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Metabolite profiling of Indonesian cacao using Gas Chromatography-Mass Spectrometry

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Abstract. Indonesia as the world third largest cacao producer is also well known for its fine cacao from Trinitario group. Fine cacao breeding will support the sustainability of Indonesian specialty cocoa products. However, information on the distinction between metabolite profile of Indonesian fine and bulk cacao are still limited. The objective of this study was to compare the metabolite profile of Indonesian fine and bulk cacaos using Gas Chromatography-Mass Spectrometry (GC-MS) method with methanol-formic acid-water extraction. Four Indonesian fine cacaos (i.e. DR 2, DRC 16, PNT 16, PNT 17) and bulk cacaos (i.e. MCC 02, KW 516, KW 617, SUL 01) were used. Measurements were conducted on untargeted metabolite profile, theobromine (T), caffeine (C), T/C and total fat content. Forty-nine peaks were detected and 23 putative metabolites with high reliability were identified. Principal component analysis separated fine and bulk cacaos into two different groups. Hierarchical cluster analysis showed fine cacaos contained higher caffeine while bulk cacaos contained higher fatty acids (palmitic acid, stearic acid and oleic acid) content than the opposite. However only fatty acids, which represented in total fat content, showed stable result under targeted analysis. Therefore, these metabolites could be further analysed as biomarker for fine cacao selection.

1. Introduction

Cacao (*Theobroma cacao* L.) is one of valuable Indonesian estate crops. As the third largest cacao producer in the world [1] and the third top estate crops in Indonesia [2], cacao breeding program become an important issue. Information on genetic background is crucial in this program. There are three main cacao population in the world namely Criollo, Forastero and Trinitario [3]. Trinitario is a hybrid product of Criollo and Forastero. Based on their quality, Criollo or Trinitario genotypes generally produced the high-quality fine cacao while Forastero clones usually produce bulk cacao with some exception [4]. Fine cacaos contribute higher price than bulk cacaos because of its specialty and Indonesia is well-known for its “Java A” fine-flavour cacao since 1900s.

Indonesian cacao breeding was started in 1912 by CJJ van Hall at Djati Roenggo, Central Java. The main purpose of that program was to select high yielding, cacao stem borer resistant and white bean cacao [5]. Indonesian fine cacaos were Trinitarios, selected from hybrid of “Java Criollo” which originated from Venezuela and unknown parent [6]. Some commonly used clones until now are DR 1, DR 2, DR 38 and DRC 16.



Fine cacao produced lower yield, less disease resistant and less vigorous than fine cacao [7] thereby despite its higher price, plantation area of fine cacao in Indonesia is decreasing because of some limitations, especially the pest and disease problem [6]. Interdisciplinary crop improvement strategies will be able to identify traits leading to plant varieties that use fewer inputs, less land and less energy, thereby resulting in a more sustainable agricultural landscape [8]. Therefore, effort on developing tolerant fine cacao was conducted by Indonesian Coffee and Cocoa Research Institute (ICCRI) as the new era of fine cacao breeding [9]. The program will support the sustainability of Indonesian Java fine-flavour cacao as the national heritage.

Specialty products such as fine cacao is linked to their quality. The quality is often associated with metabolite characteristics of the crop. However, information on metabolite profile for the distinction of Indonesian fine and bulk cacao are still limited. This information could be assigned by metabolomics, a relatively new emerging technology. Metabolomics is considered as the connector between genotype and phenotype because metabolites are the final product from a cellular regulation process. It is also considered as basic respond of biological system to environment and genetic changes [10]. Therefore, metabolomics is assumed will be able to describe the phenotype of the observed character.

To date, untargeted cacao metabolomics analysis performed on several issues such as detection of primary and secondary metabolism on polyphenol accumulation and respond to stress [11] and metabolite changes identification during fermentation [12]. However, none of them specifically differentiate fine and bulk cacaos. The information on those metabolites will be the basic of genetic diversity analysis as a crucial factor in breeding program. This study reported the difference of Indonesian fine and bulk cacao by Gas Chromatography-Mass Spectrometry. The quantitative analysis of identified metabolites was also investigated to validate the GC-MS result.

2. Materials and Methods

2.1. Genetic Materials

Eight Indonesian cacaos were used in this experiment. Those are four fine cacaos (DR 2, DRC 16, PNT 16, PNT 17) and four bulk cacaos (SUL 01, MCC 02, KW 617, KW 516) with three biological replications. Harvesting time conducted at mature stage. These clones were the collection of Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia.

2.2. Chemicals

Methanol, formic acid, petroleum ether, acetone, acetonitrile and hexane were purchased from Merck KGaA (Darmstadt, Germany). Standard of theobromine and caffeine were from Sigma-Aldrich (St. Louis, USA).

2.3. GC-MS

2.3.1. Extraction.

Cacao seeds were de-pulping, quenched in liquid nitrogen and kept in -80 °C for further use. Extraction referred to [11] with modification. Seeds were grinded using mortar and pestle with liquid nitrogen. Fifty to one hundred mg powder was used. Extraction solution consisted of 750 μ L methanol:water:formic acid (70:28:2). Powder were diluted in 1.5 mL microtube extraction solution, vortexed, incubated 30 min on ice, vortexed every 6 min and centrifugated 10 min in 14.000 rpm. Supernatant were placed in a new microtube. This process was repeated once and the new supernatant were collected with the first one. This mixture was dried in a fume hood for 2 h, in a vacuum pump for 2 h and overnight lyophilization in a freeze drier.

2.3.2. GC-MS Analysis.

The dried methanol phases were re-dissolved in 500 μ L methanol, sonicated for 10 min, vortexed and centrifugated 1 min in 14.000 rpm [11]. Supernatant were used for GC-MS analysis. Gas chromatography Mass Spectrometry (GC-MS) analysis was performed on Agilent 7890 Gas

Chromatograph with autosampler and coupled to Agilent 5975 mass selective detector (Agilent Technologies) with Agilent HP Ultra 2 capillary column (30 m length, 0.20 mm diameter, 0.11 μm film) and operating in EI mode at 70 eV. Five microlitres sample was injected in split mode 8:1 (v/v). Injection temperature was 250 °C, carrier gas flow (He) was held at constant flow 1.2 mL/min. The initial oven temperature was 80 °C hold for 0 min, ramped at 3 °C/min to 150 °C hold for 1 min and finally ramped 20 °C/min to 280 °C hold for 26 min.

2.4. *Theobromine and Caffeine Content*

Cacao seeds were milled (IKA) to obtain cocoa liquor. Three grams of cocoa liquor was defatted with petroleum ether (60-80 °C) in a Soxhlet for 4 h. The cocoa powder was air-dried and stored in -20 °C [13]. Before injected, 1 g defatted cocoa powder was extracted with 80 mL mixture of acetone:water (80:20), sonicated, centrifugated and supernatant was kept for further use. This extraction was conducted twice [14]. Supernatant was filtered using PVDF before injected to LC-MS.

Determination of theobromine and caffeine concentration determined using LC-MS 2020 (Shimadzu)[15]. Five microlitres samples were injected on Waters C-18 column, temperature 40 °C. A mobile phase of water/acetonitrile (85:15, v/v) at a flow rate of 0.8 mL min⁻¹ and UV detection at 254 nm were applied. Total run time 5 min. Quantification was performed by external calibration with standard solutions of theobromine (50, 100, 120, 150, 200 and 250 ppm) and caffeine (20, 40, 80, 120 and 160 ppm).

2.5. *Total Fat Content*

Analysis of total fat content conducted with SNI 01-2891-1992 method[16]. Two grams of samples were grounded, dried and placed in the thimble. Thimble placed in the soxhlet extractor and extracted for 6 hours with 150 mL of hexane. The extracted fat dried in an oven (105 °C) for 1 hour. The resulting residue, or crude fat, is determined by weight after drying.

2.6. *Data Analysis*

2.6.1. *GC-MS.*

Metabolite identification was analyzed using MS-Chemstation G1701-DA with WILEY spectral libraries. Data analysis were conducted by Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) using R Studio ver. 1.1.442 [17]. The HCA analysis was based on Euclidean distances with Ward clustering algorithm. Data were transformed to log base 2 prior to analysis.

2.6.2. *Theobromine, Caffeine and Total Fat Content.*

Analysis were conducted using analysis of variance and post-hoc test by Duncan's multiple range test [18] using SAS ver. 9.4.

3. **Result and Discussion**

3.1. *Identification of metabolites*

Total of 49 peaks were identified in GC-MS metabolite analysis but only 23 putative metabolites (table 1) were further analysed because of their high-quality chromatogram. The identified metabolites are mostly fatty acid (palmitic acid, stearic acid, oleic acid, margaric acid, cis-vaccenic acid, elaidic acid) and alkaloid (theobromine, caffeine, paraxanthine and xanthine). Reference [19] mentioned that cacao powder contained high level of fatty acid. Palmitic acid, stearic acid, oleic acid were the most important fatty acids in unroasted cocoa beans [20]. Meanwhile major alkaloids found in cacao seeds (cotyledons and embryonic axis), was theobromine and caffeine [21].

Table 1. List of metabolites assigned in cacao methanol-formic acid-water extract by GC-MS.

Code	Name	Formula	Class
C01	13-Methyloxacyclo tetradecane-2,11-dione	C ₁₄ H ₂₄ O ₃	Ester amide
C02	Margaric acid	C ₁₇ H ₃₂ O ₂	Unsaturated fatty acid
C03	Butylated hydroxytoluene	C ₁₅ H ₂₄ O	Phenol
C04	Caffeine	C ₈ H ₁₀ N ₄ O ₂	Alkaloid
C05	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	Unsaturated fatty acid
C06	Cytidine	C ₉ H ₁₃ N ₃ O ₅	Glycosylamines
C07	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)	C ₁₄ H ₂₄ O ₃	Flavonoid
C08	Elaidic acid	C ₁₈ H ₃₄ O ₂	Unsaturated fatty acid
C09	Gibberellic acid	C ₁₉ H ₂₂ O ₆	Pentacyclic diterpene
C10	Hexamethylcyclotrisiloxane	C ₆ H ₁₈ O ₃ Si ₃	Organosilicon
C11	Hydroxymethylfurfural	C ₆ H ₆ O ₃	Aldehydes (furans)
C12	Melamine	C ₃ H ₆ N ₆	Amines
C13	Methylbutylpropylamine	C ₈ H ₁₉ N	Amines
C14	N-Ethylformamide	C ₃ H ₇ NO	Formamides
C15	Stearic acid	CH ₃ (CH ₂) ₁₆ COOH	Saturated fatty acid
C16	Octane-1-D, 4-(methyl-D)	C ₉ H ₂₀	Organophosphorous compound
C17	(9Z)-9-Octadecenal	C ₁₈ H ₃₄ O	Fatty aldehydes
C18	Oleic acid	C ₁₈ H ₃₄ O ₂	Unsaturated fatty acid
C19	O-Propan-2-yl ethyl sulfanylmethanethioate	C ₆ H ₁₂ OS ₂	-
C20	Palmitic acid	C ₁₆ H ₃₂ O ₂	Saturated fatty acid
C21	Paraxanthine	C ₇ H ₈ N ₄ O ₂	Alkaloid
C22	Xanthine	C ₅ H ₄ N ₄ O ₂	Alkaloid
C23	Theobromine	C ₇ H ₈ N ₄ O ₂	Alkaloid

3.2. Principal Component Analysis and Hierarchical Cluster Analysis

Principal Component Analysis (PCA) plot explained 82.8% of total variance where PC 1 contributed 67.3% and PC 2 contributed 15.5% (Figure 1). Fine cacaos (DR 2, DRC 16, PNT 16, PNT 17) were separated from bulk cacaos (KW 516, KW 617, MCC 02, SUL 01) based on metabolite percentage peak area of the GC-MS chromatogram.

Metabolite marker for cacao types were further analysed in hierarchical clustering analysis (HCA) showed in heat map graphic (Figure 2). Cacao clones were divided into two distinct cluster based on Euclidian distance. The first cluster consisted of fine cacaos (DR 2, DRC 16, PNT 16, PNT 17) and the second consisted of bulk cacaos (KW 516, KW 617, MCC 02, SUL 01). This grouping strengthened the PCA result.

Metabolites were separated into two clusters. Cluster 1 consisted of theobromine, caffeine and palmitic acid which were found in both bulk and fine cacaos meanwhile the second consisted of the rest of the metabolites (Figure 2). These metabolites showed different correlation with certain clones. Metabolites found mostly in bulk cacaos are fatty acids (8-heptadecenoic acid, stearic acid, oleic acid), butylated hydroxytoluene, gibberellic acid and hydroxymethylfurfural), while cytidine was mostly found in fine cacaos. In this experiment, only saturated fatty acid (palmitic acid) was detected in fine cacaos. In Criollo cacao, which was a fine cacao type, saturated fatty acids performed higher value than the unsaturated, with palmitic and stearic acids as the main fractions [22]. Characterization of bulk cacao bean from 12 region of South Sulawesi, Indonesia showed their dominant fatty acids were stearic acid, oleic acid and palmitic acid [23].

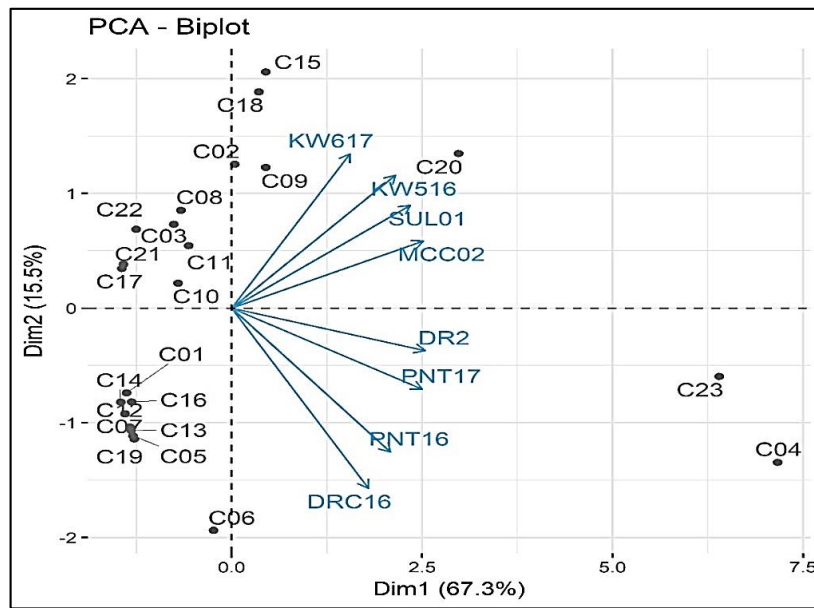


Figure 1. Distribution of 23 metabolites and eight cacao clones in principal component analysis bi-plot showed the correlation between specific metabolites and cacao types. PC1, 67.3% explained variance. PC2, 15.5% explained variance.

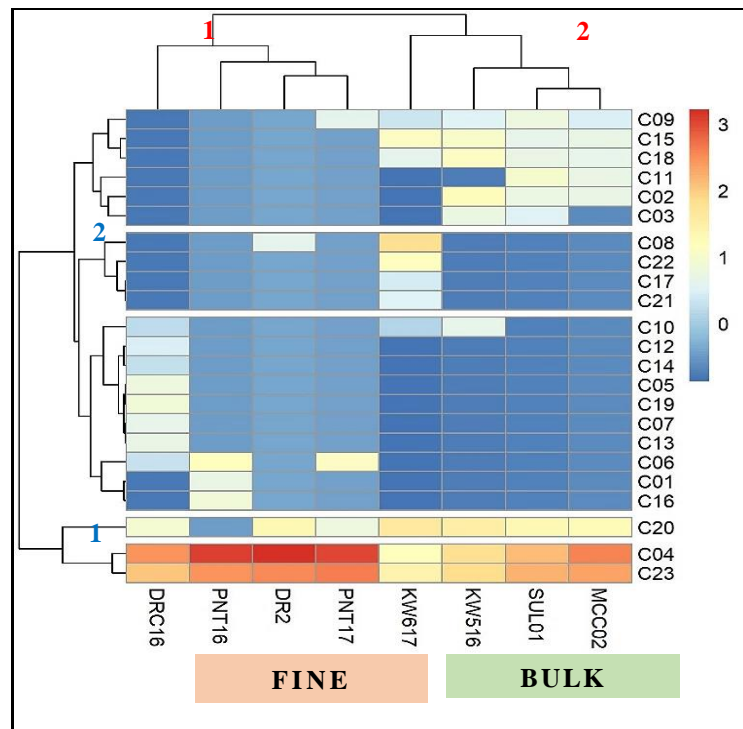


Figure 2. Several metabolites were able to differentiate bulk and fine cacao based on hierarchical cluster analysis using Euclidian distance (C04= caffeine, C15= stearic acid, C18= oleic acid, C20= palmitic acid, other codes were in Table 1)

Metabolites that could be used as marker should be able to perform distinct trend on different cacao types. Therefore, only four putative metabolites were recommended as metabolite marker. Candidates for fine cacao metabolite marker was caffeine meanwhile fatty acid (palmitic acid, oleic acid

and stearic acid) were the candidate for bulk cacao metabolite marker. However, these metabolites need further analysis in targeted metabolomics.

3.3. Theobromine, Caffeine and Total Fat Content

Metabolite marker candidates from GC-MS analysis were analysed to validate their correlation with cacao types. In general, result of the GC-MS analysis was in accordance with targeted metabolite analysis. Boxplot of caffeine content and theobromine/caffeine value showed no significant differences between fine and bulk cacaos (figure 3a, 3d). Fine cacaos contained higher theobromine content than bulk cacaos (figure 3b). Fine cacaos caffeine contents and theobromine/caffeine value were more variable than bulk cacaos. Opposite trend showed by theobromine content where fine cacaos performed smaller variability than bulk cacaos.

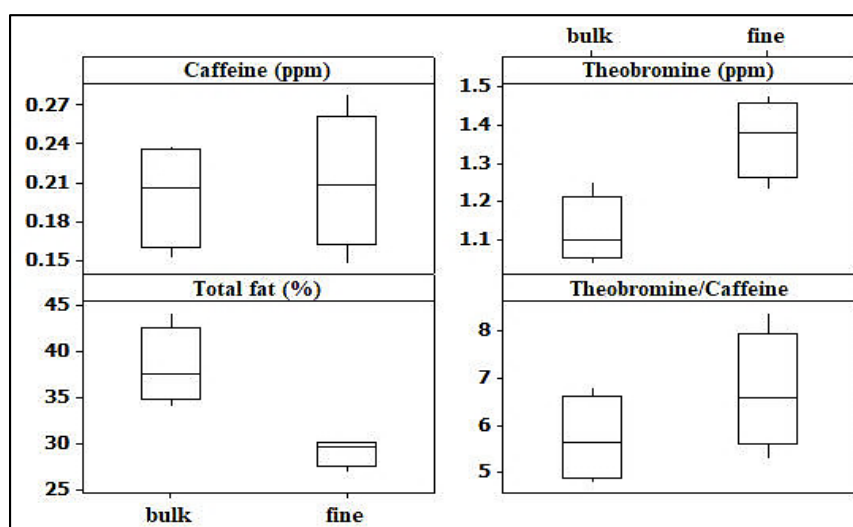


Figure 3. Boxplots showing different respond of fine and bulk cacao in their (a) caffeine, (b) theobromine, (c) theobromine/caffeine, (d) total fat content.

Boxplot of total fat content showed significant difference between fine and bulk cacaos where bulk cacaos contained higher total fat than fine cacaos (Figure 3c). This result is similar to [24] where Amazonian region cacaos contained higher fat levels than Trinitario-Criollo and Bahian genotypes. Amazonian region cacaos are Forastero cacao which categorized as bulk cacaos while Trinitario-Criollo categorized as fine cacaos.

Total fat content of the bulk and fine cacao was around 30%. Generally, the fat content of cacao bean was 40-50% [25]. However, some study reported the range of fresh cocoa bean total fat content was approximately 30%–32% [26] and 20-38.1% [23]. Total fat content variability in bulk cacaos was higher than in fine cacaos (figure 3c). Khan et al. [27] reported that Upper Amazon Forastero group had higher butterfat content in cotyledons compared to Refractario and Trinitario groups. The variability pointed to different cacao clones in each cacao types were explained by analysis of variance and post-hoc test result.

Cacao clones performed different respond in caffeine, theobromine, total fat content and theobromine/caffeine value (table 2). Highest total fat content showed by MCC 02 (bulk) and the lowest was by PNT 17 (fine). Duncan Multiple Range Test of total fat content showed that all cacao clones segregated differently according to their types. This trend was in accordance with the GC-MS result where fatty acids (stearic acid, oleic acid, palmitic acid) of the bulk cacaos were higher than the bulk cacaos (figure 2).

Among cocoa compounds, caffeine and theobromine are involved in cocoa flavour development [28]. The highest caffeine and theobromine content in this study showed by DR 2 which was a fine cacao. Caffeine content of each cacao clones in cacao type showed different trend. Not all bulk cacaos

contain low caffeine content, such as KW 617 which was not significantly different from DR 2. Otherwise, not all fine cacaos contain high caffeine content, such as PNT 17 which showed the lowest value. However, in general fine cacaos contain higher caffeine content.

Table 2. Bulk and fine cacao clones performed different trend on caffeine, theobromine, theobromine/caffeine and total fat content^a.

Cacao Type	Clone	Caffeine (ppm)		Theobromine (ppm)		Theobromine/Caffeine		Total Fat (%)	
Bulk	KW516	0.183 ± 0.002	d	1.103 ± 0.073	cd	6.018 ± 0.267	bc	37.17 ± 0.833	b
	KW617	0.238 ± 0.004	ab	1.251 ± 0.055	bc	5.038 ± 0.009	cd	34.03 ± 0.674	c
	MCC02	0.229 ± 0.006	bc	1.095 ± 0.060	cd	4.550 ± 0.912	d	44.26 ± 0.748	a
	SUL01	0.152 ± 0.004	e	1.037 ± 0.029	d	6.831 ± 0.026	b	37.97 ± 0.984	b
Fine	DR2	0.279 ± 0.007	a	1.479 ± 0.060	a	5.318 ± 0.194	cd	29.38 ± 1.627	d
	DRC16	0.209 ± 0.011	c	1.394 ± 0.015	ab	6.709 ± 0.002	b	30.12 ± 0.355	d
	PNT16	0.208 ± 0.006	c	1.365 ± 0.080	ab	6.579 ± 0.034	b	30.08 ± 1.046	d
	PNT17	0.147 ± 0.002	e	1.232 ± 0.003	bc	8.336 ± 0.007	a	26.91 ± 0.055	e

^aData are expressed as the mean of triplicate ± SE. Means followed by the same letter at the same column are not significantly different ($P = 0.05$) as determined by Duncan's Multiple Range Test.

High variability of theobromine content in bulk cacaos was because of KW 617 value. Its theobromine content even larger than fine cacao PNT 17. The result of theobromine analysis (table 2) was not linear to those of GC-MS where no significant difference showed by bulk and fine cacaos (figure 2). This trend was also showed by caffeine content where there was no linear relationship between GC-MS and caffeine analysis result.

Study on the correlation between cocoa genotype and theobromine/caffeine mentioned that Criollo (fine) has lower theobromine concentration and the most caffeine content, opposite to the Forastero (bulk), which have more theobromine content and less caffeine concentration[29]. Trinitario which was the hybrid of Criollo and Forastero showed intermediate T/C levels.

Theobromine content, caffeine content and T/C in this study which were not as distinct as total fat content were assumed connected to their ancestor. Indonesian fine cacao is Trinitario types which were a hybrid between Criollo (fine cacao) and Forastero (bulk cacao) [6]. Fine cacao showing the lowest T/C level was DR2 which originated from the biparental cross of Venezuelan Cundeamor and local Java Criollo [30]. This clone was developed at Central Java, Indonesia in 1912 by van der Hall [31]. Meanwhile bulk cacao showing high T/C level was SUL 01. Study on parentage analysis of Sulawesi cacaos mentioned that parent of Sulawesi cacao clones mainly hybrids of Trinitario and two Upper Amazon Forastero groups[32]. Their Simple Sequence Repeat based parentage analysis revealed that the parent of SUL 01 was Nanay, which was bulk cacao of upper Amazon Forastero.

4. Conclusions

Twenty-three metabolites of eight cacao clones, extracted with methanol-formic acid-water by GC-MS analysis, were identified. These metabolites clustered the cacao clones into 2 different groups according to their types, i.e. bulk and fine, respectively. Three metabolites namely stearic acid, oleic acid and palmitic acid, which were fatty acid, related to bulk cacaos. The trend of the fatty acids was in

accordance to the analysis result of total fat content. Fine cacaos contained lower total fat content than bulk cacaos. These metabolites could be further analyzed as selection criteria of cacao plant breeding program in order to support the sustainability of Indonesian Java fine flavor cocoa.

References

- [1]. ICCO 2017 Production of Cocoa Beans 2015/2016. Quarterly Bulletin of Cocoa Statistics, Vol. XLIII (3) Cocoa year 2016/17 (Abidjan: ICCO)
- [2]. Badan Pusat Statistik 2017 Ekspor Komoditi Pertanian Berdasarkan Kode HS, Sub Sektor Perkebunan Januari-Desember 2016 (Jakarta: BPS)
- [3]. Bartley B G D 2005 The Genetic Diversity of Cacao and Its Utilization (London: CABI Publishing) p 341
- [4]. Smulders M J M, Esselink D, Amores F, Ramos G, Sukha D A, Butler D R, Vosman B and van Loo E N 2008 *INGENIC Newsletters* **12** 1-13
- [5]. Mawardi S Winarno H Suhemdi D 1994 Proc Intl Workshop on Cocoa Breeding Strategies 18–19 Oct 1994 Kuala Lumpur Malaysia (Reading: INGENIC) pp 81-8
- [6]. Susilo A W, Anita-Sari I and Mawardi S 2013 Prosiding Simposium Ekspo Kakao 2012 (Jember: Pusat Penelitian Kopi dan Kakao Indonesia) pp 110-21
- [7]. Wood GA, Lass RA 2008 *Cocoa* (New Jersey: John Wiley & Sons)
- [8]. Brummer E C, Barber W T, Collier S M, Cox T S, Johnson R, Murray S C, Olsen R T, Pratt R C, and Thro A M 2011 *Frontiers Ecol. Environ.* **9** 561-8
- [9]. Anita-Sari I, Susilo A W and Mawardi S. 2015a *Kakao: Sejarah, Botani, Proses Produksi, Pengolahan dan Perdagangan* (Yogyakarta: Gadjah Mada University Press) pp 36-61
- [10]. Fiehn O 2002 *Plant Mol. Biol.* **48** 155–171
- [11]. Wang L, Nägele T, Doerfler H, Fragner L, Chaturvedi P, Nukarinen E, Bellaire A, Huber W, Weizmann J, Engelmeier D 2016 *Plant J.* **87** 318-32
- [12]. Mayorga-Gross A L, Quirós-Guerrero L M, Fourny G and Vaillant F 2016 *Food Res. Int.* **89** 901-9
- [13]. Luna F, Crouzillat D, Cirou L and Bucheli P 2002 *J Agric. Food Chem.* **50** 3527-32
- [14]. Nazaruddin R, Yatim A M, Said M and Hok HC 2001 *Malays. J Anal. Sci.* **7** 377-86
- [15]. Camu N, De Winter T, Addo SK, Takrama JS, Bernaert H and De Vuyst L 2008 Fermentation of cocoa beans: influence of microbial activities and polyphenol concentrations on the flavour of chocolate *J Sci. Food Agric.* **88** 2288-97
- [16]. Badan Standardisasi Nasional 1992 Standar Nasional Indonesia (SNI) 01-2891-1992: Cara Uji Makanan dan Minuman (Jakarta: BSN)
- [17]. Everitt B S and Hothorn T 2010 *Analysis Using R.* (Boca Raton: CRC Press)
- [18]. Gomez K A and Gomez A A 1984 *Statistical Procedures for Agricultural Research* (New Jersey: John Wiley & Sons)
- [19]. Shadkani F and Helleur R 2009 *J Chromatogr. A* **1216** 5903-10
- [20]. Torres-Moreno M, Torrescasana E, Salas-Salvadó J and Blanch C 2015 *Food Chem.* **166** 125-32
- [21]. Xin-Qiang Z, Koyama Y, Nagai C, Ashihara H 2004 Biosynthesis, accumulation and degradation of theobromine in developing *Theobroma cacao* fruits *J Plant Physiol.* **161** 363-9
- [22]. Liendo R, Padilla F C and Quintana A 1997 *Food Res. Int.* **30** 727-31
- [23]. Ristanti E Y, Suprapti S and Anggraeni D 2016 *J Industri Hasil Perkebunan* **11** 15-
- [24]. Pires J L, Cascardo J C M, Lambert S V and Figueira A 1998 *Euphytica* **103** 115–21
- [25]. Afoakwa E O, Paterson A, Fowler M and Ryan A 2008 *Crit. Rev, Food Sci. Nutr.* **48** 840-57
- [26]. Lopez A S and Dimick P S 1995 *Enzymes, Biomass, Food and Feed* (Weinheim: VCH) pp. 561–77
- [27]. Khan N, Motilal L A, Sukha D A, Bekele F L, Iwaro A D, Bidaisee G G, Umaharan P, Grierson L H and Zhang D 2008 *Plant Genet. Resour.* **6** 175-186
- [28]. Davrieux F, Assemat S, Sukha D A, Bastianelli D Boulanger RE et al. 2007 Near Infrared Spectroscopy. Proc 12th Intl Conf on Near Infrared Spectroscopy (Chichester: IM Publications) pp 382–6

- [29]. Carrillo L C, Londoño-Londoño J and Gil A 2014 Food Res. Int. **60** 273-80
- [30]. Susilo AW, Zhang D, Motilal LA, Mischke S and Meinhardt LW 2011 Trop. Agric. Devel. **55** 84-92
- [31]. Hall C J van 1913 Eerste verslag van de cacao-selectie Meded Proefstn Mid. Java **10**p 45
- [32]. Dinarti D, Susilo A W, Meinhardt L W, Ji K, Motilal L A, Mischke S and Zhang D 2015 Breed.Sci. **65** 438-46

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