

Sensory profiles of cocoa genotypes in Indonesia

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Manuscript received: 28 October 2021. Revision accepted: 12 December 2021.

Abstract. Sari IA, Murti RH, Misnawi, Putra ETS, Susilo AW. 2022. Sensory profiles of cocoa genotypes in Indonesia. *Biodiversitas* 23: 648-654. The Cocoa flavor is influenced by genetic factors and has an important role in increasing the added value of beans as specialty products. For this reason, superior genotypes with specific and unique flavors should be identified as fine flavor cocoa (FFC) products. This study was conducted to obtain genotypes with sensory quality and specific profiles. Ten clones under three groups were used: Forastero (KW 516 and KEE 2), Trinitario with purple beans (ICCRI 03, ICCRI 07, ICCRI 09, MCC 02, Sulawesi 1, Sulawesi 2, and TSH 858), and Trinitario with white beans (DR 2). Genotypes were assigned using a completely randomized block design with three replications. A sensory test was performed by three trained and certified panelists using the Indonesian Coffee and Cocoa Research Institute (ICCRI) and Guittard Chocolate Company standards. The attributes, acidity, bitterness, astringency, fresh fruit, browned fruit, floral aroma, woody aroma, spicy, nutty taste, and browned/roasted appearance of cocoa were evaluated on a scale of 1-10. Data were examined through ANOVA, flavor character profiling, PCA Biplot, and dendrogram analysis in STAR 2.0.1, Microsoft Excel 2019, and R Studio. Results showed that the genotypes significantly influenced the chemical content of the beans, and each genotype had a specific flavor character. The genotypes were classified into three groups based on flavor characters via PCA biplot and dendrogram analysis. Two groups of aromatic genotypes had the potential to develop FFC products with weak (DR 2) and strong cocoa taste (ICCRI 09, ICCRI 03, ICCRI 07, MCC 02, and TSH 858). Conversely, the nonaromatic cocoa groups with strong taste were KEE 02, KW 516, Sulawesi 1, and Sulawesi 2. Therefore, the intensity of floral and nutty aromas significantly affected the overall quality of cocoa flavor.

Keywords: Fermented bean, fine flavor cocoa, germplasm, sensory analysis, *Theobroma cacao* L.

INTRODUCTION

Cocoa is the third-most important commodity, following oil palm and rubber (Nugraheni et al. 2013; Oktaviani et al. 2014), occupying >90% of plantations managed by farmers. In Indonesia, the quality of cocoa beans is lower than that in other countries because physical attributes are prioritized as standards (SNI 2323: 2008 Amendment 2010). A previous study showed that the increase of the added value of cocoa as a specialty product or fine flavor cocoa (FFC) is determined by flavor quality. The FFC is characterized by balanced chocolate taste with unique flavors, such as fruity (fresh and browned fruit), floral, herbal, and woody aromas (ICCO 2020). It is influenced by variety type, genetic origin, morphological characteristics of plants, flavor, chemical characteristics of beans, color of beans and nibs, fermentation degree, drying, acidity, and taste defects (ICCO 2020). New and unique fine cocoa flavor sources are in demand by global fine flavor chocolate industry (Qin et al. 2017). Approximately 5%-7% FFC of the world cocoa production is needed, and this demand increases yearly; one of the 17 FFC producer countries is Indonesia (ICCO 2020).

Indonesia is the sixth-highest cocoa producer in the world. In 2019, it produced 2×10^5 tons (4.26%) of cocoa, and 1% was a specialty product. It can supply FFC of up to 10% of the total national production (ICCO 2020). Initially,

Indonesia is known not only as a producer of FFC based on FFC genotypes but also as a producer of special-origin cocoa; in particular, Jembrana in Bali, West Sumba in East Nusa Tenggara, Payakumbuh in West Sumatra, and Soppeng in South Sulawesi are internationally recognized as FFC producers.

Cocoa beans have unique characters based on genetics, origin, certification, and flavors (Muñoz et al. 2019; Kadow 2020). Fresh ones influence the quality of dry beans. Fresh beans consist of two main components: pulp and cotyledons, which affect the formation of flavor precursors in fermentation, drying, and roasting. The pulp comprises 12% monosaccharides and disaccharides, 2% citric or organic acids, esters, aldehydes, methyl ketones, alcohols, and terpenes (Kadow et al. 2013). Moreover, fresh beans have an astringent taste because of their phenolic content, especially anthocyanins. During fermentation, the pulp is degraded to form lactic acid and acetic acid and generate heat (Kadow et al. 2013). Consequently, beans die, and proteins and carbohydrates decompose into peptides and amino acids, lowering sugar levels as precursors of chocolate aroma (Afoakwa et al. 2008).

Aroma is an important cocoa bean attribute that determines its quality (Alayo-Casto et al. 2019). It is produced through complex biochemical processes and chemical reactions in the post-harvest period.

Preconditioning, fermentation, drying and industrial processes such as roasting, grinding and product formulation are crucial (Hernandez et al. 2019). Differences influence this process in genotype, chemical content, environmental conditions, cultivation, and other stages (Aprotosoie et al. 2016). Chemical compounds cause the flavor with three sensations: taste and odor (Yin et al. 2017). The protein, carbohydrate, polyphenol, and fat contents and enzymatic activity of beans are affected by genotype. The production of flavor precursors formed during fermentation and other processing operations is influenced by the chemical composition of fermented pulp and beans. In turn, the formation of organoleptic quality is directly affected by these precursors (De Vuyst and Weckx 2016). Therefore, organoleptic quality influences the final flavor of products.

Sensory or organoleptic analysis is a scientific tool used to identify differences in flavor attributes and test consumers' acceptance of a cocoa product (Leite et al. 2013). The present study aims to provide each genotype's taste and flavor characteristics and select clones that have specific and unique aromas and may be produced as FFC.

MATERIALS AND METHODS

Study area

This study was conducted at Kaliwining Research Station, the Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia which had climate types C to D according to Schmidt and Ferguson and an altitude of 45 m above sea level. Fruits were harvested and processed from November 2020 to January 2021. Ten genotypes with different genetic origins from the cocoa germplasm collection of the ICCRI were used: KW 516 (Forastero var Amelonado), KEE 2 (Introduced Forastero), ICCRI 03, ICCRI 07, ICCRI 09, MCC 02, Sulawesi 1, Sulawesi 2, TSH 858 (Trinitario with purple beans), and DR 2 (Trinitario with white beans). They were assigned using a completely randomized block design with three blocks as replications.

Procedures

Pulp/bean ratio

The pulp/wet bean ratio was calculated by comparing the pulp weight with the bean in 15 pod samples per genotype.

Fermentation

Beans of 100-150 pods per genotype were fermented in a 70 cm × 70 cm × 50 cm box developed by the ICCRI for 96 h and then turned 48th h. After 96 h, the beans were moved and spread on a tarpaulin with a thickness of two bean layers, and dried in a dryer until the moisture content was 7%.

Polyphenol content analysis

Total polyphenol was determined through spectrophotometry by previously described methods (Singleton and Rossi 1965) using fermented bean samples. A fat-free sample and 40 mL of 80% acetone were placed in a glass beaker and sonicated for 30 min. During sonication, the solution was kept cold by filling the vessel with ice water, and the extract was obtained via vacuum filtration using Whatman filter paper no. 1. Then, 1 mL of the extract was placed in a 100 mL glass beaker and dissolved with 70 mL of distilled water. The extracted polyphenols were reacted with 5 mL of 0.2 N Follin Ciocalteau reagent for 2 min. Subsequently, 15 mL of saturated Na₂CO₃ solution was added to stabilize the color formed and left for approximately 2 h. The absorbance at 765 nm was measured. The standard catechins (+/-) with known concentrations were used for calculations.

Fermentation index analysis

Fermentation index (FI) of cocoa beans was measured following Romero-Cortes et al. (2013). Cocoa powder (~50 mg) was mixed with 5 mL of acidic methanolic solution (3% hydrochloric acid, HCl). The solution was vortexed and stored in a refrigerator for 18 h. Reddish/brownish solution was then centrifuged at 2383g/3500rpm for 10 min. The absorbance of the supernatant obtained was measured using a Thermo Fisher Spectro Nic 200 at 460 and 530 nm. The FI was calculated by dividing the absorbance of 460 nm to 530 nm.

Fat content analysis

The fat content was measured at the Post-Harvest Laboratory of the ICCRI using a total fat analysis procedure based on the Indonesia National Standard (SNI) for cocoa beans (SNI 2010). The sample used was composed of 3-5 g of beans ground to a maximum size of 150 microns and poured into a 300 mL beaker. Fat was hydrolyzed and extracted by the procedure recommended by the SNI.

Table 1. Ten cocoa genotypes with different genetic backgrounds

| Genotype | Description |
|------------|--|
| DR 2 | Trinitario, white beans, selection result from the Criollo half-sib population |
| ICCRI 03 | Trinitario, purple beans, selection result from the Trinitario population |
| ICCRI 09 | Trinitario, purple beans, selection result from the Trinitario population |
| MCC 02 | Trinitario, purple beans, exploration result in the Sulawesi region |
| Sulawesi 1 | Trinitario, purple beans, exploration result in the Sulawesi region |
| Sulawesi 2 | Trinitario, purple beans, exploration result in the Sulawesi region |
| TSH 858 | Trinitario, purple beans, introduction clone |
| ICCRI 07 | Trinitario, purple beans, exploration result in North Sumatra |
| KEE 2 | Forastero, purple beans, introduction clone from PNG |
| KW 516 | Forastero, purple beans, exploration result in North Sumatra |

Paste processing and flavor test

Cocoa paste was used as a sample in a flavor test. It was prepared by manually peeling 500 g of beans to separate the cotyledons from the shell. Then, the cotyledons were roasted at 120°C for 12 min, crushed, and mashed with a grinder for 15 min (Misnawi and Ariza 2011). The paste was packed and stored at 5°C. Afterward, the samples were served individually to three trained panelists at 40°C without sugar. After testing each sample, the panelists were instructed to drink water and eat plain biscuits to neutralize the sense of taste and avoid bias.

Sensory analysis

Three trained and certified panelists carried out sensory analysis at the ICCRI using the flavor test standards developed by the ICCRI and Guittard Chocolate Company. The following attributes were evaluated with a scale of 1 to 10: cocoa, acidity, bitterness, astringency, fresh fruit, browned fruit, floral, woody, spicy, nutty, sweet, browned/roasted appearance, dusty, meaty, putrid, smoky, mouldy and global quality. The higher the value, the stronger the attribute.

Data analysis

Data were analyzed using one-way ANOVA via STAR 2.0.1 (IRRI), and Duncan's test was further carried out when a significant difference was found. The flavor quality profile was illustrated with a spider chart in Microsoft Excel 2019. The relationship between genotype and flavor character was examined using a PCA biplot in Microsoft Excel 2019. Flavor-based clustering was performed with a dendrogram through R Studio.

RESULTS AND DISCUSSION

The pulp/bean ratio was significantly different among the 10 genotypes tested (Table 2). KW 516 and KEE 2 clones belonging to the Forastero group had the highest pulp/bean ratio, whereas DR 2 (Trinitario with white

beans) had the lowest. A thick pulp content affects the duration of fermentation, the Forastero needs a longer fermentation time than Criollo and Trinitario. Alayo-Castro et al. (2019) found that Forastero and Criollo groups require 5-7 and 3-4 days of fermentation, respectively. A high pulp content blocks air exchange; consequently, the bean mass becomes more anaerobic, and its sugar content increases. Thus, the amount of acid in cotyledons increases after fermentation (Afoakwa et al. 2008).

Cocoa beans contain 30%-40% pulp (Gu et al. 2013), and pulp consists of 82%-87% of water, 10%-15% of sugar, 2%-3% of pentosan, 1%-3% of citric acid, and 1%-1.5% of pectin (Kongor et al. 2016). During fermentation, the pulp causes the death of cotyledons (Afoakwa et al. 2008); stimulates enzymatic biochemical transformation, which leads to the reduction of bitterness and astringency; and affects the formation of flavor precursors, such as free amino acids, peptides, and sugars (Brunetto et al. 2020). Moreover, volatile compounds in the pulp influence the formation of the fine aroma of cocoa beans (Kadow et al. 2013) through the migration of compounds into cotyledons during fermentation. The aromatic cocoa pulp likely affects the aroma development of cocoa beans (Chetschik et al. 2017) because of several compounds, including monoterpenes, methyl ketones, alcohol, and esters (Kadow et al. 2013).

Fermentation is one of the important stages determining flavor precursor formation (Batista et al. 2015; Fang et al. 2019). The fermentation index does not appear to be related to the pulp/bean ratio. In this study, the fermentation index of cocoa beans varied. The DR 2 and Sulawesi 2 had the highest significantly different indices with values of 1.97 and 1.40, respectively. By contrast, KW 516 and MCC 02 had the lowest index of 0.90 (<1; Table 2). The fermentation index measures the red or purple absorbance formed at two wavelengths. The two waves are then compared for the final determination of fermentation, with an index of 1 indicating the perfect level (Iflah and Tresniawati 2016).

Table 2. Analysis of pulp content, fermentation index, polyphenol content, and fat content of 10 cocoa genotypes

| Clone | Ratio pulp/Wet bean (%) | Fermentation index | Polyphenol content (%) | Fat content (%) |
|------------|-------------------------|--------------------|------------------------|-----------------|
| DR 2 | 21.37 f | 1.97 a | 5.93 c | 52.77 bcd |
| ICCRI 03 | 37.70 cd | 1.00 e | 6.23 bc | 47.63 ef |
| ICCRI 07 | 43.90 b | 1.30 c | 4.93 def | 46.33 f |
| ICCRI 09 | 35.30 d | 1.30 c | 4.80 ef | 50.23 de |
| KEE 02 | 45.20 ab | 1.10 d | 5.30 d | 56.00 ab |
| KW 516 | 48.70 a | 0.90 f | 6.63 ab | 53.73 bc |
| MCC 02 | 37.17 d | 0.90 f | 6.87 a | 55.50 ab |
| Sulawesi 1 | 32.97 de | 1.07 d | 4.33 g | 51.73 cd |
| Sulawesi 2 | 42.07 bc | 1.40 b | 4.50 fg | 57.17 a |
| TSH 858 | 29.67 e | 1.10 d | 5.10 de | 57.90 a |
| Average | 37.40 | 1.20 | 5.46 | 52.90 |
| CV (%) | 6.97 | 2.15 | 4.59 | 3.49 |
| F value | 29.10** | 452.72** | 38.29** | 13.66** |

Note: Numbers within the column with the same letter indicate that results are not significantly different at 5% level according to Duncan's test, ** denotes highly significant differences at 1% level

Fermented beans are indicated by 3/4 or more of the cotyledon slice surface, which is brown, hollow, and characteristically aromatic; conversely, unfermented beans are denoted by 1/2 or more of the slice surface, which is slate gray or grayish blue with a dense texture (SNI 2010). In addition, color or anthocyanin content is used as an indicator to estimate the fermentation results of cocoa beans. The anthocyanins were drastically degraded through fermentation (De Taeye et al. 2016). Therefore, the fermentation index, which shows the fermentation level, also affects the decrease in the astringent taste of cocoa beans. Fermentation causes a decrease in the intensity of bitter, sour, and astringent tastes (Alayo-Castro et al. 2019), so the higher the fermentation index is, the greater the taste intensity will be.

The clones MCC 02 and KW 516 with the lowest fermentation index caused of the highest polyphenol content of 6.87 and 6.63, respectively. Moreover, a low fermentation index indicated that an ideal fermentation process did not occur, as characterized by cocoa beans' high color intensity and anthocyanin content. The fermentation index of cocoa beans could not be directly related to the polyphenol profile (Febrianto and Zhu 2019). The polyphenol content depends on the intensity of anthocyanins that contribute to bitter and astringent tastes (Abhay et al. 2016). Polyphenols affect the astringency intensity of beans during fermentation and processing operations (Dwiatmoko et al. 2018; Febrianto and Zhu 2020). Indeed, they remarkably influence flavor and determine the intensity of bitter and astringent sensory of cocoa. Leite et al. (2013) also demonstrated that polyphenols are positively correlated with astringent, bitter, and green tastes. This finding is consistent with our results that KW 516 which had higher polyphenol content, showed a stronger astringency taste. Astringency is a dry taste in the mouth for a long time with a response to the lining that shrinks and is generally a contribution of tannin compounds. The amount of polyphenol content in beans varies in terms of the maturity level of fruits, varieties/cultivars, and growing environments (Oracz et al. 2015). Oracz et al. (2015) stated that the polyphenol content of the Forastero group is higher than those of Trinitario and Criollo groups. We also showed that the polyphenol content of cocoa beans was highly dependent on genotype. Similarly, Afoakwa et al. (2008) indicated that differences in genotypes significantly determine the amounts of polyphenols. However, this finding is in contrast with the fat analysis results. In particular, Sulawesi 2 and TSH 858 clones had the highest contents of 57.17% and 57.9%, which were not significantly different from KEE 2. Conversely, ICCRI 07 had the lowest fat content (46.33%) and inconsistent with that of ICCRI 03.

Each genotype had unique flavor characters (Figure 1). The clones in the Forastero group, namely, KW 516 and KEE 02 (Figure 1), were dominated by the main attributes of cocoa, such as bitterness, astringency, and browned/roasted aroma, but they showed no aromatic characters. The Forastero group also exhibits a strong basic

chocolate taste (Aprotosoae et al. 2016). It is used to manufacture cocoa mass, cocoa powder, cocoa butter, and milk/dark chocolate.

The DR 2, ICCRI 03, ICCRI 09, ICCRI 07, and MCC 02 had aromatic characters with a strong cocoa taste (Figure 1). Similarly, DR 2 showed potential for manufacturing products with floral, woody, nutty, and browned/roasted aromas. The ICCRI 07 could be used for fresh and browned fruit, woody, and nutty aromas. The ICCRI 09 and ICCRI 03 could be utilized for products with floral and browned/roasted aromas. MCC 02 could be products with fresh fruit, browned fruit, woody, nutty, and browned/roasted aromas. Sulawesi 1 and Sulawesi 2 were similar to those of KEE 02 and KW 516. By comparison, TSH 858 was dominantly characterized by fresh fruit, woody, and nutty aromas. The sensory test showed that ICCRI 03, ICCRI 09, and ICCRI 07 had a high global quality, equivalent to cocoa beans from Ghana and Ivory Coast as products at the international market level but not classified as FCCs. The Criollo, Trinitario, and national types are classified as "fine" or flavor cocoa and perceived as aromatic or smooth with fruity, floral, spicy, nutty, molasses, and caramel notes (Afoakwa et al. 2008). They are mainly used to manufacture dark chocolate, representing 5% to 10% of the global cocoa market (Seguine et al. 2014). A flavor character is specifically based on genotypes, growing conditions, and processing (Afoakwa et al. 2008). Genotypes affect the quality and intensity of flavor (Afoakwa et al. 2008) with a unique character. With all of its diversity, Flavor is extremely hard to include within a breeding program for cocoa; however, the reason and the fear of not having flavor as breeding criteria likely leads to the loss of previous chocolate flavor diversity (Seguine et al. 2014). With the importance of cocoa genotype on quality, studies have analyzed the genetic diversity of cocoa materials to optimize genetic resource utilization and propose core collections for breeding program development (Osorio-Guarin et al. 2017).

Sensory analysis is performed to identify differences in flavor attributes and test consumers' acceptance of a cocoa product (Leite et al. 2013) to provide statistically valid data (Sukha et al. 2017). In the PCA biplot analysis of the 10 genotypes in our study, the flavor profile maps based on taste and aroma differed. In the analysis of the taste characteristics of the 10 cocoa genotypes (Figure 2A), the Forastero group (KEE 02 and KW 516) had strong bitterness and astringency. The Trinitario group had strong cocoa and acidity taste, whereas DR 2 had the weakest taste character. The aromatic map (Figure 2B) revealed that each of the 10 genotypes had unique aroma potential. ICCRI 09, ICCRI 07, MCC 02, TSH 858, and DR 2 had aromatic potential with different intensities and complex aroma characters with varied dominance. ICCRI 09 was dominated by floral, slightly fresh fruit, browned fruit, sweet, and nutty aromas. Floral and fresh fruit aromas dominated the ICCRI 03.

The ICCRI 07 was dominated by fresh and browned fruit aroma, whereas MCC 02 was mainly characterized by browned and roasted fruit aroma. These aroma characters belong to the fine flavor group. The Forastero group produces beans with a lower aromatic potential; therefore, it has a lower quality (Aprotosoae et al. 2016). According to the ICCO session, cocoa products included in the FFC

category have a unique flavor quality characterized by the presence of fruity (fresh and browned fruit), floral, herbal, and woody aromas with a balanced chocolate taste (ICCO 2020). Kadow et al. (2013) indicated that bulk and FFC in trading are characterized by special aromas such as floral and fruity.

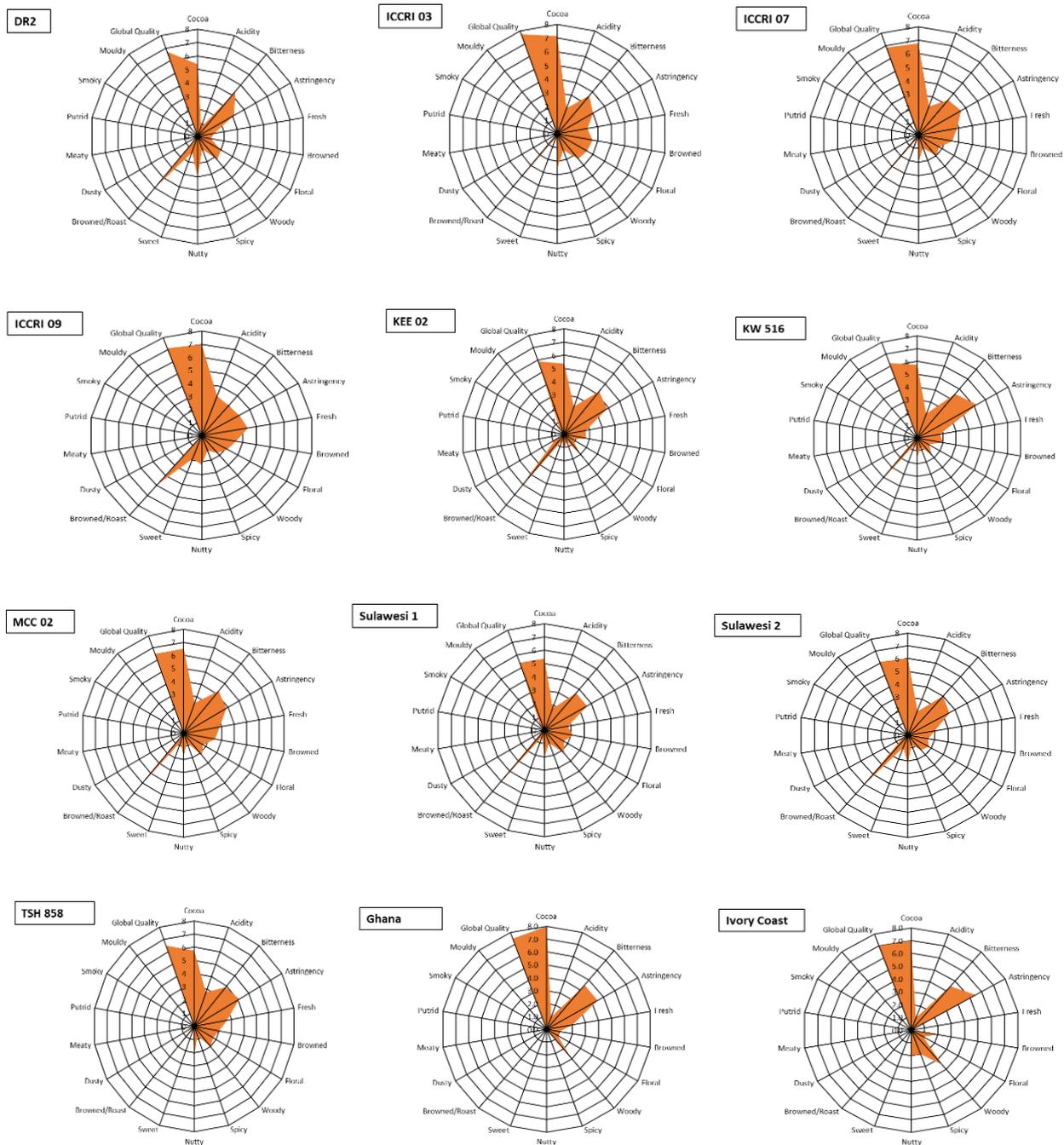


Figure 1. Sensory profiles of 10 Indonesian genotypes and references from Ghana and the Ivory Coast

Cluster analysis based on taste and aroma (Figure 3) showed three flavor groups from the 10 cocoa genotypes. The flavor is the total organoleptic impression, including tactual, gustatory, and olfactory aspects, influencing taste and aroma (Yin et al. 2017). In our study, DR 2 was in a different group with nine other genotypes. The DR 2 has a different character with others particularly in bean color and this character affect the flavor. Furthermore, the taste character or main attributes dominated KEE 02, KW 516, Sulawesi 1, and Sulawesi 2. In contrast, ICCRI 09, ICCRI 03, ICCRI 07, MCC 02, and TSH 858 were mainly characterized by aroma attributes and could be developed as FFC products. The aroma and taste of chocolate depend

on the origin and genotype of cocoa beans (Alayo-Castro et al. 2019). The Forastero group has a strong flavor character, whereas fine cocoa has a highly aromatic and smooth character (Afoakwa et al. 2008). The Forastero group also shows a strong basic chocolate flavor and is classified as bulk, basic, or ordinary cocoa grade; as such, it has a lower quality and needs a longer fermentation period to generate the flavor precursor (Muñoz et al. 2019). By contrast, the flavor of fine cocoa products is described as soft and highly aromatic (Seguine et al. 2014). The DR 2, ICCRI 03, ICCRI 07, ICCRI 09, MCC 02, and TSH 858 in the Trinitario group exhibited aromatic flavors and thus showed potential for the development of FFC products.



Figure 2. PCA Biplot on the (A) taste and (B) aroma of 10 cocoa genotypes: FF, Fresh Fruit; BF, Browned Fruit; FLO, Floral; WOO, Woody; SPC, Spicy; NUT, Nutty; SWT, Sweet; BRR, Browned/Roast; DUS, Dirty/Dusty; PUT, Putrid/Over Fermented; MLD, Moldy; and GQ, Global Quality

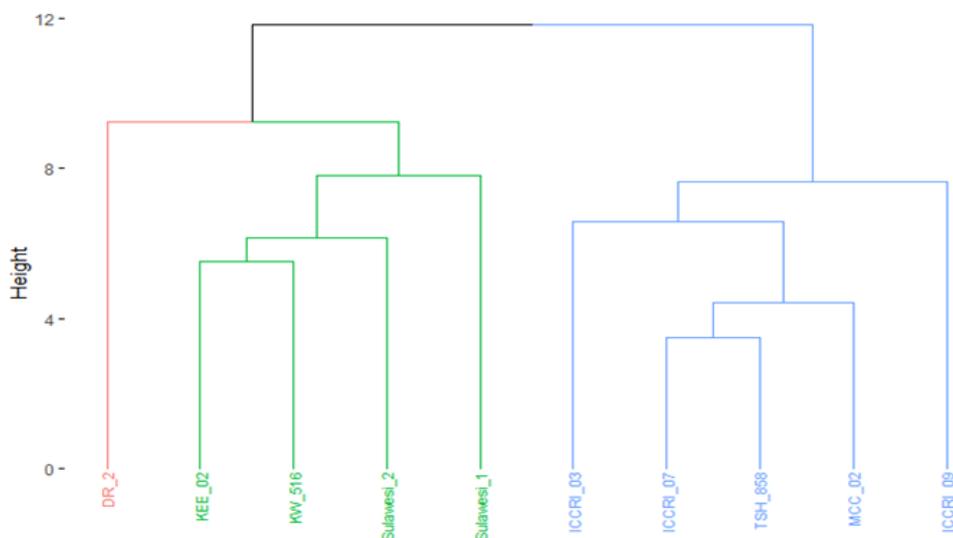


Figure 3. Clustering analysis of 10 genotypes based on flavor components

In conclusion, each cocoa genotype had unique chemical content and flavor character. The Forastero group exhibited nonaromatic characteristics and had strong astringency and bitter taste. The Trinitario group showed aromatic characters and had strong cocoa and acidity taste. The genotypes with potential for developing FFC products were the aromatic groups, namely, DR 2, ICCRI 03, ICCRI 07, ICCRI 09, MCC 02, and TSH 858.

ACKNOWLEDGEMENTS

The author would like to thank Bayu Setyawan, Ariza Budi Tunjungsari, Rosyidatul Putri, Avan Nurdiansyah, Fitratin, Sukarmin, Rudi Hartoyo, Ninik Kusmiansih, Husna, and Arik Ermawan from ICCRI for their research assistance.

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