of the cocoa beans from a different duration of pod storage and fermentation.

The second major group is hydrocarbons with 34 compounds. Dodecane, eicosane, heneicosane, hexadecane, octacosane and tetratriacontane were among the hydrocarbons detected. Most of the compounds detected have been found to develop at the beginning of the fermentation, from 0 to 72 hours (day zero to three) of fermentation. While, twelve hydrocarbons were detected specifically for the batch of pods without storage, five for the pod storage for two days, whereas another seven for the pod storage for four days and five for the pod storage for six days.
Besides esters and hydrocarbons, 32 acids compound were identified in the dried cocoa beans from different durations of the pod storage and fermentation. The compounds have been derived from sugar metabolism in the pulp layer by microbial during fermentation (Aprotosoaie *et al*., 2016) and the numbers were observed high in the dried cocoa beans after 96 hours and 48 hours of fermentation from the pods without pod storage and pod storage for six days, respectively.

Table 1: The number of chromatographic peaks at the different durations of pod storage and fermentation

Pod storage (days)	Fermentation duration (hours)					
	0	24	48	72	96	120
0	43	23	26	31	43	23
2	19	30	34	28	19	30
4	36	29	43	26	36	29
6	40	40	44	46	40	40

Cocoa specific aroma compounds
Among all the 293 volatile compounds identified, 35 compounds have been claimed or might be associated with cocoa specific aroma (Table 2). Rodriguez-Campos *et al.*, (2012) reported ethyl palmitate has an

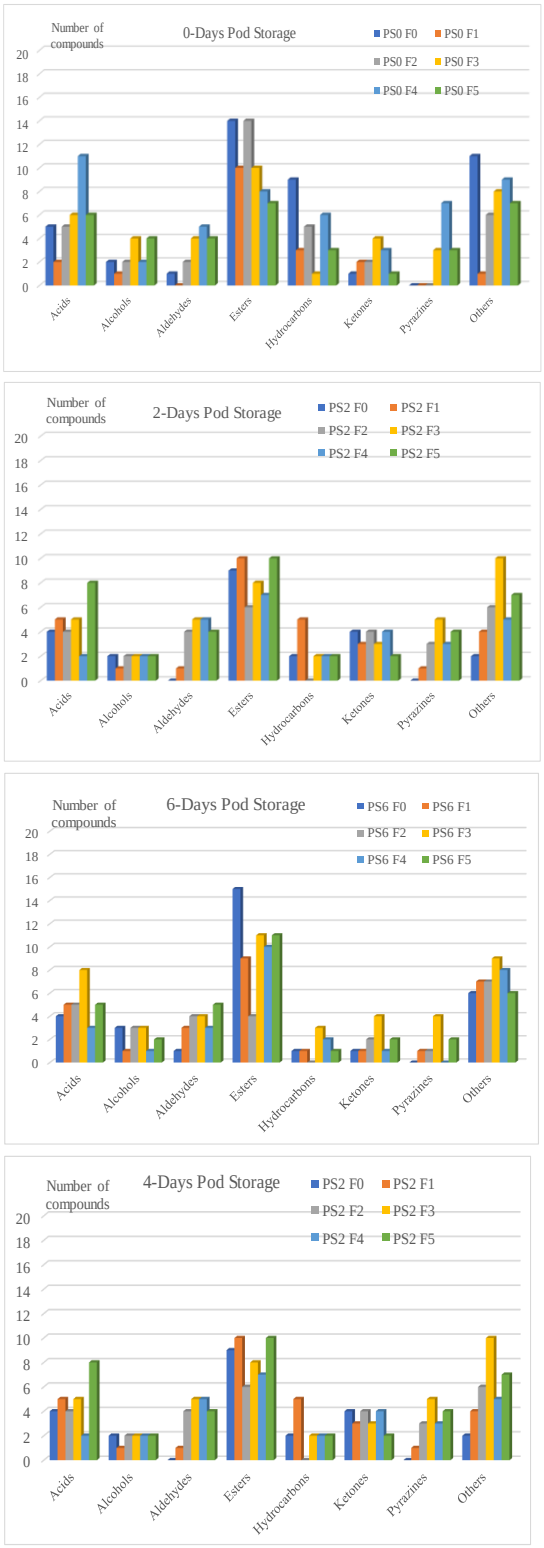

Figure 1: The chemical group of compounds from different durations of pod storage and fermentation.

aroma of waxy green which is suggested as an undesirable flavour during sensory. However, the compound is used in the wine or coffee industry for enhancing the flavour of their products and could be increased by the microbial enzymatic treatment (Li *et al.,* 2020). On the other hand, 2-phenethyl acetate is attributed to aroma of fruity floral and reported to be one of the important compounds to differentiate 'fine' cocoa beans (Bonvechi, 2005). While, ethyl laurate has a honey floral of aroma attribute, whereas ethyl cinnamate (sweet and cinnamon-like) and ethyl phenylacetate (sweet like honey) were also important compounds and these compounds could not be detected in the cocoa beans from the pod storage for six days. The study revealed that fermentation duration has an important effect on the formation of esters compared to pod storage. This find out is supported by the fact that the esters are synthesized from alcohol during the aerobic phase of fermentation (Barišić *et al.,* 2019).

Six of the 32 identified acids compounds are reported to be associated with cocoa specific aroma (Table 2). Acids compound are reported to be derived from sugar metabolism in the pulp layer by microbial during fermentation. Acetic and lactic acids are predominantly formed as fermentation products which are responsible to reduce the cotyledon pH to approximately 4.5–5.5.

Thus, enabling the enzymatic reactions especially aspartic endoprotease and carboxypeptidase required during the primary processing of cocoa beans (Barišić et al., 2019; Voigt *et al*., 2016). Moreover, this study revealed that short-chain carboxylic acids dominated the acidic compounds and in contrast to other researchers who detected acetic acid in their studies.

Aldehydes are essential not only as aroma compounds but also for further reactions for the formation of pyrazines. In line with the report by other researchers, fermentation and drying of cocoa beans will produce low concentrations of aldehydes. This study detected a total of 16 aldehydes with benzeneacetaldehyde as the most abundant compound. The benzeneacetaldehyde or known as phenylacetaldehyde has been described with a honey-like aroma (Frauendorfer and Schieberle, 2008) and is associated with chocolate-like notes (Misnawi and Ariza, 2011). The compound could not be detected in the dried unfermented cocoa beans except for the pod storage for six days, which might be one of the important aromas for cocoa. Whereas, benzaldehyde has been classified by Bonvechi (2005) as bitter pungent of sensory perception and detected in fourteen samples. In addition, α-ethylidene benzeneacetaldehyde which is known as cocoa butenal has been identified in the dried cocoa beans which were fermented for 48 and 72 hours from the pods without storage.

A high abundance of ketones is reported to be favourable for cocoa quality. However, the study found only two of 27 detected ketones compounds could be associated with cocoa specific aroma. 2-nonadecanone, 1-(4,5-dihydro-1,3-thiazol-2-yl) ethanone, 1-(1H-pyrrol-2-yl) ethanone, and 2-heptadecanone were detected in more than seven batches of cocoa beans from the different duration of pod storage and fermentation. Meanwhile, alcohol compounds are known as a result of microbial activity during fermentation and from heat degradation of amino acids (Aprotosoaie et al., 2016). The compounds are reported to be responsible for the fruity and floral aroma, hence higher content of alcohol compounds are desirable in obtaining the flowery and candy notes of cocoa products (Rodriguez-Campos et al., 2012). Similar to ketones, the study detected small numbers of alcohols (14 compounds) with only phenylethyl alcohol and 2-heptanol that can be associated with cocoa specific aroma. The phenylethyl alcohol or also known as 2-phenylethanol has caramel-like, alcohol-like as well as sweet, honey and floral quality of aroma (Aprotosoaie et al., 2016; Misnawi and Ariza, 2011) was detected in all the dried cocoa beans and has been recognized as one of the most active aroma compounds in dried and fermented cocoa (Rodriguez-Campos *et al.*, 2012).

Pyrazines are responsible for desire aromas such as nutty, chocolate-like, roasty, and caramel (Rodriguez-Campos *et al*., 2012; Bonvechi, 2005) and representing about 40% of the aroma in roasted cocoa. The compounds were proposed to be one of the most important volatile compounds and specifically be found in dried fermented cocoa beans. About 80 pyrazines have been reported to contribute to cocoa flavour but this study only discovered 10 compounds. Three of the compounds were associated with cocoa specific flavour. However, the numbers of pyrazine detected in this study were higher as compared to study by Rodriguez-Campos *et al*., (2012).

CONCLUSIONS

Volatile compounds in Malaysian cocoa beans which undergo different durations of pod storage and fermentation were successfully profiled and identified. The distribution of detected compounds demonstrated that the duration of fermentation can be a significant factor in the changes of volatile compounds in Malaysian dried cocoa beans. Thirty of the identified compounds are reported to be associated with cocoa specific aroma. The effect of different durations of pod storage and fermentation on the volatile compound should be extended with specific clones and compared with sensory evaluation.

Table 2: The number of chromatographic peaks at the different durations of pod storage and fermentation

Compounds	Odour description[a]	PS0						PS2						PS4						PS6					
		F0	F1	F2	F3	F4	F5	F0	F1	F2	F3	F4	F5	F0	F1	F2	F3	F4	F5	F0	F1	F2	F3	F4	F5
Ester																									
Ethyl palmitate	Mild waxy, milky balsamic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Ethyl myristate	Waxy, soapy	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+					+	+	+
Phenethyl acetate	fruity, sweet		+	+	+	+	+		+	+	+		+		+	+	+				+	+	+	+	
Ethyl laurate	Fruity, floral	+	+	+	+	+	+		+	+			+		+	+	+	+							
Ethyl phenylacetate	Fruity, sweet			+	+				+				+			+	+	+							
Ethyl cinnamate	Sweet, cinnamon-like	+		+					+									+	+						
Methyl tetradecanoate	Sweet, waxy	+			+											+				+					
Isoamyl acetate	Fruity, banana			+											+	+	+	+							
Methyl 2-methylpentanoate	Fruity, sweet, apple-like						+				+					+									+
β-Phenylethyl butyrate	Musty sweet floral yeasty	+											+											+	+
Isobutyl benzoate	balsam, cherry		+																	+	+				
Methyl benzoate	Strong fruity				+						+							+							
Butyl benzoate	floral, balsam	+																					+		
Methyl cinnamate	Balsamic, strawberry			+					+																
Ethyl decanoate	Pear, grape			+													+								
Methyl 2-methylbutyrate	fruity tutti frutti green apple			+																			+		
Acid																									
Phenylacetic acid	Sweet, honey-like					+		+		+	+	+	+	+			+			+	+	+	+	+	+
Octanoic Acid	Sweaty, rancid-like	+		+	+	+	+		+	+	+		+			+	+						+		
2-Ethylbutanoic acid	Acidic fruity				+	+				+					+			+					+		
3-Methylbutanoic acid	rancid cheese, sweaty, putrid				+								+			+							+		
Dodecanoic acid	Metal					+							+				+						+		
n-Decanoic acid	Rancid, fatty					+	+																		

Aldehyde																										
Benzeneacetaldehyde	Honey floral			+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
Benzaldehyde	Bitter	+			+	+	+				+		+	+	+	+	+	+	+				+		+	
5-Methyl-2-phenyl-2-hexenal	Cocoa					+					+	+				+	+	+			+	+	+	+	+	
2-methylbutanal	Chocolate malty					+	+			+				+	+				+							
α- ethylidene benzeneacetaldehyde	Cocoa honey			+	+																					
3-methylbutanal	Nutty chocolate aroma															+	+	+				+			+	
Ketone																										
1-(4,5-Dihydro-1,3-thiazol-2-yl)ethanone	Roasted caramel				+	+				+	+	+						+	+		+		+			
2-Pentanone	Fruity				+									+												
Alcohol																										
Phenylethyl Alcohol	Caramel-like	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2-heptanol	Fruity, citrus, herbal																	+	+							
Pyrazine																										
Tetramethyl pyrazine	Fermented mild cocoa				+		+		+	+	+	+	+				+	+					+		+	
Trimethyl pyrazine	Roasted peanut				+		+			+	+	+	+		+			+					+		+	
2-ethyl-3-methyl pyrazine	Roasted nutty					+												+			+					

[a]Source: Herrera-Rocha *et al*., 2021; Delgado-Ospina *et al*., 2020; Li *et al,* 2020; Chen *et al.,* 2020.

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