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Sensory Characterization Cocoa (*Theobroma cacao* L.) from Various Clones During Fermentation

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Abstract. *Indonesia is one of the biggest producers of cocoa bean. However, this big production of cocoa is not followed by its quality to fulfill chocolate industry requirement in flavor. Fermentation of cocoa is the most important process for producing high-quality flavor in cocoa beans. The goal of this study was to find out the fermentation effects of four clones on the sensory and chemical profile of cocoa liquor. Sensory analysis was conducted using a Quantitative Descriptive Analysis (QDA) method and the volatile components of the cocoa liquor were extracted using Solid Phase Microextraction (SPME) and then followed by identification using Gas Chromatography - Mass Spectrometry (GC-MS). Correlation between volatile components and sensory attributes were determined by OPLS (orthogonal partial least square) test. The results of this study indicate that OPLS S-plot GC-MS determination 2,3,5-trimethylpyrazine and acetic acid were found as the active compounds. Fermented and unfermented cocoa liquor from various cocoa clones had different sensory attributes. All cocoa clones of fermented cocoa beans were dominated by attributes of specific cocoa aroma, cocoa, and acidity, whereas in unfermented cocoa, they were dominated by astringent and bitter.*
Keywords: *cocoa; fermentation; GC-MS; sensory.*

Type of the Paper: Regular Article.

1. Introduction

Indonesia is a major producer of cocoa (*Theobroma cacao* L.) beans. According to Badan Pusat Statistik [1], Indonesia's estimated cocoa production in 2021 was 688.210 tons. However, this high output is out of proportion to the quality of its cocoa beans, particularly in terms of fermentation. Only 10% of cocoa beans in Indonesia are fermented and this has an impact on the quality of Indonesian cocoa exported. The most crucial step in creating high-quality flavor in cocoa beans is fermentation. When roasted, unfermented cocoa beans do not produce a distinct aroma, but they retain a strong bitter and astringent flavor. These cocoa beans are not preferred by the manufacturers of cocoa [2]. In general, fermentation process in cocoa beans is intended to take off mucilage in cocoa beans and to produce a variety of flavor precursors in cocoa [3]. In general, the process of fermentation lasts 5 days [4]. Cocoa beans can be fermented spontaneously in wooden box baskets or containers, with or without the addition of microbial starter cultures. Cocoa powder, chocolate, and other derivative goods made from cocoa beans are produced by the processing of

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cocoa beans [5].

The chemical composition and sensory characteristics of cocoa beans can be influenced by variety, post-harvest processing, geographical location, and climate. For example, cocoa trees in West Africa are less aromatic and bitter than cocoa trees in Indonesia [5]. A variety of processing and biological conditions can affect sensory quality during the processing of fresh cocoa beans to the finished products. Thus, sensory characterization studies of fermented and unfermented various cocoa clones are essential.

2. Materials and Methods

2.1. Materials

Lindak Cocoa beans clones SUL 1, SUL II, ICCRI 03, and KW 617 were acquired from Indonesian Coffee and Cocoa Research Institute (Jember, Indonesia). Cocoa beans used in this research were harvested from ripe cocoa pods. Cocoa beans were separated into two categories: unfermented (dried directly in the dry house under the sunlight) and fermented (5 days fermented) groups. Cocoa beans were fermented on a small scale using the batch insert fermentation technique. A shade net was used to cover the cocoa beans before they were either placed in a wooden box (40 cm x 40 cm x 50 cm) at 45–48°C (fermented group). After that, the nibs were manually separated after the cocoa beans being dried for 5 days. The, the nibs were roasted. After roasting cocoa nibs for 25 minutes at 116°C, they were crushed to create cocoa liquor.

2.2. Sensory evaluation

The descriptive test was performed by six trained panelists (Indonesian Coffee and Cocoa Research Institute, Jember). Panelists were given training and attribute development [6]. The sensory training consisted of an introduction to cocoa liquor products from various countries such as Ghana, Madagascar, Ecuador, Indonesia, Belize, and Costa Rica to develop panelists' understanding on sensory attributes. The attributes evaluated in the cocoa liquor were cocoa, acidity, bitterness, astringent, nutty, browned fruit, sweet, floral, fresh fruit, woody, spicy, and aroma cocoa. Samples were assessed by six trained panelists and they were given a three-digit number code. The panelists were instructed to evaluate each sample on a 10-point scale (0: not detected, 1: slightly and may not be detected if felt again, 2: detected in the sample, 3-5: describes the characteristics of the sample, 6-8: dominant, 9-10: maximum of what is felt).

2.3. Determination of volatile components with GC-MS

The method in processing cocoa liquors in this research referred to the method compiled by Kusumaningrum et al. [7]. Cocoa liquor (5 g) was put in a 40 mL capacity vial. It is was heated at 60°C for 15 minutes. The SPME needle was inserted into the vial. Next, the SPME fiber was ejected by pressing the plunger and holding it in the Z-slot. Cocoa liquor was extracted for 30

minutes with the condition of the sample being heated in a water bath at a constant temperature of 60°C. The SPME fiber was reinserted by releasing the resistance in the Z-slot and returning the plunger to its original position. Next, the SPME needle was separated from the vial and the vial was removed from the water bath. Samples were injected at 1 µL with the splitless method.

Analysis of volatile components was done with GC-MS type Shimadzu GC-2010 with a splitless injector at 260°C, with the column temperature of 60°C for 5 minutes and continued to be raised until it reached 220°C. The detector temperature consisted of interface temperature and ionSource temperature of 200 °C with RTX-50 column (30 m x 0.25 mm i.d, 0.25 µm film thickness). Helium was employed as the carrier gas with a rate of 0.78 mL min-1. Identification of compounds was done using full scan mode with database from NIST Standard Reference Database 147.

2.4. Statistical analysis

SIMCA 13.0.2 and SAS software for Windows (Version 9.1.3) were used to study the data. This study employed a completely randomized design with four clones and two treatments. Each treatment was performed twice.

3. Results and Discussion

Fermentation of cocoa bean is first of the stages in cocoa post-harvest processing. It is usually done through spontaneous fermentation in heaps, boxes, baskets, trays, or on platforms [8]. Fresh cocoa beans have various components, such as polyphenols, alkaloids (methylxanthines), carbohydrates, and protein which contribute to the formation of specific cocoa flavors during processing [9]. According to Camu et al. [10] the pigment in raw cocoa beans ranges from white to purple, depending on the quantity of anthocyanin present. Because anthocyanin is changed into anthocyanidin during fermentation, the purple color of the bead is bleached [11].

The microorganism is used for spontaneous fermentation of cocoa, as well as their physiological roles. According to Papalexandratou et al. [8], lactic acid bacteria, acetic acid bacteria, and yeast colonize the cocoa bean fermentation process, producing lactic acid, acetic acid, and ethanol. Then, these compounds are diffused into the cocoa beans, killing the embryo of the seed. Following that, complex biochemical reactions and physical processes are initiated in the cocoa beans to produce color and flavor precursors. Other microbial metabolites like pyrazines and ester may enter the cotyledons of beans and function directly as flavor compounds or as flavor precursors [10].

The volatile components detected from the retention time in each of Sulawesi 1, Sulawesi 2, ICCRI 03, and KW 617 of cocoa liquor clones which were not fermented were 29, 28, 14, and 16 respectively. Meanwhile, the number of volatile components obtained in cocoa liquor after

fermentation in each of Sulawesi 1, Sulawesi 2, ICCRI 03, and KW 617 were 32, 30, 28, and 29 respectively. Several components contained in cocoa liquor have been reported from various studies (Table 1).

There were many volatile components that appearred in fermented sample such as acetic acid, tetradecanoic acid, octadecanoic aci4exadecenoicoic acid, benzoic acid, nonanoic acid, 3-hydroxy-2 butanone, 2-methyl-1-butanol, 2,3-butanediol, 3-methyl-1-butanol, 2-phenithyl alcohol, phenyl methanol, ethylphenyl acetate, 2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyrone, tetramethylpyrazine, and 2,3,5-trimethylpyrazine. In fact, volatile components such as acetic acid and pyrazine have been found to influence the aroma and taste of cocoa [12].

Aldehyde and ketone components also give cocoa flavor, such as 3-hydroxy-2-butanone, which contribute to the attribute of caramel [13] and nutty [14]. In general, these components are *strecker* degradation of amino acids during roasting [15]. *Strecker* degradation is one of the reactions related with Maillard reaction. It gives brown colour. Alcohol components can also come from fermentation from the results of microbial activity such as 3-methyl-1-butanol and 2,3-butanediol. The alcohol component can give cocoa a sweet taste perception [15].

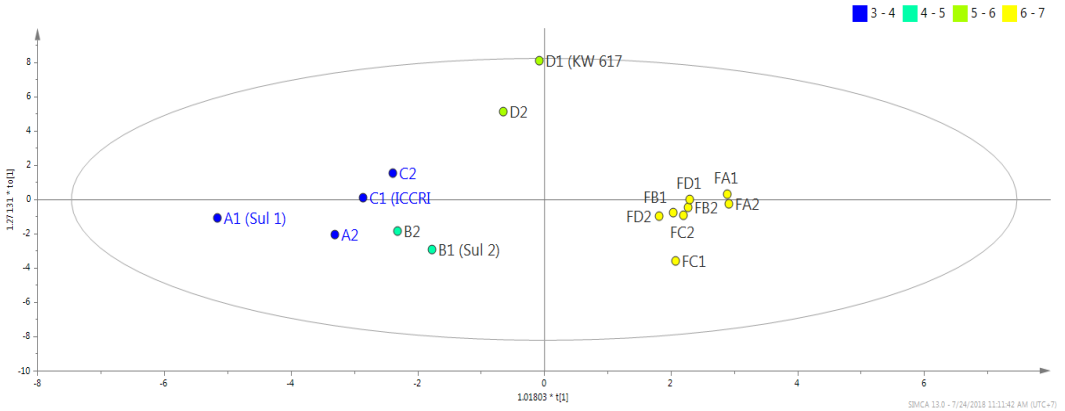
Table 1. Relative percentage of identified volatile components in cocoa liquor of Sulawesi 1, Sulawesi 2, ICCRI 03, and KW 617

Components	Reference	Unfermented (%area)				Fermented (%area)			
		a	B	c	d	A	b	c	d
Acid									
Acetic acid	[15]	10.86	18.07	0.07	0.67	38.68	42.72	39.96	37.31
Tetradecanoic acid	[14]	3.71	0.35	1.89	0.18	0.98	0.99	0.82	1.12
Octadecanoic acid	[16]	0	0.05	0	0.01	0.05	0.09	0.05	0.10
Hexadecanoic acid	[16]	2.9	0.77	0.44	1.17	1.12	0.55	0.84	1.2
2,3-methyl man-made acid	[14]	0	0	0	0	0.37	0	0	0.16
Benzoic acid	[17]	0	0.05	0	0.01	0.05	0.09	0.05	0.10
Nonanoic acid	[15]	1.13	0.60	0	0	1.76	1.90	1.65	1.18
1,2- benzenedicarboxylic acid		0.65	3.59	0.79	0.10	3.30	1.72	5.22	0
Aldehydes and Ketones									
3-hydroxy-2-butanone	[14]	1.91	3.82	0	0	4.65	4.16	6.49	5.55
2-undecanal	[18]	0.99	0	0	0	0.73	0.08	1.19	0
Alcohol									
2,3-butanediol	[15]	5.05	8.37	0	0	12.16	10.56	7.65	13.45
Linalool	[14]	0.88	0.57	0	0	0.39	0.72	0.22	0.44
2-methyl-1-butanol	[15]	0	0.20	0	0	0.55	0.42	1.33	0.93
3-methyl-1-butanol	[15]	0	0.05	0	0	0.07	0.13	0.73	0.11
Phenylmethanol	[19]	0.45	0.20	0	0	0.11	0.16	0.08	0.10
Ester									
Ethylphenyl acetate	[20]	1.40	0.44	0	0	0.27	0.17	0.33	0.18
Furanone dan Pyrone									
Dihydro-2(3H)-furanone	[13]	1.47	1.22	0	0	0	0	0	0
2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyrone	[15]	1.22	0.46	0	0	0.58	0.50	0.50	0.53
Pyrazine									
Tetramethylpyrazine	[13,14,21]	1.05	1.11	0	0	1.82	1.50	2.57	1.38
2,3,5-trimethylpyrazine	[15]	0	0	0	0	3.52	3.90	2.56	2.71

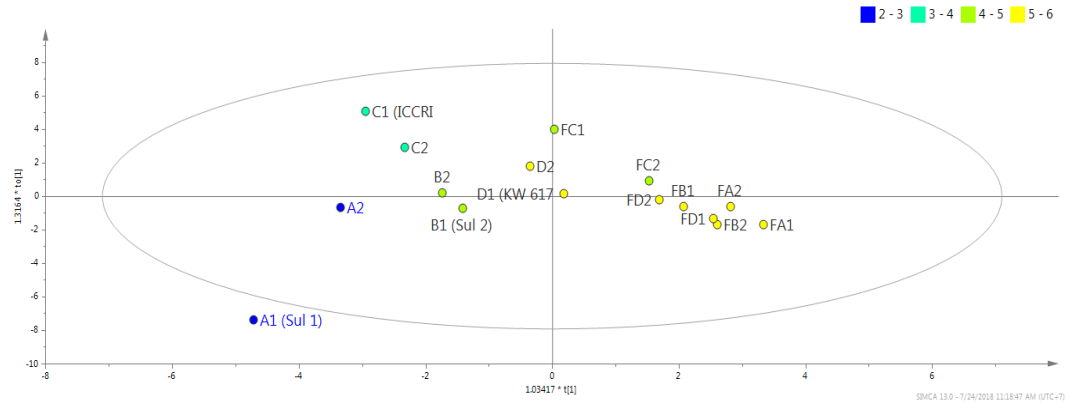
Note: a: Sulawesi 1, b: Sulawesi 2, c: ICCRI 03, d: KW 617

The results of the OPLS plot score on cocoa liquor with sensory attributes of cocoa flavor is presented in Fig. 1. The analysis of sensory attributes of aroma and taste was chosen because these attributes are important components produced from fermentation. The results of the score plot indicate that there was a separation of samples based on aroma and taste values in fermented and unfermented samples.

The sample with yellow color had a higher aroma and taste score, while the sample with blue color had a smaller aroma and taste score. The OPLS model can be seen with the R2Y and Q2Y scores. The R2Y score is the number of Y variables that can be explained by the model. The Q2Y score is the result of cross validation and quantitative measurement between the predicted results and the actual data [22]. The R2Y and Q2Y scores obtained in the model from Fig. 1a were 0.912 and 0.794 respectively, while the R2Y and Q2Y scores obtained in the model in Fig. 1b were 0.831 and 0.499 respectively. In the OPLS model, the range of scores from 0.5 to 1 is considered a good model [22].



(a)



(b)

Note: FA: Sulawesi 1 fermented, FB: Sulawesi 2 fermented, FC: ICCRI 03 fermented, FD: KW 617 fermented; A: Sulawesi 1 unfermented, B: Sulawesi 2 unfermented, C: ICCRI 03 unfermented, D: KW 617 unfermented. Samples are colored in a gradient from blue to yellow indicating the intensity of sensory attributes from low to high.

Fig. 1. OPLS Score plot curve (a) cocoa aroma, (b) cocoa taste in cocoa liquor from various cocoa clones

The results of the score plot can be explained through the S-plot curve. The S-plot curve was employed to find out the dominant retention time of the active and inactive components. The position of the retention times corresponds to the position of the sample on the score plot. In this

study, the more to the right the results of the OPLS S-Plot curve are, the more active the components are. The OPLS S-plot curve in terms of aroma and taste of cocoa on various types of cocoa clones can be seen in Fig. 2. The results of the S-plot curve on the sensory attributes of aroma and taste cocoa showed retention times of 14.776 and 3.772, being the dominant retention times. The active compound at retention time of 14.776 was 2,3,5-trimethylpyrazine. In addition, there was also an acetic acid compound at a retention time of 3.772.

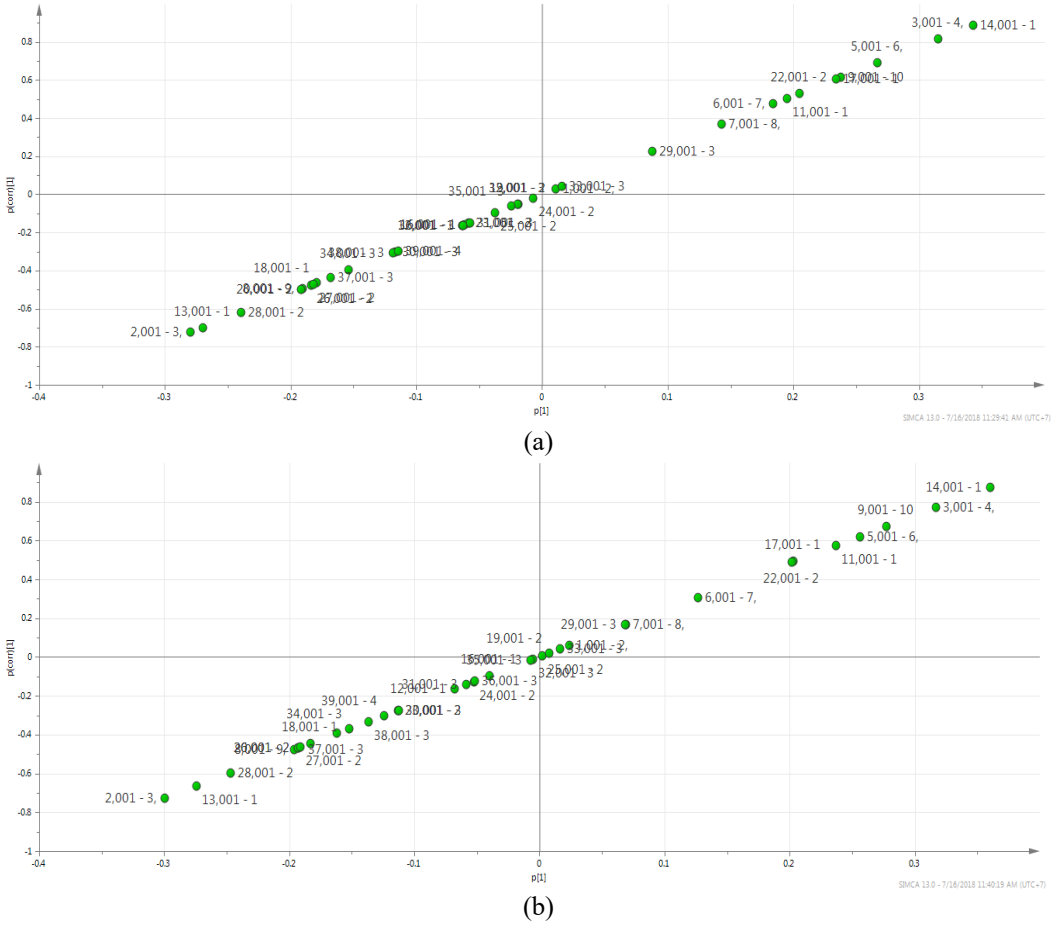


Fig. 2. OPLS S-plot curve (a) intensity of cocoa aroma, (b) intensity of cocoa taste in cocoa liquor from various cocoa clones

The results of the cocoa liquor description test can be seen in Fig. 3. Fermented and unfermented cocoa clones had different sensory profiles. The fermented cocoa liquor from the Sulawesi 2 clone had the attributes of acidity, floral, spicy, and fresh fruit. Those floral and fresh fruit flavors can come from alcohol and ester components [15], such as 2-phenylethanol [21], but in the GC-MS analysis, the components found contained 2,3-butanediol, 1 -propanol, linalool, 2-methyl-1-butanol, and ethylphenyl acetate (Table 1). In addition, the acidity component comes from the compound of 3-methyl-butanoic acid [13], in which this study found the volatile component of 2,3-methyl-abuanoic acid (Table 1).

The results of the two dominant components from the OPLS-Splot can be seen in the mass spectra in Fig. 3. Fragmentation of the peak mass spectra at 14.776 minutes resulted in a mass

spectra showing the presence of fragments with fragment values of m/z 42 and 81, and also molecular ions of m/z 122. After comparing with the literature [23,24], it can be confirmed that the compound was 2,3,5-trimethylpyrazine with the molecular formula C7H10N2. Fragmentation of the peak mass spectra at 3,772 minutes resulted in a mass spectra showing the presence of fragments with a fragment value 43 m/z and molecular ions 60 m/z. Results from the literature [25,26] also show that the compound is an acid acetate with the molecular formula CH3COOH and the result of fragmentation is CH3CO+ (losing the OH fragment).

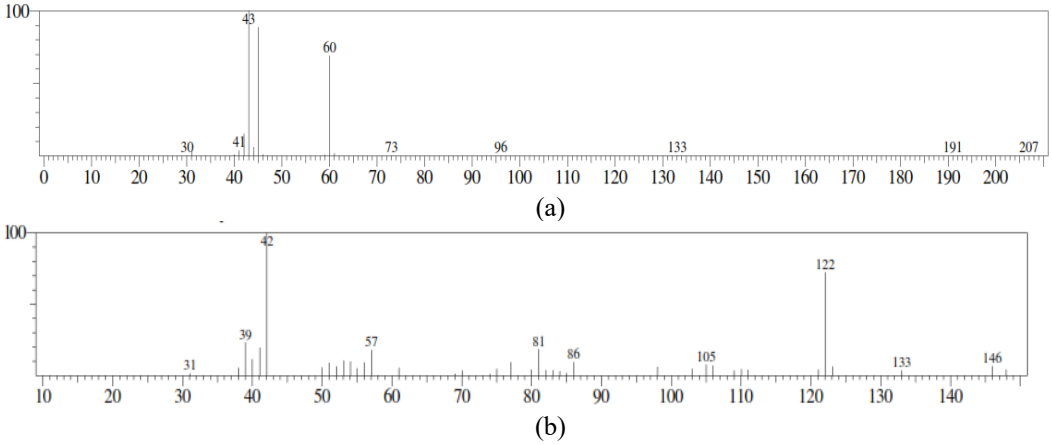
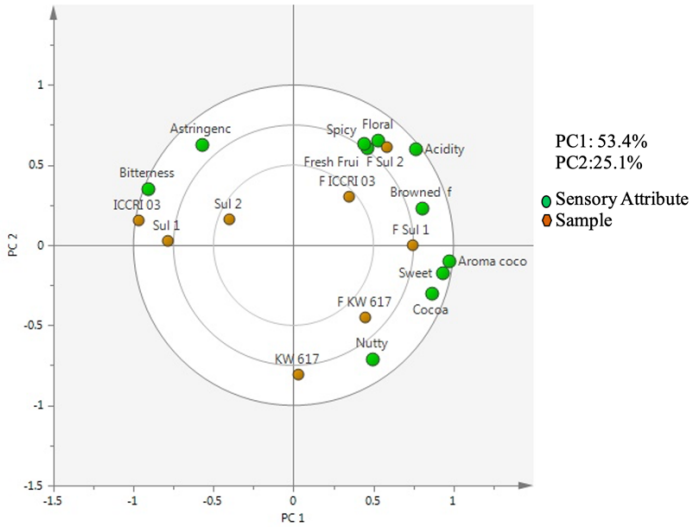


Fig. 3. Mass spectra of peak minutes to (a) 3.772, (b) 14.776 from fermented cocoa clones with GC-MS

The fermented Sulawesi 1 clone had the attributes of cocoa, cocoa aroma, sweet, and browned fruit (Fig. 4). Kusumaningrum [13] reported that the cocoa aroma and the taste of cocoa were from the components of 2,3,5-trimethylpurazine, 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, and 2,3,5,6-tetramethylpyrazine. These compounds were also present in the clones in this research, such as tetramethylpyrazine and 2,3,5-trimethylpyrazine.



Note: FA: Sulawesi 1 fermented, FB: Sulawesi 2 fermented, FC: ICCRI 03 fermented, FD: KW 617 fermented; A: Sulawesi 1 unfermented, B: Sulawesi 2 unfermented, C: ICCRI 03 unfermented, D: KW 617 unfermented

Fig. 4. PCA sensory analysis of fermented and unfermented cocoa liquor

The results of the OPLS S-plot also show the contribution of compounds such as 2,3,5-trimethylpyrazine to the taste and aroma attributes of cocoa. The sweet taste attribute was from phenylacetaldehyde, 2-phenylethanol, 2-phenylethyl acetate [14], where component such as phenylacetaldehyde was also present in the sample (Table 1). Attributes of nutty were found in fermented and unfermented clones of KW 617, where the 3-hydroxy-2-butanone component also contributed to the peanut taste attribute [14].

The cocoa liquor from unfermented clones, such as ICCRI 03, Sulawesi 1, and Sulawesi 2 were in the same quadrant, while the attributes that contribute to these clones were bitterness and astringent (Fig. 4). The bitter and astringent taste of unfermented cocoa is caused by alkaloid components such as theobromine and caffeine. Furthermore, phenolic components, pyrazine, and a variety of free amino acids and peptides give contribution to the bitter and astringent flavor [13]. According to Dwijatmoko et al. [27] Sulawesi 1, Sulawesi 2, and ICCRI 03 have higher total polyphenols, total flavonoids, epicatechin, and catechin than KW 617.

4. Conclusions

The results of the OPLS S-plot GC-MS data show that the active compounds that contributed to the aroma and taste of cocoa liquor were 2, 3, 5-trimethylpyrazine and acetic acid. The fermented and unfermented cocoa liquor of the various clones had different attributes. In the fermented clones, the dominant sensory attributes of cocoa aroma, cocoa, and acidity were higher than those that were not fermented. The non-fermented clones had higher astringent and bitter dominant sensory attributes than the fermented clones.

Abbreviations

QDA Quantitative Descriptive Analysis
SPME Solid Phase Microextraction
GC-MS Gas Chromatography - Mass Spectrometry
OPLS Orthogonal Partial Least Square

Data availability statement

Data will be shared upon request by the readers.

CRediT authorship contribution statement

Muhammad Isa Dwijatmoko: Project administration, Methodology, Writing – original draft. Budi Nurtama: Data curation. **Nancy Dwi Yuliana**: Conceptualization, Data curation, Investigation, Software. Methodology. **Misnawi**: Resource, Writing – review and editing.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

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