Malaysian Cocoa Journal 2023, Vol. 15(2)

Short Communication

APPLICATION OF GC/MS AND E-NOSE TECHNOLOGY FOR SPECIAL COCOA FLAVOR PROFILING IN MALAYSIAN DRIED COCOA BEANS

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Malaysian Cocoa J. 15 (2): 1-6 (2023)

ABSTRACT - *Cocoa aromatic compounds were important to develop premium chocolate products. The analysis of specialty cocoa flavor 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 remains challenging, because it still in development. Conventionally, this can be achieved by combining multiple microanalysis techniques such as Gas Chromatography Mass Spectrometry (GCMS), and normal sensory panel test. Investigation of simpler and quicker aromatic analytical information is anticipated. Objectives of this short communication, to focus on collection and classification of information several compound that contributed to special flavor such as flowery flavor using cocoa beans analysis and application of electronic nose (E-Nose) sensor technology to produce Malaysian Renaissance Chocolate. Dry cocoa beans sample used for analysis was taken from Cocoa Research and Development Center in Bagan Datuk, Perak. The entire sample was analyzed using E-Nose sensor technology. Two standard solutions represent linalool and 2-phenylethylesther acetic acids were used along the detection of existing volatile compounds in cocoa beans. The CBR (Case-based reasoning) intelligent classifier was used to show the result of percentage similarity between standard samples and cocoa samples. Both samples MCBC12 and MCBC15 when compared with linalool standard solution using this classifier, show a similarity percentage of 98.56% and 92.6% respectively. Meanwhile for 2-phenylethylesther acetic acids standard solution, the percentage of similarity obtained for both MCBC12 and MCBC15 samples is 97.39% and 96.65% respectively. As a conclusion, the analysis showed that each cocoa beans sample can be analyzed for special compounds using E-Nose sensor. In addition, 2-phenylethyl alcohol, linalool and benzene acetaldehyde contributed to the floral flavour. It was confirmed that cocoa beans from MCBC12 and MCBC15 contained flowery special notes using GCMS and E-Nose sensor. Within this analysis, we can develop a new unique identification and qualification strategy for accurate analysis special compounds in cocoa beans.*

***Keywords**:* Sensory, cocoa flavor, cocoa compound, microanalysis, chocolate, specialty flavor

INTRODUCTION

The analysis of pharmacological content in cocoa beans is important and crucial for quality control of chocolate product since cocoa flavor is one of the major components in chocolate industry. Accurate microanalysis remains challenging, because it still in development. Conventionally, this can be achieved by combining multiple microanalysis techniques such as *Gas Chromatography Mass Spectrometry (GCMS), High Performance Liquid Chromatography (HPLC)* and UV/Vis Spectrophotometry. Development of simpler and quicker analytical method is anticipated.

This study will focus on determination of several compounds that contributed to special flavor such as flowery flavor (linalool, 2-phenylethylcetate and 2-phenylethanol) using cocoa beans and cocoa liquor analysis and application of microanalysis via E-Nose and GCMS approaches. At the same time, we would like to share a new unique identification and qualification strategy that will discover and allow accurate identification and qualification of important compounds in cocoa beans and cocoa liquor.

Unique cocoa flavor

Cocoa beans classified as valuable food that contributed all over around the world. One of the important things that mostly researcher focus is concentrated on flavor analysis and investigated their chemical compound that useful to human being such as in pharmaceutical industries and food industries.

Special cocoa flavor is a complex process during which numerous chemical reactions occur from fresh cocoa beans, fermented cocoa beans, drying processes of cocoa beans and in chocolate processing levels. The most important processes, involving most of the reactions important for development of the proper cocoa flavor, are fermentation, drying and roasting of cocoa bean, and chocolate conching.

During fermentation, formation of important precursors occurs, which are essential for further chemical reactions in the following processes of chocolate production. Roasting is one of the most important processes.

Chocolate flavor development involves two complex processes such as fermentation and roasting. During fermentation storage protein degrade into amino acids and short oligopeptides. They can reach with reducing sugar to produce a complex mixture of compounds during roasting. These compounds are the major contributors to chocolate flavor. Cocoa flavor turn to be low when polyphenols content was high and cocoa flavor was high when polyphenols level was low (Clapperton *et al*.,1994). This is common situations when cocoa beans have been fermented and roasted for several days. Chemical changes were shown at the pre-fermentation and after fermentation process.

Besides components that contributed to chocolate flavor, seeds also contain ones that detract from chocolate flavor, notably condensed tannins and methylxanthines (*theobromine* and *caffeine*). *Tannins* reduce perceives chocolate flavor and cause astringency, and *methyxanthines* are bitter. Along the process, it is producing other cocoa flavor integrated with existing cocoa flavor aroma. It has been called as special cocoa flavor. Special cocoa flavor consisted of nutty flavor, fruity flavor, flowery flavor and spicy flavor.

E-Nose technology is one of powerful techniques that can be used as latest tool~~s~~ for cocoa compound analysis and screening special compounds. The objectives of the study are to identify and analyzing special cocoa aroma (flowery) using GCMS and eNose technology, in MCB commercial clones.

MATERIALS AND METHODS

1. *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. Two clones were used in the study, namely as MCBC12 and MCBC15.

2. *GCMS analysis*

Volatile compounds in two cocoa beans (from MCBC12 and MCBC15) 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.

3. *Odor profiling using eNose technology*

Four (4) samples of cocoa were given and data collection and analysis on these four samples were conducted. Two (2) samples of the cocoa samples are the standard sample 1 (standard 1) that represents linalool compound and standard 2 (2-phenylethylester acetic acid). While another two samples are MCBC12 and MCBC15 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 which standard either standard 1 or standard 2.

RESULT AND DISCUSSIONS

Currently, two clones from MCB, namely MCBC12 and MCBC15 with high flavor notes were selected to be analyzed using E-Nose and GCMS. Dried cocoa beans samples were analyzed using E-Nose and GCMS to detect several compounds that contribute to special aromatic flavors.

The analysis from both clones have successfully classified /identified as a reference unique flavor which are linalool and 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 flavor of some cocoa varieties.

a. GCMS results

Flavor 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, *linalool* was found in MCBC12 (3) sample while 2-*phenylethylester acetic acid* was found in MCBC12 (2) and all MCBC15 samples.

Table 1 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 MCBC12 and MCBC15 contained phenylethyl alcohol. Rottiers et al., (2019) reported that *phenylethyl alcohol* or also known as 2-*phenylethylester acetic acid* 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). MCBC12 mostly exhibit compound of *benzyl alcohol*, α-*methyl-benzene methanol*, *phenylethyl alcohol* and, also *linalool*. Meanwhile, MCBC15 majorly occupied with *benzyl alcohol, phenylethyl alcohol* and 2-*phenoxy-ethanol*.

In this analysis of MCBC12 and MCBC15, two standards have been chosen as a reference unique flavor which are *linalool* and 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 flavor of some cocoa varieties.

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Table 1: Summary of chemical composition for two samples of cocoa beans (MCBC12 and MCBC15 using GC/MS

Compounds	Molecular formula	Peak Area (%)					
		MCB C12 1	MCB C12 2	MCB C12 3	MCB C15 1	MCB C15 2	MCB C15 3
Alcohol							
benzyl alcohol	C_7H_8O	0.62	0.62	-	0.74	0.62	0.50
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	4.76	5.12	4.63
(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	$C_8H_{10}O_2$	-	-	-	14.13	11.83	15.26
nicotinyl alcohol	$C_{10}H_{13}NO_7$	-	-	-	-	0.74	-
Aldehyde							
nonanal	$C_9H_{18}O$	5.88	9.04	4.83	1.98	6.44	5.67
benzaldehyde	C_7H_6O	4.70	5.02	9.56	4.10	3.06	2.63
benzeneacetaldehyde	C_8H_8O	5.03	7.12	10.05	5.91	5.60	3.61
α-ethylidene-benzeneacetaldehyde	$C_{10}H_{10}O$	0.15	0.39	-	0.95	0.33	0.53
decanal	$C_{10}H_{20}O$	-	0.32	-	-	0.41	-
1-methyl-1H-pyrrole-2-carboxaldehyde	C_6H_7NO	-	-	-	0.69	-	-
5-Methyl-2-phenyl-2-hexenal	$C_{13}H_{16}O$	-	-	-	1.13	0.55	0.52
α-(2-methylpropylidene)-benzeneacetaldehyde	$C_{12}H_{14}O$	-	-	-	0.51	0.35	0.31
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	0.31	-	-
ethyl ester octanoic acid	$C_{10}H_{20}O_2$	1.34	1.16	0.94	0.67	0.47	0.93
ethyl ester benzeneacetic acid	$C_{10}H_{12}O_2$	0.97	1.16	1.49	1.13	0.73	0.70
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	0.31	-	-
ethyl ester dodecanoic acid	$C_{14}H_{28}O_2$	0.59	0.65	0.55	0.41	0.23	0.22
ethyl ester tetradecanoic acid	$C_{16}H_{32}O_2$	0.31	0.39		0.23	-	-
methyl ester hexadecanoic acid	$C_{17}H_{34}O_2$	0.25	-	0.26	1.48	0.77	0.65
ethyl ester hexadecanoic acid	$C_{18}H_{36}O_2$	3.24	4.05	-	3.14	1.81	1.46
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	-	3.11	1.87	1.89
pent-2-yl ester benzoic acid	$C_{12}H_{16}O_2$	-	4.20	6.38	1.77	1.20	-
phenylmethyl ester acetic acid	$C_9H_{10}O_2$	-	-	-	0.33		0.43
methyl ester benzeneacetic acid	$C_9H_{10}O_2$	-	-	-	-	0.30	0.31
ethyl oleate	$C_{20}H_{38}O_2$	0.65	0.84	-	0.36	0.31	0.22
ethyl (Z)-cinnamate	$C_{11}H_{12}O_2$	-	-	-	-	-	0.52
2-phenoxyethanol acetate	$C_{10}H_{12}O_3$	-	-	-	0.50	0.35	0.40
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	-	-	-	7.18	6.44	4.17
1-(2-pyridinyl)-1-propanone	C_8H_9NO	-	-	-	0.40	-	0.32
2,3 dihydro 3,5 dihydroxy 6 methyl 4H pyran-4-one	$C_6H_8O_4$				0.72		-
2-hydroxy-3-methyl-2-cyclopenten-1-one	$C_6H_8O_2$	-	-	-	2.99	-	1.81

b. E-Nose results

Table 2 shows the percentage similarity between standard samples and cocoa samples. From the table, it shows that the comparison between four (4) cocoa samples (Standard 1, standard 2, MCBC12 and MCBC15) using CBR intelligent classifier.

Both samples MCBC12 and MCBC15 when compared with standard 1 (linalool) using this classifier, show a similarity percentage of 98.56% and 92.6% respectively.

Meanwhile for standard 2 (2-*phenyl ethyl alcohol*), the percentage of similarity obtained for both MCBC12 and MCBC15 samples is 97.39%, and 96.65% respectively. For these two standard samples, both standard samples have a high percentage of similarity for the MCBC12 sample. However, the MCBC12 sample with standard 1 has the highest percentage of similarity between these two standard samples with a value of 98.56%.

This shows that the MCBC12 sample is similar and close to standard 1 (*Linalool*). This information will be used as an indicator to develop new specialty chocolate with special flavor notes. It was found that flowery compound can be detected from MCBC15 samples.

Currently we have successfully developed specialty chocolate with flowery compound. After this, we will develop another specialty chocolate with spicy flavor and fruity flavor.

Table 2 Similarity percentage (%) of odor-profile between different standard 1 (*Linalool*) and standard 2 (2-*phenyl ethyl alcohol*) with MCBC12 and MCBC15

Similarity Percentages (%)	Std 1 (Linalool)	Std 2	C12	C15
Std 1 (Linalool)		95.95	98.56	92.60
Std 2	95.95		97.39	96.65
MCBC12	98.56	97.39		94.04
MCBC15	92.60	96.65	94.04	

CONCLUSION

From the research results, it was found that special compounds can be detected from all cocoa samples using E-Nose and GCMS analysis. This two-compound known as "*linalool*" and "2-phenyl *ethyl alcohol*". As a summary in this research, it was found that unique cocoa flavor (flowery compound) can be detected in MCB commercial clone (MCBC12) samples collected from clones of MCBC12 and MCBC15. It was confirmed that cocoa beans from MCBC12 and MCBC15 contain flowery special notes using GCMS and E-Nose sensor.

ACKNOWLEDGEMENT

The authors express their appreciation to the Malaysian Cocoa Board's Director General, Deputy Director General R&D, the Director of Biotechnology, and the Director of Downstream for providing laboratory facilities and financial support for this research study. This investigation was supported by grant of the Development Fund of 12th Malaysian Plan (2021-2025). The authors wish to thank all who have directly and indirectly contributed to our project.

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