

## RESEARCH ARTICLE

# Cocoa volatile compounds affect aroma but not taste

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## ABSTRACT

Flavor is one of the most essential indicators for determining the quality of cocoa beans, especially fine flavor cocoa. The volatile compound in the beans affect the mechanism the flavor emergence. Therefore, this study aimed to identify the volatile compound in the aromatic and non-aromatic cocoa groups as one of FFCs indicator. It was conducted at the Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia. Furthermore, the genetic material used consisted of six aromatic cocoa genotypes, such as DR 2, ICCRI 03, ICCRI 07, ICCRI 09, MCC 02, and TSH 858 as well as four non-aromatic genotypes such as Sulawesi 1, Sulawesi 2, KW 516, and KEE 2. A completely randomized block design (RCBD) was used with three repetitions. The fermented bean samples of each genotype were analyzed of volatile compound at the Food and Science Laboratory, Jember Polytechnic, East Java and sensory test was conducted by three certified panelists from ICCRI. Data were analyzed by orthogonal contrast analysis with IRRI's STAR 2.0.1 software. While the Microsoft Excel 2019 and R Studio were used for correlation analysis and heatmaps. The results showed aldehydes, acids, esters, pyrroles, and pyrazines of aromatic cocoa bean group higher than the non-aromatic. The alanine and terpenoid compounds was only found in clones DR 2 and ICCRI 03, while the alcohol and phenol compounds dominated in the non-aromatic group. Genotypes were grouped into six groups, based on flavor and volatile compound. ICCRI 09 characterized by strong acidity with a fresh fruit, spicy and sweet aroma as well as contained aldehydes. Furthermore, ICCRI 03, MCC 02, and TSH 858 bean had weaker acidity and aroma than ICCRI 09 and contain terpenoids. ICCRI 07 had a nutty and woody aroma and was dominated by furan and pyrazine compounds. While Sulawesi 1 had a putrid aroma and alkaloids. The genotype groups of KW 516, KEE2, and Sulawesi 2 had astringent taste, dirty/dusty and moldy aroma as well as contained alcohol and phenol.

**Keywords:** Aromatic; Flavor; Non-aromatic; *Theobroma cacao* L; Volatile compounds

## INTRODUCTION

Cocoa is an important plantation commodity and it is the primary raw material for chocolate products. The demand is constantly increasing, especially for products with specific flavor, which is considered as an important attribute of the cocoa bean quality (Aprotosoai et al., 2016). In international trade, two categories of the beans are bulk and fine flavor cocoa (FFC) (Cevallos-cevallos et al., 2018). The main character of the FFC group is the presence of specific aromas such as floral, fruity, and woody hence they have higher quality at a premium price (Rottiers et al., 2019).

Fresh cocoa beans are characterized by astringent and bitter taste caused by the high content of polyphenols and methylxanthine (Cevallos-cevallos et al., 2018), and the flavor is formed during the post-harvest sequential processes of fermentation, drying, and

roasting (Cevallos-cevallos et al., 2018). In fermentation process, the cocoa bean formed flavor precursors. Volatile compounds are synthesized through enzymatic reactions and microbial activity impact under aerobic and anaerobic conditions during the fermentation process (Rottiers et al., 2019). Volatile compounds such as alcohol, aldehydes, ketones, organic acids, esters, pyrazines, and phenols contribute to the final flavor of cocoa beans (Cevallos-cevallos et al., 2018). The composition and quantity of volatile compound are influenced by clones/genotype and environmental conditions (Aprotosoai et al., 2016) as reported by Caligiani et al. (2016) and De Vuyst & Weckx (2016) in which genotype effected the amount of protein, carbohydrates, polyphenols, and enzyme activity. The chemical composition affects the production of flavor precursors that play a direct role in the formation of the organoleptic quality in chocolate especially the flavor.

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Anita-Sari et al., (2022) identified DR2, ICCRI 03, ICCRI 07, ICCRI 09, MCC 02, and TSH 858 as Indonesian aromatic cocoa, and Sulawesi 1, Sulawesi 2, KW 516, and KEE 2 as non-aromatic. The both of groups have different non-volatile compound content. Meanwhile, Rottiers et al. (2019) found the sensory character is related to the volatile compound content and Cevallos-cevallos et al. (2018) result indicated the flavor differences in bulk cocoa and FFC caused by the volatile compounds beans, which affect the mechanism for the flavor formation (Alvarez et al., 2016). Therefore, this study aimed to describe the differences of volatile compounds in aromatic and non-aromatic groups and for the future will use as indicator in the selection program to select potential genotypes as FFCs.

## MATERIALS AND METHODS

### Study area

The study was conducted at the Food and Science Laboratory, Jember Polytechnic, East Java, Indonesia. Meanwhile, the pod samples were obtained from Kaliwining Research Station, Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java with climate type C to D according to Schmidt and Fergusson with an altitude of 45 meters above sea level. Sample preparation was performed at the Post Harvest Laboratory, ICCRI. The cocoa genotypes used consisted of six aromatic genotypes of DR2, ICCRI 03, ICCRI 07, ICCRI 09, TSH 858, and MCC 02, and four non-aromatic genotypes of Sulawesi 1, Sulawesi 2, KW 516, and KEE 2 (Anita-Sari et al. 2022).

### Sample preparation of cocoa bean and liquor

Wet cocoa beans were obtained from approximately 100-150 freshly harvested pods. There was no storage treatment on the pods, and no pulp removal was conducted on the cocoa beans. The fermentation method was according to ICCRI, using mini wooden boxes with 10 kg capacity. It was carried out naturally as the common practice of the industries with no addition of starter culture for 4 days and turning of the fermentation mass was at every 48 h. The paste samples were prepared based on the method of Misnawi and Ariza (2011) from 500 grams of cocoa beans. Subsequently, the cotyledons were roasted at a temperature of 120°C for 12 minutes. They were mashed for 15 minutes, and the paste was packed and stored at 5°C.

### Sensory analysis

Sensory analysis was performed by standard flavor test developed by ICCRI and Guitard Chocolate. The samples were served simultaneously at a temperature of 40-60°C without sugar. At the change, panelists were given plain biscuits and drinking water to neutralize the sense of flavor; hence, there was no bias. The flavor attributes, including

bitterness, astringency, acidity, cocoa, floral, fresh fruit, browned fruit, spicy, woody, nutty, and browned/roast, were evaluated on a scale of 1-10.

### Volatile compound analysis

Extraction was conducted by the modified method of Wang et al. (2016), and the beans were ground until smooth using a mortar that was given liquid nitrogen to obtain a sample of 50 to 100 mg. The samples were dissolved in 750 µl of extraction solution consisting of methanol, water, formic acid in the ratio of 70:28:2 in a 1.5 mL microtube and then vortexed until well mixed. Meanwhile, the homogeneous mixture was incubated for 30 minutes on ice, then vortexed every 6 minutes, and centrifuged for 10 minutes at a speed of 14,000 rpm. The supernatant was separated into a new microtube, and the extraction procedure was repeated. The supernatant was collected with the first extraction results then dried in a fume hood for 2 hours, in a vacuum pump for 2 hours then lyophilized in a freeze drier overnight and the analysis of non-target metabolites was conducted in two repetitions.

GC-MS analysis was performed to determine volatile metabolites, while the dry methanol phase was redissolved in 500 µl of 100% methanol solution, sonicated for 10 minutes then homogenized and centrifuged at 14,000 rpm for 1 minute. The supernatant was taken for use in the GC-MS analysis, and the ready-to-injection solution was stored in a refrigerator at 4°C. Instrument parameters according to the method of An et al. (2014). Furthermore, the column used was Agilent HP-5MS of 30 m length, 0.25 mm diameter, 0.25 µm film. A total of 1 L sample was injected using a 25:1 (v/v) split mode with a temperature of 230°C, and the carrier gas rate (He) used was 1.12 mL/min with a linear speed of 39 cm/s. The column temperature was set at 80°C for 2 minutes, increased to 15 to 310°C/min, and held for 6 minutes. The transfer line and ion source temperatures were 250 and 200°C, while the electron ionization (EI) was conducted at 0.94 kV. Additionally, spectra were recorded at 10,000 u/s (check value) and a mass range of 85–500 m/z.

### Data analysis

The relationships between variables were tested using Spearman correlation analysis, with Microsoft Excel 2019 program. The relationships between genotype and flavor characteristics was analyzed using PCA biplot while flavor-based genotype clustering was performed using dendrogram and heatmap analysis. The software used were STAR 2.0.1 from IRRI for analysis of variance, Microsoft Excel 2019, as well as R Studio for correlation, PCA biplot, and dendrogram analyses.

## RESULTS AND DISCUSSION

Total of 32 volatile compounds belonging to ten groups of alcohols, aldehydes, acids, esters, terpenoids, furans, furanones, pyrans, pyrroles, pyrazines, alkaloids, and alanines. This amount was less than Loannone et al. (2015)'s study, where more than 600 volatile compounds including hydrocarbons, alcohols, aldehydes, ketones, esters, pyrazines, pyridine furans, pyranones, lactones, and sulfur were identified.

Volatile compounds are aroma precursors produced during the fermentation and drying processes (Aprotosoai et al., 2016). These compounds were play a role in the formation of cocoa bean flavor. There was no significant difference in the aromatic and non-aromatic cocoa groups, even with a large mean difference. This is due to variations in content within each group, hence it is necessary to pay close attention to the content of each genotype. Furthermore, there were differences in the types of volatile compounds in the two groups as shown in Table 1. The aromatic group contains aldehydes, acids, esters, furans, furanones, pyrans, pyrroles, and pyrazines which tend to be higher than the non-aromatic. In contrast, the non-aromatic group contains higher alcohol and phenol than the aromatic. Terpenoid and alanine compounds are found in the aromatic group and not found in the non-aromatic. The formation of aromatics is not only determined by one compound but involves several compounds simultaneously and forms a special aroma, different from one another. The non-aromatic group contains alkaloid compounds that are not found in the aromatic. According to Alvarez et al. (2016), pyrazine compounds (tetramethylpyrazine) contribute to the chocolate aroma, while acetylpyrrole gives an astringent, bitter, and roasted aroma. Meanwhile, the pyrazine content is influenced by climate, environmental conditions, fruit ripeness level, variety type, and processing (Alvarez et al., 2016).

**Table 1: Volatile compounds in aromatic and non-aromatic cocoa clones based on t-test between cocoa clone groups**

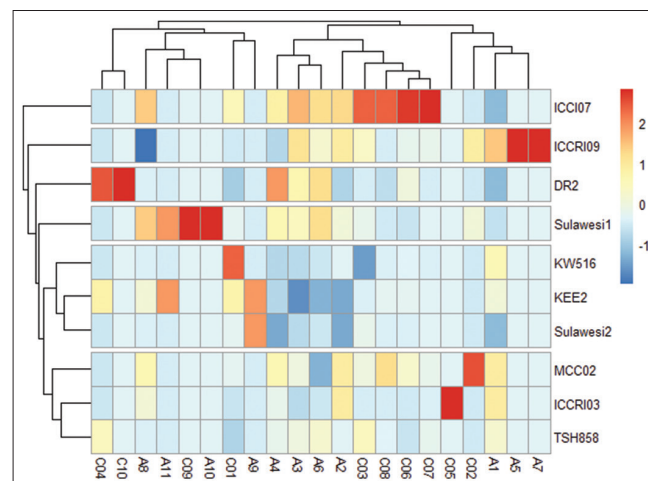
Traits	Rataan + standard error		Uji t
	Kelompok aromatik	Kelompok non aromatik	
Alkohol & Phenol	6.30 + 1.90	15.60 + 5.40	tn
Aldehydes	0.26 + 0.18	0.06 + 0.06	tn
Acids	61.10 + 8.60	41.90 + 6.60	tn
Ester	0.19 + 0.14	0.09 + 0.09	tn
Terpenoid	0.08 + 0.00	0.00 + 0.00	tn
Furan, furanone, pyran	2.38 + 1.40	0.53 + 0.20	tn
Pyrolle	3.38 + 2.50	0.58 + 0.12	tn
Pyrazines	2.98 + 1.60	0.93 + 0.16	tn
Alkaloid	0.00 + 0.00	0.65 + 0.00	tn
Alanine	0.30 + 0.00	0.00 + 0.00	tn

\*\*significant different at  $\alpha$  1%, \* significant different at  $\alpha$  5%, tn non-significant different at  $\alpha$  5%

The results show that each genotype within and between groups has different volatile compound composition and content (Table 2), affecting the formation of cocoa bean aroma, hence each genotype has a specific flavor character. Anita-Sari et al. (2022) stated that each genotype of Indonesian cocoa has different flavor characteristics, both belonging to the aromatic and non-aromatic groups.

The results show that the DR 2 clone has the lowest alcohol and phenol content as well as the highest ester content compared to others and produces alanine compounds (Table 2). The ester content affects the formation of fruity, floral, and honey aromas (Owusu et al., 2011; Moreira et al., 2018). Meanwhile, alanine content was associated with an increase in the intensity of the woody aroma (Table 3). This is consistent with a previous study that stated that DR 2 has a floral and fruity sensory character (Anita-Sari et al., 2022). This clone contains linalool compounds, and according to Alvarez et al. (2016) is a precursor of floral aroma. Anita-Sari et al (2022) stated that ICCRI 03 has a sensory character dominated by a floral aroma. The Sulawesi 1 clone, which is a non-aromatic group, contains alkaloids and caffeine, which contribute to the bitter aroma. Theobromine and caffeine alkaloids play a role in the formation of cocoa flavor, but with different effects (Franco et al., 2013).

Volatile compound, in term of type and content ddi not significantly affect the formation of cocoa bean taste (cocoa, acidity, astringency, bitterness) (Table 4), but show a correlation with aroma (Table 3). The results show that the acid content was related to the intensity of the floral aroma, while the alkaloid was



**Fig 1.** Volatile compounds and aroma of 10 cocoa clones based on hierarchical clustering analysis. C01: alcohol & Phenol. C02: Aldehydes. C03: acids. C04: Esther. C05: Terpenoids. C06: Furan. furanone. pyran. C07: Pyrroles. C08: Pyrazines. C09: Alkaloids. C10: Alanine. A1: Fresh Fruits. A2: Brownded Fruit. A3: Floral. A4: Woody. A5: Spicy. A6: Nutty. A7: Sweet. A8: Brownded/Roast. A9: Dirty/Dusty. A10: Putrid/Over Fermented. A11: Moldy.

**Table 2: Content and types of volatile compounds in 10 cocoa genotypes**

Code	Compound	Percentage area (%)									
		Aromatic						Non-Aromatic			
		ICCRI 03	ICCI 07	ICCRI 09	MCC 02	TSH 858	DR 2	Sul 1	Sul 2	KW 516	KEE2
C01	Alkohol & Phenol	<b>5.02</b>	<b>14.67</b>	<b>5.7</b>	<b>7.69</b>	<b>2.72</b>	<b>2.02</b>	<b>7.59</b>	<b>7.92</b>	<b>30.42</b>	<b>16.49</b>
	2.3-Butanediol	5.02	14.67	-	5.29	-	-	6.64	4.09	30.04	9.24
	Benzenethanol	-	-	-	2.40	2.15	0.95	0.95	2.03	0.38	-
	1.3-Butanediol	-	-	2.53	-	0.57	-	-	-	-	7.25
	1-Pentanol	-	-	0.51	-	-	-	-	-	-	-
	Ethanol	-	-	2.30	-	-	-	-	1.8	-	-
	Octanol	-	-	0.36	-	-	-	-	-	-	-
	1-Hexanol	-	-	-	-	-	1.07	-	-	-	-
C02	Aldehyde	-	-	<b>0.49</b>	<b>1.07</b>	-	-	<b>0.23</b>	-	-	-
	Benzaldehyde	-	-	0.49	-	-	-	0.23	-	-	-
C03	Acids	<b>44.58</b>	<b>100.85</b>	<b>60.98</b>	<b>53.13</b>	<b>63.02</b>	<b>44.32</b>	<b>50.31</b>	<b>49.92</b>	<b>22.49</b>	<b>44.91</b>
	Acetic acid	36.32	35.98	53.04	36.5	54.37	42.53	40.15	38.69	19.45	42.3
	Octadecanoic acid	-	-	-	-	-	1.79	-	5.82	-	1.05
	2-Methyl propanoic acid	2.99	39.68	2.75	6.80	1.81	-	1.64	1.34	2.92	1.56
	Butanoic acid	5.07	-	5.19	0.97	6.29	-	-	4.07	-	-
	Butanoic acid. 4-chloro-	-	12.99	-	8.40	-	-	0.42	-	-	-
	Valeric acid	-	-	-	0.46	-	-	7.02	-	-	-
	Pentanoic acid. 4-methyl	-	-	-	-	-	-	-	-	-	-
	Hexadecanoic acid	0.20	12.2	-	-	0.55	-	0.43	-	-	-
C04	Ester	-	-	-	-	<b>0.30</b>	-	-	-	-	<b>0.36</b>
	Ethyl ester	-	-	-	-	0.30	-	-	-	-	-
	10-Methyl ester	-	-	-	-	-	0.85	-	-	-	0.36
C05	Terpenoid	<b>0.5</b>	-	-	-	-	-	-	-	-	-
	Linalool	0.5	-	-	-	-	-	-	-	-	-
C06	Furan, furanone, pyran	<b>0.24</b>	<b>9.04</b>	<b>1.03</b>	<b>2.25</b>	-	<b>1.7</b>	-	<b>0.53</b>	<b>0.65</b>	<b>0.94</b>
	2-Furanone	0.24	-	-	-	-	0.22	-	-	-	0.39
	2.3-Pyran	-	-	1.03	-	-	-	-	0.53	0.11	-
	2.3-Dihydro-pyran	-	9.04	-	1.21	-	0.38	-	-	-	-
	Furan	-	-	-	1.04	-	-	-	-	0.15	-
	2-Butanone	-	-	-	-	-	-	-	-	-	0.55
	3.4-Pyran	-	-	-	-	-	1.10	-	-	0.11	-
	Furan. tetrahydro-e methyl	-	-	-	-	-	-	-	-	0.28	-
C07	Pyrolle	<b>0.44</b>	<b>15.86</b>	<b>1.41</b>	<b>1.46</b>	<b>1.08</b>	-	<b>0.69</b>	<b>0.76</b>	<b>0.23</b>	<b>0.65</b>
	Acetylpyrrole	-	-	-	1.46	-	-	0.69	-	-	0.65
	Pyrrrol	0.44	15.86	1.41	-	1.08	-	-	0.76	0.23	-
C08	Pyrazines	<b>0.8</b>	<b>9.55</b>	<b>0.69</b>	<b>5.87</b>	<b>0.99</b>	-	<b>0.59</b>	<b>0.86</b>	<b>0.94</b>	<b>1.34</b>
	Tetramethylprazine	0.8	9.55	0.69	5.87	0.99	-	0.59	0.86	0.94	1.34
C09	Alkaloid	-	-	-	-	-	-	<b>2.58</b>	-	-	-
	Caffeine	-	-	-	-	-	-	2.58	-	-	-
C10	Others	-	-	-	-	-	-	-	-	-	-
	Alanine	-	-	-	-	-	1.81	-	-	-	-

C01: alkohol & Phenol. C02: Aldehydes. C03: acids. C04: Ester. C05: Terpenoid. C06: Furan. furanone. pyran. C07: Pyrolle. C08: Pyrazines. C09: Alkaloid. C10: Alanine.

related to the intensity of putrid, over fermented, and moldy aroma. Dirty aroma will be weakened by the intensity of the browned fruit and floral aroma, which will strengthen intensity of the nutty aroma. Meanwhile, spice strengthen by the sweet aroma, even though the

browned aroma will be weakened. Table 5 showed that each volatile compound has a different sensory or aroma character. The composition and intensity in a genotype will certainly produce a different and specific flavor of cocoa beans.

**Table 3: Pearson correlation of aroma profiles and volatile compounds in 10 cocoa bean genotypes**

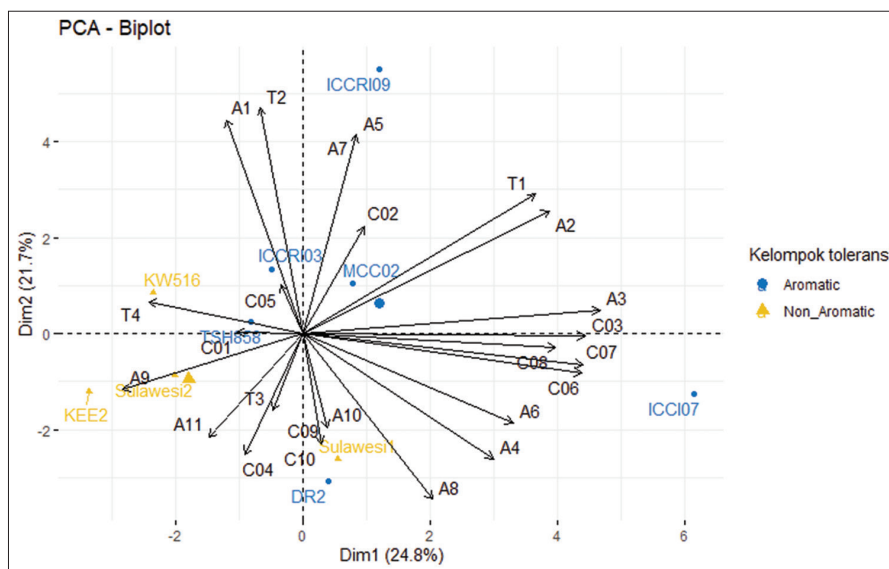
	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	
C02	-0.19																				
C03	-0.28	0.04																			
C04	-0.30	-0.30	-0.16																		
C05	-0.21	-0.17	-0.16	-0.19																	
C06	0.18	0.01	0.79**	-0.10	-0.19																
C07	0.16	-0.10	0.87**	-0.23	-0.14	0.96**															
C08	0.17	0.30	0.75*	-0.32	-0.16	0.91**	0.87*														
C09	-0.10	0.02	-0.06	-0.19	-0.11	-0.21	-0.11	-0.18													
C10	-0.33	-0.17	-0.16	0.87**	-0.11	0.01	-0.17	-0.25	-0.11												
A1	0.07	0.50	-0.29	-0.36	0.33	-0.37	-0.37	-0.17	-0.23	-0.42											
A2	-0.11	0.47	0.50	-0.52	0.33	0.47	0.49	0.57	0.02	-0.29	0.43										
A3	-0.31	0.20	0.69*	-0.02	-0.26	0.59	0.59	0.45	0.16	0.24	-0.19	0.61									
A4	-0.35	0.14	0.28	0.50	-0.06	0.40	0.28	0.31	0.23	0.66*	-0.36	0.29	0.56								
A5	-0.18	0.31	0.13	-0.19	-0.11	-0.08	-0.06	-0.17	-0.11	-0.11	0.52	0.33	0.40	-0.27							
A6	-0.29	-0.33	0.46	0.25	-0.19	0.36	0.41	0.09	0.44	0.44	-0.54	0.19	0.78**	0.60	0.08						
A7	-0.18	0.31	0.13	-0.19	-0.11	-0.08	-0.06	-0.17	-0.11	-0.11	0.52	0.33	0.40	-0.27	1.00**	0.08					
A8	0.12	-0.01	0.33	-0.17	0.04	0.46	0.48	0.57	0.49	-0.15	-0.46	0.19	0.08	0.47	-0.70*	0.21	-0.70*				
A9	0.13	-0.26	-0.16	0.07	-0.17	-0.18	-0.17	-0.18	-0.17	-0.17	-0.30	-0.72*	-0.63*	-0.57	-0.17	-0.48	-0.17	-0.08			
A10	-0.10	0.02	-0.06	-0.19	-0.11	-0.21	-0.11	-0.18	1.00**	-0.11	-0.23	0.02	0.16	0.23	-0.11	0.44	-0.11	0.49	-0.17		
A11	0.13	-0.12	-0.15	0.07	-0.17	-0.23	-0.17	-0.21	0.67*	-0.17	-0.16	-0.35	-0.32	-0.03	-0.17	-0.01	-0.17	0.40	0.38	0.67*	

C01: alkohol & Phenol. C02: Aldehydes. C03: acids. C04: Ester. C05: Terpenoid. C06: Furan. furanone. pyran. C07: Pyrrole. C08: Pyrazines. C09: Alkaloid. C10: Alanine. A1: Fresh Fruit. A2: Browned Fruit. A3: Floral. A4: Woody. A5: Spicy. A6: Nutty. A7: Sweet. A8: Browned/Roast. A9: Dirty/Dusty. A10: Putrid/Over Fermented. A11: Mouldy. \*\* significant different at p < 0.01. Sample size n on all character = 10 samples

**Table 4: Pearson correlation of taste profiles and volatile compounds in 10 cocoa bean genotypes**

	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	Cocoa	Acidity	Bitterness
C02	-0.19												
C03	-0.28	0.04											
C04	-0.30	-0.30	-0.16										
C05	-0.21	-0.17	-0.16	-0.19									
C06	0.18	0.01	0.79**	-0.10	-0.19								
C07	0.16	-0.10	0.87**	-0.23	-0.14	0.96**							
C08	0.17	0.30	0.75*	-0.32	-0.16	0.91**	0.87**						
C09	-0.10	0.02	-0.06	-0.19	-0.11	-0.21	-0.11	-0.18					
C10	-0.33	-0.17	-0.16	0.87**	-0.11	0.01	-0.17	-0.25	-0.11				
Cocoa	-0.15	0.30	0.48	-0.38	0.34	0.52	0.49	0.48	-0.33	-0.06			
Acidity	0.04	0.27	-0.03	-0.32	-0.02	-0.28	-0.20	-0.20	-0.26	-0.44	0.24		
Bitterness	-0.06	0.09	-0.11	0.48	-0.19	0.13	-0.04	0.16	-0.57	0.52	-0.07	-0.38	
Astringency	0.48	0.10	-0.46	-0.09	0.01	-0.24	-0.28	-0.02	-0.39	-0.22	-0.25	0.19	0.46

C01: alkohol & Phenol. C02: Aldehydes. C03: acids. C04: Ester. C05: Terpenoid. C06: Furan. furanone. pyran. C07: Pyrrole. C08: Pyrazines. C09: Alkaloid. C10: Alanine. \*\* significant different at p < 0.01. Sample size n on all character = 10 samples.



**Fig 2.** PCA biplot based on volatile compound and sensory of 10 cocoa clones. C01: alkohol & phenol. C02: aldehydes. C03: acids. C04: ester. C05: terpenoid. C06: furan. furanone. pyran. C07: pyrrole. C08: pyrazines. C09: alkaloid. C10: alanine. T1: cocoa. T2: acidity. T3: bitterness. T4: astringency. A1: fresh fruit. A2: browned fruit. A3: floral. A4: woody. A5: spicy. A6: nutty. A7: sweet. A8: browned/roast. A9: dirty/dusty. A10: putrid/over fermented. A11: mouldy.



**Table 5: The identification of volatile compounds based on GC-MS analysis and the aroma character caused by volatile compounds in cocoa beans**

Compound	Formula	Aroma	References
<b>Alcohol</b>			
2.3 Butanediol	C4 H10 O2	Fruit, onion	Cevallos-cevallos et al.,2018
Benzenethanol	C8 H10 O	Honey, soice, rose, lilac	Cevallos-cevallos et al.,2018
1-Pentanol	C12 H23 N O2	Medicine, fruit, wine	Cevallos-cevallos et al.,2018
Ethanol	C3 H4 O3	Sweet	Cevallos-cevallos et al.,2018
Octanol	-	Flower	Cevallos-cevallos et al.,2018
1-Hexanol	C7 H16 O	Fruity, herbal	Alvarez et al., 2016
<b>Aldehyde</b>			
Benzaldehyde	C7 H6 O	Cherry, candy, almond, burnt sugar	Cevallos-cevallos et al.,2018
<b>Acids</b>			
Acetic acid	C2 H4 O2	Pungent, stinging sour	Cevallos-cevallos et al.,2018
Octadecanoic acid	C18 H36 O2	NF	-
2-Methyl propanoic acid	C4 H8 O2	Pungent, stinging sour	Cevallos-cevallos et al.,2018
Butanoic acid	C5 H10 O2	NF	-
Butanoic acid. 4-chloro-	C4 H7 CL O2	NF	-
Valeric acid	C5 H10 O2	NF	-
Pentanoic acid. 4-methyl	C6 H12 O2	Sweat	Cevallos-cevallos et al.,2018
Hexadecanoic acid	C18H36O2	Fatty	Cevallos-cevallos et al.,2018
<b>Ester</b>			
Ethyl ester	C5 H10 O3	Fruity	Alvarez et al., 2016
<b>Terpenoid</b>			
Linalool	C10H18O	Flower, lavender	Cevallos-cevallos et al.,2018
<b>Furan, furanone, pyran</b>			
2-Furanone	C4 H6 O2	NF	-
2.3-Pyran	-	NF	-
2.3-Dihydro-pyran	-	NF	-
Furan		NF	Cevallos-cevallos et al.,2018
2-Butanone	C4 H8 O2	Cocoa	Alvarez et al., 2016
3.4-Pyran	C6 H8 O2	NF	-
Furan. tetrahydro-e methyl	C5 H10 O	NF	-
<b>Pyrolle</b>			
Acetylpyrrole	C6 H7 N O	Chocolate, bitter, roasted	Alvarez et al., 2016
Pyrrrol	C6 H7 N O	Nutty, almond	Alvarez et al., 2016
<b>Pyrazines</b>			
Tetramethylprazine	C8 H12 N2	Chocolate, cocoa	Alvarez et al., 2016
<b>Alkaloid</b>			
Caffeine	C8 H10 N4 O2	Bitter	Alvarez et al., 2016
<b>Others</b>			
Alanine	C3H7NO2	Sweet	-

\*= based on reference

Clustering analysis based on volatile compound content and flavor (aroma) shows the occurrence of six genotype groups. ICCRI 07 is characterized by browned fruit aroma as well as acid, furan, furanone, pyran, and pyrazine compounds. Furthermore, ICCRI 09 is dominated by fresh fruit, spicy, and sweet aroma as well as aldehyde compounds. MCC 02, ICCRI 03, and TSH 858 are dominated by spicy aroma and contain aldehyde and terpenoid compounds. The volatile compound groups in these genotypes are associated with flowery, fruity, caramel, spicy, and nutty aromas (Owusu et al., 2012). Pyrazines, such as methylpyrazines, are key compounds in the formation of specific flavors in cocoa (Munoz et al., 2019). Linalool was only detected in

ICCRI 03 as a derivative of DR 2 and this compound was found mostly in Arriba cocoa (De Vuyst and Weckx, 2016). Moreover, DR 2 as a derivative of the Criollo group has a woody aroma character, and contains alanine and ester compounds found in the Criollo group (Ascrizzi et al., 2017; Munoz et al., 2019). Sulawesi 1 has a putrid, moldy aroma character and contains alkaloid compounds, while KW 516, KEE 2, and Sulawesi 2 contain alcohol compounds and a dusty/dirty aroma.

The PCA analysis show six genotype groups with different taste and aroma, and volatile compound content, such as ICCRI 09 of strong acidity with fresh fruit, spicy and sweet

aromas and dominated by aldehydes. Furthermore, ICCRI 03, MCC 02 and TSH 858 clones showed weaker acidity and aroma than ICCRI 09 and dominated by aldehydes and terpenoids. ICCRI 07 was characterized by nutty and woody aroma, contains furan, furanine, pyran, and pyrazines, while DR 2 contains esters and alanine. Sulawesi 1 was characterized by putrid aroma, and contains alkaloid compounds. Meanwhile, KW 516, KEE 2, and Sulawesi 2 were characterized by astringency taste, dirty/dusty and moldy aroma, as well as contain alcohol and phenol.

## CONCLUSION

Volatile compounds, in term of content and composition, were differ in aromatic and non-aromatic genotypes. The intensity and composition of volatile compounds were significantly affects final aroma of cocoa beans, but never in taste. Aromatic group of cocoa have higher aldehydes, acids, esters, furan-furanone-pyran, pyrrole, alanine, and pyrazine compounds when compared to non-aromatic group. The opposite condition was happen in alcohol and phenol content.

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## Author Contributions

Indah Anita-Sari, Rudi Hari Murti, Misnawi, Eka Tarwaca Susila Putra and Agung Wahyu Susilo conceived the experiments, Indah Anita-Sari, Rudi Hari Murti, Misnawi, and Eka Tarwaca Susila Putra conducted the experiments, Indah Anita-Sari, Bayu Setyawan and Miftahur Rizqi Akbar analyzed the results, Indah Anita-Sari and Bayu Setyawan prepared all figures and tables. All author reviewed the manuscript.

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