DEVELOPMENT OF SPECIALTY CHOCOLATE FOR FRUITY FLAVOR

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ABSTRACT - *Development of high-quality chocolate from premium cocoa beans becoming new challenging in world chocolate demand today. Application of new approaches in research and development for detection of special flavor will becoming new trend for improvement of chocolate production. The analysis of pharmacological and flavor content in cocoa beans is important and crucial for quality control of chocolate product since cocoa flavor are one of the major components in chocolate industry. Accurate microanalysis in cocoa remains challenging, because it still in develops. Microanalysis technique was achieved by combining multiple microanalysis techniques such as GCMS (Gas Chromatography Mass Spectrometry), LCMS (Liquid Chromatography Mass Spectrometry) and our new approach such as E-Nose (Electronic Nose sensor). Development of simpler and quicker analytical method is anticipated to produce chocolate based on flavor content from analysis. This innovation will focus on determination of several compound that contribute to special flavor using cocoa beans analysis and application of microanalysis techniques. At the same time, we would like to share a new unique identification and qualification strategy that will discover and allow accurate important compound in cocoa beans and also creating special flavor with new Malaysian Cocoa Signature with fruity flavor. Now, we are going to introduced to you our new specialty chocolate with fruity flavor note.*

Keywords**:** Specialty flavor, specialty chocolate, fruity flavor, e-nose, GCMS, cocoa flavor, microanalysis, compound, chocolate

INTRODUCTION

The pharmacological content analysis of cocoa beans plays a pivotal role in ensuring the quality control of chocolate products. Given that cocoa flavor is a cornerstone in the chocolate industry, accurate microanalysis of these compounds is essential, though still challenging due to the developmental nature of current methods (Smit, 2011).

Traditionally, this analysis has been accomplished by employing multiple microanalytical techniques such as Gas Chromatography-Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC), and UV/Vis Spectrophotometry (Afoakwa, 2010). However, there is an ongoing need for the development of simpler and quicker analytical methods (Gautier *et al.,* 2020).
This study focuses on the identification and quantification of several key compounds that contribute to unique cocoa flavors, particularly those associated with a fruity profile, such as linalool, 2-phenylethyl acetate, and 2-phenylethanol (Nijssen *et al.,* 2010). By analyzing cocoa beans and cocoa liquor using both E-Nose and GCMS approaches, we aim to refine the microanalysis techniques used in the industry.

Additionally, we propose a new strategy for uniquely identifying and qualifying significant compounds within cocoa beans and cocoa liquor, which promises to enhance the precision of flavor profiling in chocolate production (Jinap *et al.,* 2019).

Specialty cocoa flavor

Cocoa beans, a globally treasured food product, are the subject of extensive research, particularly in the realm of flavor analysis. Researchers are keen to investigate the chemical compounds within cocoa that not only contribute to its distinctive flavor but also offer potential benefits in pharmaceutical and food industries (Beckett, 2009).

The development of special cocoa flavor is a complex process involving multiple chemical reactions that occur from the moment the beans are harvested through fermentation, drying, and roasting, to the final stages of chocolate processing. Among these, fermentation, drying, and roasting are crucial stages where most of the flavor-defining reactions take place (Afoakwa *et al.,* 2008).

During fermentation, essential precursors form, setting the stage for the complex chemical interactions that occur during roasting. Roasting is particularly significant as it catalyzes reactions between storage proteins, which degrade into amino acids and short oligopeptides, and reducing sugars. This interaction produces a complex array of compounds, which are the primary contributors to the distinctive chocolate flavor (Fowler, 2016).

Interestingly, the balance of polyphenols plays a crucial role in flavor intensity. High polyphenol content often correlates with a lower cocoa flavor intensity, whereas a reduction in polyphenols tends to enhance the cocoa flavor. This phenomenon is typically observed in cocoa beans that have undergone extended fermentation and roasting periods, with significant chemical changes noted both before and after fermentation (Clapperton *et al.,* 1994).

In addition to the desirable flavor compounds, cocoa seeds also contain elements that can detract from the flavor, such as condensed tannins and methylxanthines (theobromine and caffeine). Tannins can reduce the perceived intensity of chocolate flavor by introducing astringency, while methylxanthines contribute a bitter taste (Afoakwa, 2016). Despite these challenges, the processing of cocoa integrates these various compounds to produce what is often referred to as a "special cocoa flavor," which includes nutty, fruity, flowery, and spicy notes (Smit, 2011).

Fruity flavor

Cocoa beans are renowned for their complex flavor profile, which includes a range of unique tastes such as nutty, spicy, flowery, and fruity flavors. Among these, the fruity flavor is particularly interesting due to its impact on the overall sensory experience of chocolate.

Fruity flavors in cocoa beans are developed through a combination of natural compounds that form during the fermentation, drying, and roasting processes. These compounds include esters, alcohols, aldehydes, and acids, which contribute to the aroma and taste of the final product. For example, fruity notes like those of berries, citrus, or tropical fruits often stem from the presence of compounds such as 2-phenylethyl acetate and ethyl 3-methylbutanoate, which are known for their fruity aromatic characteristics (Afoakwa *et al.,* 2008).

The presence and intensity of fruity flavors can vary depending on several factors, including the variety of cocoa bean, the region in which it is grown, and the specific fermentation and drying practices used. For instance, cocoa beans from certain regions in South America are known for their pronounced fruity notes, often described as berry-like or citrusy, due to the specific environmental conditions and post-harvest processing techniques employed.

Moreover, the development of fruity flavors is closely tied to the fermentation process. During fermentation, microbial activity leads to the breakdown of pulp sugars into acids, alcohols, and esters, which are key contributors to the fruity flavor profile. Proper control of fermentation time and temperature is crucial to optimizing these flavors, as over-fermentation or under-fermentation can either mask or fail to develop the desired fruity notes (Jinap *et al.,* 2019).

The fruity flavor in cocoa is a result of intricate biochemical processes influenced by the type of cocoa bean, environmental factors, and processing methods. Understanding and controlling these variables is essential for producing high-quality chocolate with a well-balanced and distinctive fruity flavor profile.

The study employs E-Nose technology, a cutting-edge tool for analyzing and screening cocoa compounds, particularly those that contribute to unique flavors. This research aims to identify and analyze special cocoa aromas, particularly fruity notes, using both GCMS and E-Nose technology, specifically focusing on MCB commercial clones (Gautier *et al.,* 2020). This approach not only aims to enhance the precision of flavor profiling but also to contribute to the overall understanding of cocoa's complex flavor chemistry.

MATERIALS AND METHODS

Sample collection

Dry cocoa beans samples from different location focusing on farmer with strong record of Cocoa of Excellence were collected for this study from Cocoa Research and Development Center in Bagan Datuk. MCBC12 clones was used in the study.

GCMS analysis

Volatile compounds in MCBC12 cocoa beans samples were identified by means of gas chromatography-mass spectrometry (GCMS).

The grinded cocoa bean samples were transferred to a 4-mL glass vial, and the SPME device with PDMS/DVB/CAR) was used to expose the fiber in the upper space of the vial at 90 °C for 30 minutes to completely absorb the analytes. Subsequently, the fiber was withdrawn and transferred immediately to the gas chromatograph injector port at 250 °C for thermal desorption.

The fiber was initially heated for 30 min at 250 °C for conditioning purposes. Then, thermal desorption was performed at the GC injection port of Agilent 7890B coupled with MS 5977A instrument for 10 min at 250 °C. The parameter of the analysis using GC/MS was programmed as 5 min at 40 °C, with a 10 °C/min ramp rate, and increased to 240 °C for 2 min using DB-5MS capillary column. The result of analysis revealed several compounds (alcohols, aldehydes, alkane, alkene, ester, ketone, oxime, pyrazine and saequiterpene) were recognized in the samples.

RESULTS AND DISCUSSIONS

GCMS results

All three sample (three replication) for MCBC12 cocoa beans samples were shown in figure 1 to figure 3. This result consists three replications of MCBC12 cocoa clones to compared with standard sample (2-phenylethylester acetic acid). The result of analysis revealed several compounds (alcohols, aldehydes, alkane, alkene, ester, ketone, oxime, pyrazine and sesquiterpene) were recognised in the samples (Table 1).

Odor profiling using e-nose technology

Four (3) samples of cocoa were given and data collection and analysis on these four samples were conducted. One (1) samples of the cocoa samples are the standard sample 1 (standard 1) that represents (2-phenylethylester acetic acid).

Raw data for each sample was collected using an e-nose device to extract the unique features found in each sample. These data are then processed using signal processing techniques to produce odor profiles These odor-profiles were compared and classified using case-based reasoning (CBR) artificial intelligent technique to identify the sample similar to standard sample.

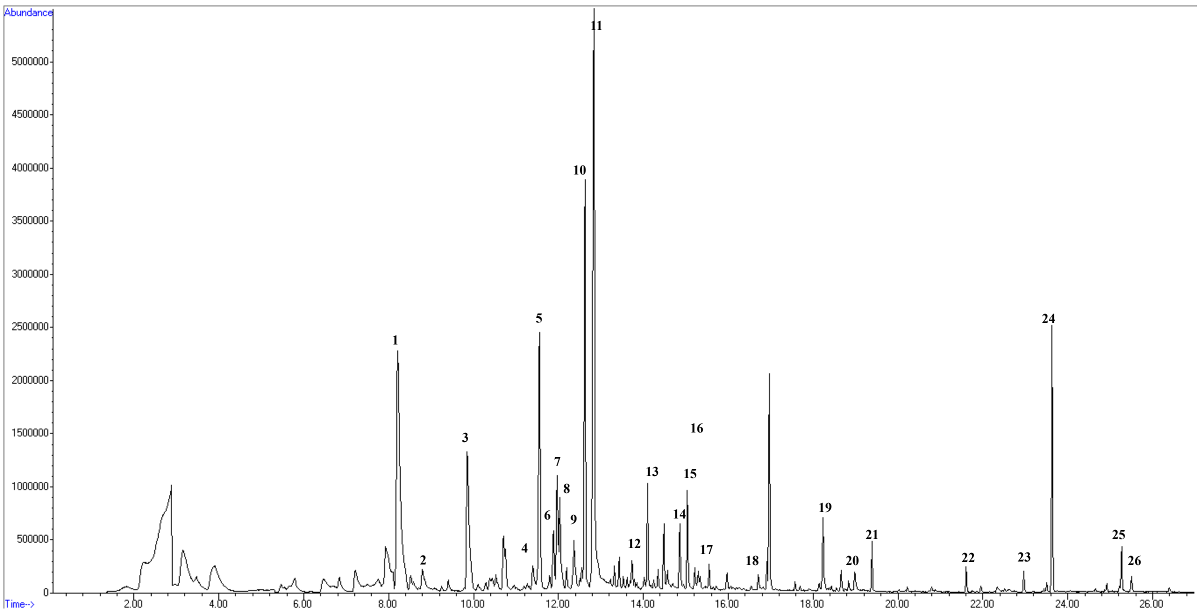


Figure 1: Chromatogram of the analysis by GC/MS for Cocoa Bean (MCBC12-1)

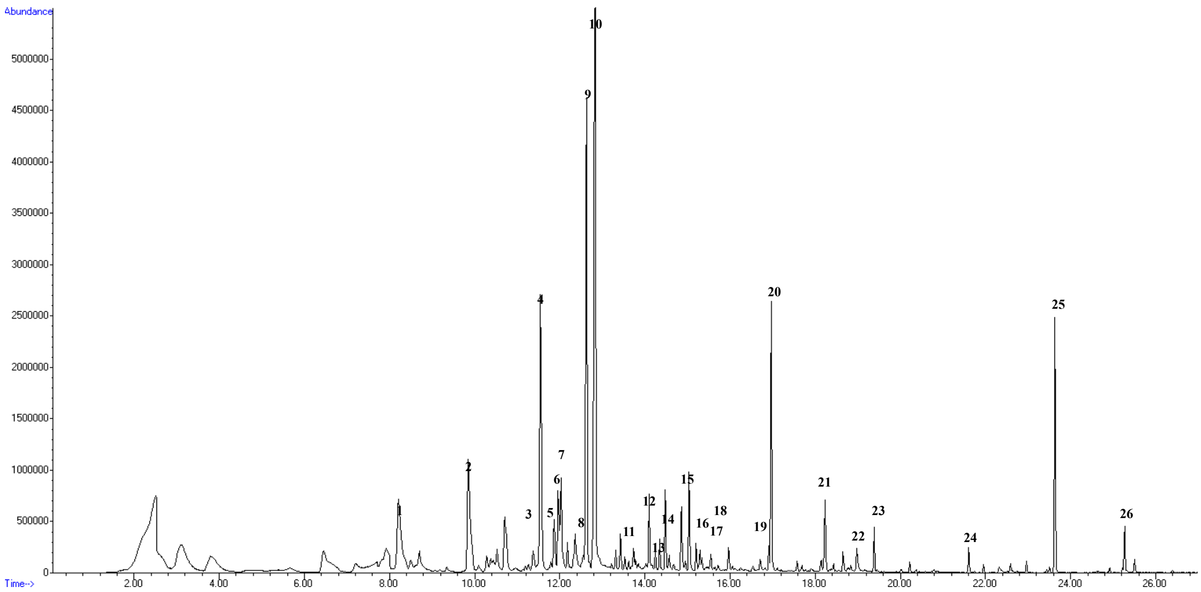


Figure 2: Chromatogram of the analysis by GC/MS for Cocoa Bean (MCBC12-2)

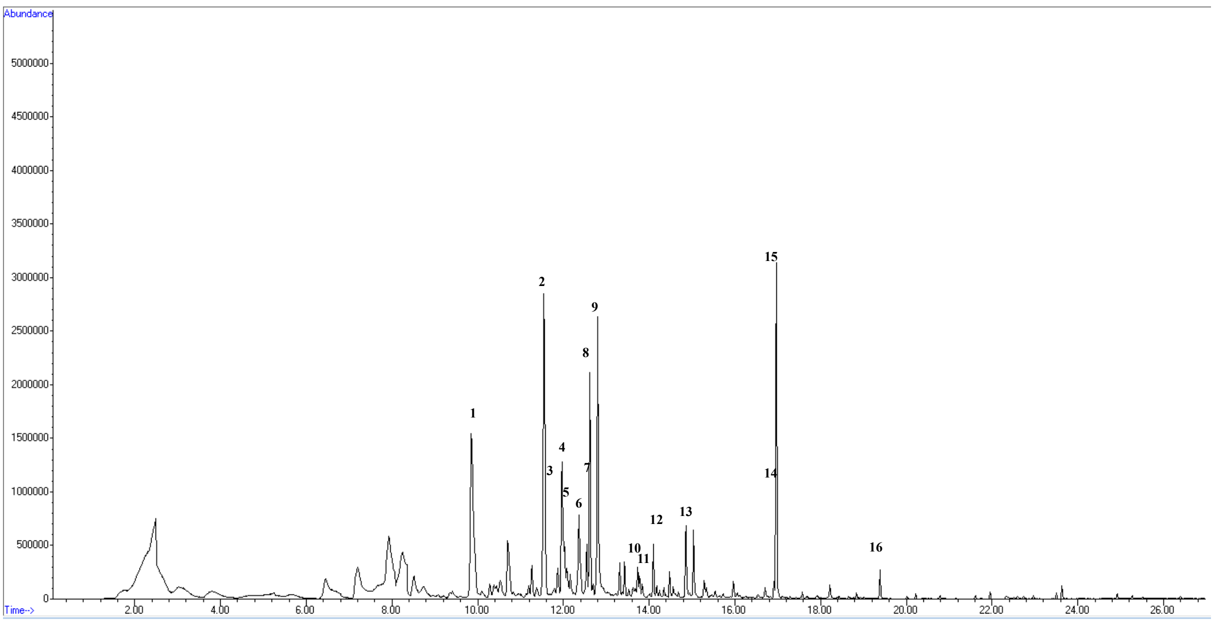


Figure 3: Chromatogram of the analysis by GC/MS for Cocoa Bean (MCBC12-3)

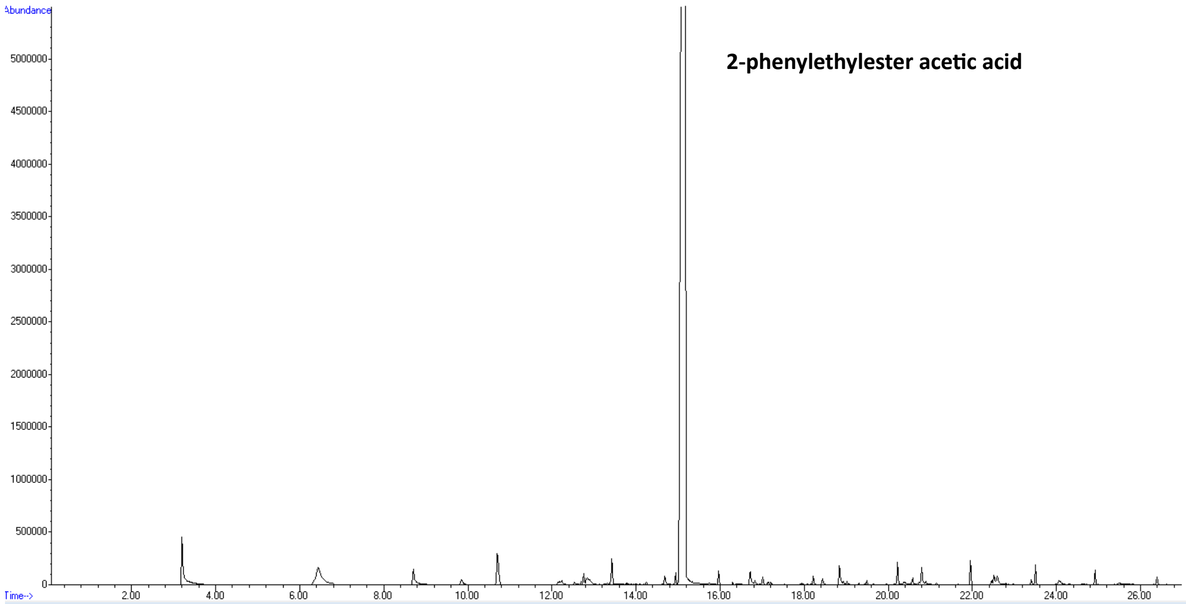


Figure 4: Chromatogram of the analysis by GC/MS for 2-phenylethylester acetic acid (standard solution)

Table 1: Summary of chemical composition for MCBC12 samples of cocoa bean using PDMS/DVB/CAR fiber identified by GC/MS

Compounds	Molecular formula	Peak Area (%)		
		MCBC12 1	MCBC12 2	MCBC12 3
Alcohol				
benzyl alcohol	C_7H_8O	0.62	0.62	-
α-methyl-benzenemethanol	$C_8H_{10}O$	1.14	-	0.86
1-octanol	$C_8H_{18}O$	1.89	-	-
phenylethyl alcohol	$C_8H_{10}O$	15.98	17.17	7.81
(R)α-methyl-benzenemethanol	$C_8H_{10}O$	-	1.26	-
linalool	$C_{10}H_{18}O$	-	-	1.24
(3R,6S)-2,2,6-trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	$C_{10}H_{18}O_2$	0.35	-	-
2-phenoxy-ethanol	$C_8H_{10}O_2$	-	-	-
nicotinyl alcohol	$C_{10}H_{13}NO_7$	-	-	-
Aldehyde				
nonanal	$C_9H_{18}O$	5.88	9.04	4.83
benzaldehyde	C_7H_6O	4.70	5.02	9.56
benzeneacetaldehyde	C_8H_8O	5.03	7.12	10.05
α-ethylidene-benzeneacetaldehyde	$C_{10}H_{10}O$	0.15	0.39	-
decanal	$C_{10}H_{20}O$	-	0.32	-
1-methyl-1H-pyrrole-2-carboxaldehyde	C_6H_7NO	-	-	-
5-Methyl-2-phenyl-2-hexenal	$C_{13}H_{16}O$	-	-	-
α-(2-methylpropylidene)-benzeneacetaldehyde	$C_{12}H_{14}O$	-	-	-
Alkane				
pentyl-cyclopropane	C_8H1_6	-	2.21	1.28
Ester				
ethyl ester benzoic acid	$C_9H_{10}O_2$	0.34	0.28	0.56
ethyl ester octanoic acid	$C_{10}H_{20}O_2$	1.34	1.16	0.94
ethyl ester benzeneacetic acid	$C_{10}H_{12}O_2$	0.97	1.16	1.49
ethyl ester nonanoic acid	$C_{11}H_{22}O_2$	0.33	0.28	-
ethyl ester decanoic acid	$C_{12}H_{24}O_2$	0.32	0.35	0.27
ethyl ester dodecanoic acid	$C_{14}H_{28}O_2$	0.59	0.65	0.55
ethyl ester tetradecanoic acid	$C_{16}H_{32}O_2$	0.31	0.39	-
methyl ester hexadecanoic acid	$C_{17}H_{34}O_2$	0.25	-	0.26
ethyl ester hexadecanoic acid	$C_{18}H_{36}O_2$	3.24	4.05	-
ethyl ester octadecanoic acid	$C_{20}H_{40}O_2$	0.22	-	-
2-phenylethyl ester acetic acid	$C_{10}H_{12}O_2$	-	1.79	-
pent-2-yl ester benzoic acid	$C_{12}H_{16}O_2$	-	4.20	6.38
phenylmethyl ester acetic acid	$C_9H_{10}O_2$	-	-	-
methyl ester benzeneacetic acid	$C_9H_{10}O_2$	-	-	-
ethyl oleate	$C_{20}H_{38}O_2$	0.65	0.84	-
ethyl (Z)-cinnamate	$C_{11}H_{12}O_2$	-	-	-
2-phenoxyethanol acetate	$C_{10}H_{12}O_3$	-	-	-
Ketone				
5,6-dihydro-6-propyl-2H-pyran-2-one	$C_8H_{12}O_2$	0.26	0.47	-
acetophenone	C_8H_8O	2.27	2.02	4.37
1-(1H-pyrrol-2-yl)-ethanone	C_6H_7NO	-	-	-
1-(2-pyridinyl)-1-propanone	C_8H_9NO	-	-	-
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	-	-	-
2-hydroxy-3-methyl-2-cyclopenten-1-one	$C_6H_8O_2$	-	-	-
Oxime				
methoxy-phenyl-oxime	$C_8H_9NO_2$	0.68	-	-
Pyrazine				
3-ethyl-2,5-dimethyl-pyrazine	$C_9H_{14}N_2$	-	-	-
tetramethyl-pyrazine	$C_8H_{12}N_2$	-	-	-
3,5-diethyl-2-methyl-pyrazine	$C_9H_{14}N_2$	-	-	-
trimethyl-pyrazine	$C_7H_{10}N_2$	-	-	-
3-ethyl-2,5-dimethyl-pyrazine	$C_9H_{14}N_2$	-	-	-
Sesquiterpene				
trans-.alpha.-bergamotene	$C_{15}H_{24}$	-	-	-
aristolochene	$C_{15}H_{24}$	1.17	1.40	-
Other				
styrene	C_8H_8	10.87	4.50	-
3,4-dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one	$C_{10}H_{10}O_3$	0.40	0.51	-
ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	$C_{13}H_{22}O_4$	1.22	1.17	2.86
(3aS,8aS)-6,8a-dimethyl-3-(propan-2-ylidene)-1,2,3,3a,4,5,8,8a-octahydroazulene	$C_{15}H_{24}$	-	-	-
1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene	$C_{15}H_{22}$	-	-	-

Table 1 and Figure 1-3 shows a number of alcoholic compounds present in the samples. High alcohol concentrations are favorable in order to obtain cocoa products with candy and flowery notes (Rodriguez-Campos *et al.*, 2012). For alcohol group, all sample contained phenylethyl alcohol. Rottiers *et al.*, (2019) reported that phenylethyl alcohol or also known as 2-phenylethanol was most abundantly present in cocoa and its concentration significantly increased over fermentation. Phenylethyl alcohol exhibits floral, honey-like and spice notes while benzyl alcohol contributes to floral flavor of the samples (Rodriguez-Campos *et al.*, 2012).

This samples shown mostly exhibit compound of benzyl alcohol, α-methyl-benzenemethanol, phenylethyl alcohol and also linalool. Three sample shows the present of 4 compounds for aldehyde group, which are nonanal, benzaldehyde, benzeneacetaldehyde and α-ethylidene-benzeneacetaldehyde. Benzaldehyde was associated with sweet chocolate and nutty-like flavor of cocoa while nonanal has citrus-like flavor (Rottiers *et al.*, 2019; Schweiggert-Weisz *et al.*, 2021).

Pentyl-cyclopropane of alkane group and acetophenone of ketone group are consistently found in sample.The ester group compounds consistently found in sample are ethyl ester benzoic acid, ethyl ester octanoic acid, ethyl ester benzeneacetic acid, ethyl ester decanoic acid, ethyl ester dodecanoic acid.

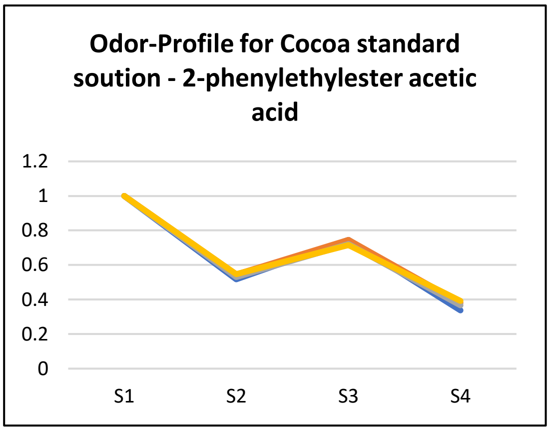
In this analysis of one standard have been chosen as a reference unique flavour which are 2-phenylethylester acetic acid. Ziegleder (1990) stated that linalool, which is the major terpene component in cocoa contributes markedly to the flowery and tea-like flavour of some cocoa varieties. Flavour grade beans contain relatively high concentrations of linalool and basic grade cocoas possess. 2-phenylethylester acetic acid contributes to the fruity and floral taste (Mohamed, 2019). Based on the results of GCMS analysis 2-phenylethylester was found in MCBC12-2 sample.

The results shown, several volatile compounds detected in the cocoa bean samples contributes to the fruity flavour including phenylethyl alcohol, benzeneacetaldehyde and benzyl alcohol. Nutty or almond-like odor may be associated with benzaldehyde. Besides exhibiting fruity odor, phenylethyl alcohol was also reported to have spice taste.

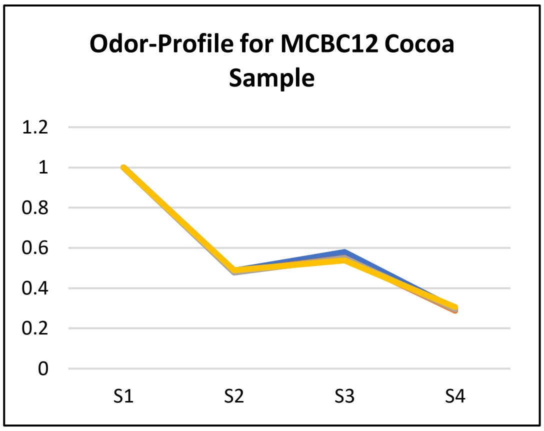
E-nose Sensor Result and Analysis

Odor Profiling

Three (3) MCBC12 samples of cocoa were given and data collection and analysis on these 3 samples were conducted. One samples of the cocoa samples are the standard sample that represents 2-phenylethylester acetic acid. While other samples are MCBC12 cocoa samples. Raw data for each sample was collected using an e-nose device to extract the unique features found in each sample. These data are then processed using signal processing techniques to produce odor profiles These odor-profiles were compared and classified using case-based reasoning (CBR) artificial intelligent technique to identify the sample similar to standard solution (2-phenylethylester acetic acid).



(a)



(b)

Figure 5: Odor-profile for (a) Cocoa standard solution (2-phenylethylester acetic acid), (b) MCBC12 cocoa sample

CBR Artificial Intelligent

In this section, CBR technique was used to compare and identify each of cocoa odor-profile (sample 1 – sample 4). Table 2 shows the similarity percentage (%) of odor-profile between different cocoa sample (sample 1 – sample 4). Based on the fundamental of CBR classification, the value of similarity percentage that nearest to 100% when compared between two cases, are two cases that contain very similar features and can be considered as two identical cases. This indicates that the highest percentage similarity value between standard sample and cocoa samples.

Table 2: Similarity percentages (%) of odor-profile between different cocoa standard solution (2-phenylethylester acetic acid) with MCBC12 cocoa samples.

Similarity Percentages (%)	**Standard (2-phenylethylester acetic acid)**	**MCBC12**
Standard (2-phenylethylester acetic acid)		97.39
MCBC12	97.39	

Table 2 shows the percentage similarity between standard samples and cocoa samples. From the table, it shows that the comparison between 3 cocoa samples using CBR intelligent classifier. Three replicate MCBC12 samples when compared with standard solution (2-phenylethylester acetic acid) using this classifier, show a similarity percentage of 97.39% and 97.39% respectively. For these std samples, standard samples have a high percentage of similarity for the MCBC12 sample. This shows that the MCBC12 sample is similar and close to standard solution (2-phenylethylester acetic acid).

CONCULSIONS

From the research results, it was found that special compounds can be detected from all cocoa samples using E-Nose and GCMS analysis. This compound known "2-phenyl *ethyl alcohol*". As a summary in this research, it was found that unique cocoa flavor (fruiyy compound) can be detected in MCB commercial clone (MCBC12). It was confirmed that cocoa beans from MCBC12 and MCBC12 contain fruity special notes using GCMS and E-Nose sensor. Based on this result chocolate with fruity flavor was develop specialty chocolate.

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