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Chemical and sensory properties of single-origin chocolate made from cocoa beans with different fermentation durations

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Abstract

Cocoa is an important agricultural commodity, with global demand rising due to its central role in chocolate production. As the world's third-largest cocoa producer, Indonesia plays a significant role in the global market, with cocoa beans as an essential export commodity. Recently, single-origin chocolate has gained popularity due to its unique sensory qualities, particularly its aroma and flavor, which are influenced by volatile compounds produced during fermentation. This study investigating the proximate composition, volatile compounds, and aroma profiles of single-origin chocolate derived from cocoa beans subjected to different fermentation treatments or durations: nonfermented/NF, half-fermented/HF (3-day fermentation), and fully fermented/FF (6-day fermentation). The proximate composition of the cocoa beans, nibs, and chocolate products was analyzed, while the chocolate products were further examined for volatile compounds using GC–MS and sensory aroma profiles through descriptive analysis. The length of the fermentation process affects the chemical and sensory characteristics of single-origin chocolate. Fully fermented cocoa beans and nibs contain the highest fat and protein, along with the least carbohydrates. Chocolate that is unfermented, partially fermented, or fully fermented can be identified by the distinct differences in their volatile compound profiles. These results underscore the critical role of fermentation timing in developing desirable chemical and aromatic characteristics, providing valuable insights for enhancing the production of high-quality single-origin chocolate.

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1. Introduction

Cocoa beans, derived from mature fruits of the cocoa tree (*Theobroma cacao* L.), have gained significant attention due to their health benefits and growing global market. Indonesia is the world's third-largest cocoa producer and the leading producer in the Asia Pacific region(1). As a key export commodity, cocoa holds substantial economic importance and serves as a primary source of livelihood for many farmers. Despite its potential, cocoa production in Indonesia faces several challenges, particularly related to quality. Ariningsih (2) highlighted that poor quality is one of the main factors undermining the competitiveness and market value of Indonesian cocoa beans.

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Cocoa beans are notably the main ingredient in chocolate manufacturing. The sensory quality of chocolate products is influenced by many factors, such as cocoa cultivation, postharvest treatment, and chocolate processing (3). In addition, understanding the characteristics of cocoa origin and improving processing technology leads to higher quality and flavor development in cocoa products. Single-origin cocoa, which is produced or originates from certain geographical areas, has shown potential due to its uniqueness and marketing potential.

Aroma and flavor are important sensory attributes of chocolate that determine consumer acceptance and market demand. The distinctive aroma of cocoa beans, followed by postharvest treatment and processing techniques, is a crucial factor affecting the final flavor of cocoa products. Currently, there is an increasing demand for fine-flavored cocoa due to its sensory uniqueness, particularly in terms of flavor and aroma (4,5). These chocolate flavors are influenced not only by the cultivar but also by the postharvest treatment and manufacturing stage.

In cocoa postharvest treatments, fermentation is often mentioned as the key to quality. It is an initial and crucial step for the formation of flavor precursors, reducing the bitterness and astringency levels and color development of cocoa beans (6). The fermentation outcome is influenced by the pulp composition, which serves as a substrate for yeast and bacteria to produce flavor precursors (7). These precursors are essential for creating the pleasant flavor of the final cocoa products (8).

The method and duration of cocoa bean fermentation are the main variables used to determine flavor precursors. An appropriate fermentation method (box or heap) and duration of fermentation lead to the optimal formation of flavor precursors. The breakdown of sugars from the pulp of cocoa beans after 72 h resulted in increasing levels of some organic acids, such as propanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid and acetic acid (7,8). These organic acids are responsible for odor-active compounds in cocoa (9). In addition, some derivates of amino acids are produced during fermentation, including 3-methylbutanol, phenylacetaldehyde, 2-methyl-3-(methyldithio) furan, 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine (10).

Fermentation duration is critical, affecting the chemical, physical, and sensory properties of cocoa beans. Optimal fermentation duration appears to depend on various factors, including the fermentation method, initial bean maturity, environmental conditions, and other treatments such as enzyme supplementation. Hence, tailoring fermentation duration can be a useful strategy in cocoa processing to optimize final product quality.

Studies on cocoa fermentation have indicated that a period of 6 to 8 days is typically ideal for producing high-quality beans (11,12). Nonetheless, fermenting for six days may result in over-fermentation in some cases (13). When using wooden fermentation boxes, the process may be essentially complete by the third day (around 40±2 hours), based on observed increases in temperature, reductions in cotyledon pH, and the development of acidic notes in sensory evaluations (14). It is also crucial to consider that fermentation outcomes can vary depending on other factors, such as the specific cocoa variety used. For instance, Forastero beans require a longer fermentation time, typically 5 to 6 days, compared to Criollo beans, which ferment in just 1 to 3 days (8). Therefore, it is important to further investigate the effects of fermentation duration.

Since cocoa fermentation is complex yet difficult and requires more effort, cocoa farmers tend to omit fermentation (15), with the reasonable purpose of securing their cash

flow or quick cash money. This practice may result in lower-quality cocoa beans. Unfermented cocoa beans have undergone little development of cocoa and chocolate flavors, whereas over-fermented cocoa beans are related to increasing pH values, hammy and putrid flavors, and darkening or blackening beans (4,9).

This study aims to demonstrate that varying fermentation durations can significantly influence the quality of cocoa beans. Specifically, it investigates the effects of simple, widely applicable fermentation methods of differing durations, i.e., non-fermented (NF), half-fermented (HF), and fully fermented (FF), on the chemical and sensory properties of single-origin cocoa and its derived chocolate products. The novelty of this research lies in its focus on alternative fermentation durations implemented by local farmers, particularly the assessment of shorter fermentation processes applied to local cocoa varieties. Evaluating the balance between processing efficiency and product quality is essential for improving cocoa production practices. Despite ongoing efforts to establish quality improvement guidelines, implementing standardized fermentation practices remains difficult due to the complex variables involved in the process. The findings from this study are expected to offer practical insights and recommendations that can be adopted by local cocoa farmers to enhance bean quality while optimizing fermentation time.

2. Materials and Methods

2.1. Sample and Preparation

Single-origin cocoa beans (*Forastero*), nonfermented (NF), half fermented (HF, 3 days fermentation), and fully fermented (FF, 6 days fermentation), were obtained from cocoa farmers in Kulon Progo, Yogyakarta. Fermentation was carried out by farmers using commercial cocoa fermentation practices in wooden boxes. Sucrose was purchased from a local supermarket. The cocoa beans were further roasted and processed into chocolate in collaboration with Pawon Gendis, a local chocolate enterprise based in Kulon Progo, Yogyakarta. Roasting was performed via a rotating oven (locally made with a capacity of 3 kg) at 120-150°C for 25-35 min. Dark chocolate was made with a composition of cocoa and sugar of 70% and 30%, respectively. For chocolate production, a *melanger* (Wonder Premier Grinder) with a 10-hour grinding time was used. Manual tempering was performed in an oven where the chocolate was heated to 50°C to start the process, after which it was stirred and held for 9 minutes. The chocolate paste was then brought to 32°C by gradually lowering the temperature with the use of ice water. This procedure was carried out until the temperature reached 27°C. The temperature was maintained at this level for nine minutes. The cooling process was performed at room temperature. The temperature of the chocolate was increased to 32°C at the conclusion of the tempering process. The tempered chocolates were poured into a mold (13 cm × 3 cm × 0.5 cm) before being vibrated for 3 minutes via a vibrating table to release air bubbles. The chocolate product was immediately cooled at 12°C for 1 hour, demolded, and further stored at 20°C.

The samples, not fermented, half fermented, and fully fermented cocoa beans, nibs, and chocolate, were stored for proximate analysis. The chocolate products were further evaluated for volatile compounds and sensory characteristics (aroma intensity).

2.2. Proximate Analysis

The moisture, ash, and protein contents of the samples were determined on the basis of the Association of Official Analytical Chemists (16). The loss of drying (moisture) of the cacao product was measured via the gravimetric method by drying the samples to a constant weight at 100 °C in a convective hot air oven (AOAC method 931.04), the ash content was analyzed via AOAC 972.15, and the protein content was determined via the Kjeldahl method (AOAC method 970.22). The total fat content of each sample was analyzed via the Weibull–Stoldt method, where the sample was acid hydrolyzed before further Soxhlet extraction. The total fat content was calculated gravimetrically after the extract had been dried to a constant weight. The carbohydrate contents were calculated by difference.

2.3. Volatile Compound Analysis

The analytical method for aroma analysis used headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC–MS) (17). Analyses were conducted in triplicate.

2.3.1. Sampling and Extraction

A sample of 2 g of chocolate was placed into a 22 mL SPME vial + 0.2 µl 0.001% ISTD (ISTD: 2,4,6-trimethyl pyridine). The SPEE fiber used was 2 cm long gray, 50/30 µm DVB/Car/PDMS (Supelco Inc., Bellefonte, PA.), and extraction was performed at 60°C for 30 minutes. After extraction, the SPME fiber was moved to the splitless injection port of the GC for manual injection. The PTV injection port for SPME sampling was equipped with a 0.75 mm I.D. borosilicate glass SPME inlet liner (Sigma–Aldrich Co., LLC).

2.3.2. GC–MS Conditions

GC–MS analysis was performed via an Agilent 7890A GC coupled with an Agilent 5975C MS instrument (Inert XL, EI/CI MSD) equipped with a J&W instrument (Agilent Technologies Inc., California, USA). A DB-WAX column (30 m × 250 µm × 0.25 µm) was used in splitless mode, and the inlet temperature was 250°C. The oven program was set at 35°C for 0 min, ramped at 4°C/minute to 182°C for 0 min, and then 7°C/minute to 240°C for 5 minutes. The transfer line temperature was 250°C. The gas carrier was helium (ultrahigh purity), with a flow rate of 1 mL/minute, an average velocity of 24.794 cm/second, a pressure of 11.325 psi, and a total runtime of 50.036 min. The MS source was 230°C, the maximum temperature was 250°C, the MS quad was 150°C, the maximum temperature was 200°C, and the scan range was 29–550 amu (MS, 70 eV). Data were collected from the Enhanced ChemStation software MSD ChemStation G1701EA revision E.02.02. Identification was performed via the NIST 14 MS Database, and linear retention index (LRI) calculations were performed on n-alkane mixtures. Semi-quantification was performed with an internal standard (ISTD).

2.4. Sensory Evaluation

2.4.1. Panelist Screening and Training

The sensory panel was screened based on the requirements of the study. The 9 selected panelists were trained in several sensory sessions of 90 minutes per session for at least 7 training sessions. The training involves performance analysis (ability to discriminate chocolate samples, reproducibility, and consistency), followed by a series of focus group discussions (FGDs), briefing, and introductions to chocolate aroma, references, and samples, as well as the method used. Sensory descriptive analysis was introduced to the sensory panel (18), with a focus on the chocolate aroma generated during FGD.

2.4.2. Formal Evaluation

A 2.5-gram sample of crushed chocolate was prepared in a covered plastic cup. The panelists were asked to evaluate the aroma of the samples via the sensory descriptive analysis method (18)by sniffing the samples. The panelists evaluated and scored the intensity of the aroma attributes. The 0 – 15 line scales with 0.5 increments were used to score selected aroma attributes in the samples (18). The analysis was performed in triplicate. Sensory evaluation design and data collection were performed via RedJade Sensory Software (Martinez, CA, US). This research adhered to the Ethical Clearance Reg. :008/KEPK-POLKESMA/2021.

2.5. Statistical Data Analysis

The data were collected and tabulated in Microsoft Excel 2013. Descriptive statistics, analysis of variance (ANOVA), and post hoc analysis (Fisher's least significance difference test) at the 95% confidence interval were performed by using Minitab 17 Statistical Software (Minitab Inc., State College, Pennsylvania, USA). Multivariate analysis and principal component analysis were performed via XLSTAT version 2015 (Addinsoft, New York, USA).

3. Results and Discussion

3.1. Proximate Composition

The proximate analysis (Table 1) revealed that full fermentation tended to produce higher-moisture cocoa beans ($p<0.05$). The moisture content of cocoa beans is within the approximate range of 7–8%, where the optimum moisture content of cocoa beans was reported by Aprotosoaie et al. (19) to be between 7–7.5%. A moisture content above 7.5% has the potential for mold and microbial growth in cocoa beans, but a moisture content that is too low below 5% may increase the brittleness of cocoa beans (20). The moisture content in the range of 7% - 8% met the standard (21). Table 1 also indicates that cocoa nibs that have been roasted, as well as chocolate products, significantly decreased ($p<0.05$) the moisture content. Compared with those of half-fermented nibs and nonfermented nibs, which have moisture contents of 1.57% and 1.60%, respectively, the moisture content of nibs with full fermentation treatment is the lowest moisture content at 1.33%. According to Afoakwa et al. (22), fermentation and drying can reduce the moisture content of cocoa beans before they are processed into nibs. In addition, the roasting process to produce nibs, which involves high temperatures for a certain period, can reduce the remaining moisture content after the drying process (4). Roasting cocoa beans into nibs at temperatures of 110–160°C for 5–120 minutes can reduce the moisture content of the nibs to 1–2% (20). For chocolate products, the moisture content also seems lower, in the range of 0.53%-1.27%. Chocolate production involves more complex processing steps, such as conching. The conching process, which uses a relatively high temperature of approximately 70–82°C (6), is capable of reducing the moisture content of dark chocolate to less than 1.5% (23).

In addition to affecting the moisture content, fermentation duration also significantly influenced the ash content of the samples ($p<0.05$). In nibs and chocolate products, the longer the fermentation duration is, the lower the ash content, which is consistent with the findings of Afoakwa et al. (8). Conversely, in cocoa beans, fermentation produces beans with a relatively high ash content. Notably, in addition to being influenced by fermentation time, ash content is also affected by drying and roasting temperatures. In chocolate products, the

overall ash content from the fermentation treatments had the lowest ash percentage (ranging from 2.51 - 1.89%) compared with the nibs from all fermentation treatments (ranging from 3.60 - 3.04%). This finding is consistent with the research of Ajala and Ojewande (24), who reported that a drying temperature of 85°C produced a lower ash content in cocoa beans than did drying at 50°C.

With respect to protein content, varying fermentation duration had a significant effect. Among the cocoa beans and cocoa nibs, the fully fermented samples presented the highest protein content, which was significantly different ($p<0.05$) from that of the nonfermented samples. The protein content of the cocoa beans and nibs ranged from 13.80%-14.64%. The duration of fermentation may cause protein modification and hydrolysis into amino acids and peptides through conversion into an insoluble form by polyphenol compounds and the diffusion process (6,25).

An investigation of the fat content of the samples revealed that the total fat content of the samples ranged from 35.46%-38.60%, where longer fermentation durations significantly increased the fat content of cocoa beans ($p<0.05$). The fat content of cocoa beans in the present study was lower than that reported by Afoakwa et al.(22), which ranged from 50–55% for fermented and unfermented cocoa beans. The pattern of increasing fat content with increasing fermentation duration was also expected for the nibs, which was 53.57% for the nonfermented samples, 53.66% for the half-fermented samples, and 54.01% for the fully fermented samples. However, cocoa nibs contain significantly more total fat than do cocoa beans and chocolate products. The results of this study indicate that the increase in fat content is directly proportional to the fermentation duration of the samples. These results are consistent with the findings of Servent et al.(26), who reported that the fat content of cocoa beans increases from fermentation at 0 hours to 144 hours. However, in the study by Afoakwa et al.(22), the fermentation time should not be too long because it can reduce the fat content in cocoa samples.

Table 1. The effects of fermentation on the proximate parameters of cocoa beans, cocoa nibs, and chocolate products.

Sample	Fermentation	Moisture content (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Cocoa beans	Nonfermented	7.77 ± 0.10^{b}	3.47 ± 0.06^{b}	13.80 ± 0.15^{c}	35.46 ± 0.45^{e}	39.49 ± 0.22^{d}
	Half fermented	7.08 ± 0.29^{c}	3.69 ± 0.04^{ab}	14.32 ± 0.12^{b}	37.30 ± 0.33^{d}	37.61 ± 0.24^{c}
	Fully fermented	8.05 ± 0.25^{a}	3.91 ± 0.08^{a}	14.34 ± 0.26^{b}	38.60 ± 0.43^{c}	35.07 ± 0.12^{c}
Cocoa nibs	Nonfermentated	1.60±0.03d	3.60±0.04ab	14.32±0.19b	53.57±0.48a	26.92±2.65a
	Half fermented	1.57±0.02d	3.82±0.03a	14.59±0.17ab	53.66±0.06a	26.36±0.29b
	Fully fermented	1.33±0.01e	3.04±0.02c	14.64±0.17a	54.01±0.43a	26.98±2.10a
Chocolate	Nonfermentated	0.53±0.03f	2.51±0.04d	9.46±0.18c	38.89±0.11c	48.61±0.14e
	Half fermented	1.24±0.03e	2.56±0.01d	9.33±0.16c	39.54±0.22b	47.34±0.22e
	Fully fermented	1.27±0.05e	1.89±0.55e	9.41±0.13c	39.08±0.27bc	48.05±0.30e

Notes: Different notations indicate significant differences ($p<0.05$)

The fat content of cocoa beans is considered important as one of the determinants of the quality index of the produced product. Cocoa fat has unique characteristics from its

triglyceride composition, which consists of 55% POS, 5% POP, and 20% SOS, and has a melting point between 32 and 34°C (27). Fermentation is suggested to be necessary to improve the yield of fat from cocoa nibs (26).

The carbohydrate contents of cocoa beans, cocoa nibs, and chocolate are significantly different ($p<0.05$), at approximately 37%, 53%, and 39%, respectively. There was no significant difference in the carbohydrate content of chocolate as affected by fermentation duration. The duration of fermentation influenced the carbohydrate contents of cocoa beans and nibs, but it should be noted that the carbohydrate content in this study was different. Therefore, other components present in samples, such as fats and proteins, reduce the carbohydrate content. This could also be due to pre-harvesting variation or treatments before fermentation, such as storage of the pods, which is not currently being studied. On the basis of the findings of Afoakwa et al.(22), cocoa beans stored for 21 days before fermentation presented the highest carbohydrate content compared with those stored for 0, 7, and 14 days.

3.2. Volatile Compounds

Further investigations of volatile compounds in chocolate products were performed. Table 2 shows that 66 volatile compounds were identified in nonfermented, half fermented, and fully fermented chocolate. These compounds belong to several chemical groups, such as hydrocarbons, alcohols, alkanes, alkanes, carboxylic acids, esters, pyrazine, furans, phenols, and furanones. There are 7 volatile compounds, including nonanal, pyrazine; 2-ethyl-3,5-dimethyl-; 2-furancarboxaldehyde, 5-methyl-; propanoic acid, 2-methyl-; butyrolactone; 5-methyl-2-phenyl-2-hexenal; and 2-furancarboxaldehyde, 5-(hydroxymethyl), which significantly differ ($p<0.05$) when cocoa nibs fermented for different durations are utilized.

Figure 1 shows the principal component analysis (PCA) results, where the three different chocolate samples can be clearly distinguished in PC1 (70.93%) and PC2 (15.87%), where fully fermented chocolate can be distinguished from nonfermented chocolate on the basis of the content of chemical groups such as aldehydes, esters, and alcohols. Nonfermented chocolate contains volatile compounds such as pyrazine, 2-ethyl-3,5-dimethyl-, 2-furancarboxaldehyde, 5-methyl-, 2-furancarboxaldehyde, 5-(hydroxymethyl)-, and butyrolactone. However, nonfermented chocolate contains more hexanoic acid than half fermented chocolate or fully fermented chocolate. However, nonfermented chocolate has lower acid concentrations of acetic, propanoic, and octanoic acids. On the other hand, half fermented chocolate has higher concentrations of acetic, propanoic, and octanoic acid than does fully fermented chocolate. It is important to note that the volatile analysis performed only detects volatile acids; thus, the presence of lactic acid as the major fermentation acid cannot be confirmed, as it is a non-volatile compound.

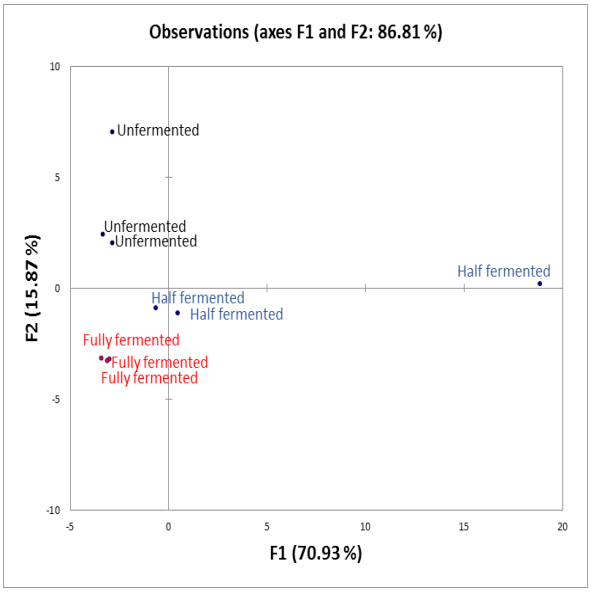

Figure 1. PC1 vs PC2 plot of volatile compounds in single-origin chocolate.

These identified aromatic compounds contributed to unique aromas in chocolate, such as sweet, earthy, caramel-like, cocoa, and sour aromas. Specifically, most of the aromatic compounds identified in nonfermented, half-fermented, and fully fermented chocolate belong to the pyrazine group, which contributes to sweet, earthy, nutty, and cocoa aromas. Fermentation indeed contributes to the formation of cocoa aroma precursors, and further processing, such as drying and roasting, translates these precursors into the unique fine aroma of cocoa (4,28,29).

3.3. Aroma Attributes

Aroma attributes were generated for chocolate products based on the FGD results. The 14 aroma attributes assessed in this study are presented in Figure 2. According to the figure, nonfermented chocolate exhibits a stronger nutty aroma compared to half-fermented and fully fermented chocolates. This nutty aroma may be attributed to volatile compounds such as 3-hydroxy-2-methyl-4-pyrone (maltol), pyrrole, pyrrole-2-carboxyaldehyde, 2-methylpyrazine, and 2,3,5-trimethylpyrazine (19).

Fully fermented chocolate exhibits a stronger cocoa aroma compared to both half-fermented and nonfermented chocolates. This aroma may be contributed by 4-methyl-2-phenyl-2-pentenal, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine (19). On the other hand, half-fermented chocolate contained a higher concentration of sour aroma compared to both fully fermented and nonfermented chocolates. Some volatile compounds, such as acetic acid and 5,6-dihydro-4-methyl-2-pyrone, may contribute to this sour/acidic aroma (19).

The aroma volatile profile has a strong influence on the sensory perception and thus acceptability of cocoa and chocolate products. Calvo et al. (30) explained that the development of this aromatic profile during cocoa production and processing is highly complex, relying on numerous biochemical reactions within the beans. These reactions are influenced by factors such as temperature and the production of organic acids.

Chocolate's rich sensory profile results from the complex interactions of various volatile compounds and their relative concentrations. Throughout fermentation, cocoa beans generate a range of volatile compounds, such as esters, pyrazines, aldehydes, and alcohols,

each adding unique flavor characteristics. Alcohols contribute creamy and sweet notes, aldehydes are linked to bitterness, and pyrazines deliver the familiar chocolate flavor (31,32). The nutty notes in non-fermented cocoa are likely due to specific pyrazines known for such notes, while the fermented cocoa may exhibit more cocoa aroma due to the presence of other specific important pyrazines. The current findings showed that fully fermented samples had higher levels of some pyrazines and esters, and lower levels of acids and phenols, which together may enhance cocoa and dried fruit aromas. In contrast, partially fermented cocoa, with the highest concentrations of acids and phenols, may present increased bitterness and astringency, since phenols were mentioned in Moreira et al. (33) to have a correlation with bitterness and astringency. It's not only the presence but also the balance of these compounds, especially between sweet and bitter elements that creates the final flavor (31). A higher proportion of alcohols and esters can enhance perceived sweetness and help balance natural bitterness.

Roasting of cocoa further transforms the volatile profile, especially through the formation of sulfur-containing compounds. The balance between these sulfur-containing compounds and pyrazines can either enrich or diminish chocolate's sensory appeal (34). These findings suggest that specific ratios of volatile compounds may act as quality indicators for cocoa (31). However, further research is needed to confirm and better understand these potential markers.

While this study specifically focused on aroma and did not evaluate the full range of sensory attributes, it is acknowledged that fermentation duration can influence more than just aroma, it can also impact taste attributes such as sweetness, bitterness, sourness, and astringency. For example, sourness and astringency contribute to a drying sensation in the mouth, while the balance between bitterness and sweetness plays a significant role in the overall eating experience and consumer preference. These factors are key to the overall enjoyment and acceptance of the chocolate. Additionally, texture and mouthfeel, although not directly addressed in this study, are also influenced by fermentation due to the formation of more fatty acids and/or compounds responsible for textural characteristics.

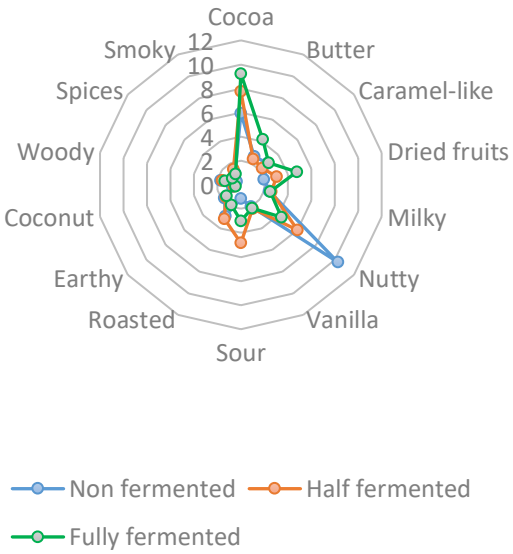

Figure 2. Aroma intensity score for single-origin chocolate.

Table 2. Volatile compound composition of chocolate products made from cocoa beans with varying fermentation durations.

Compounds	CAS No.	RT	LRI	Fully Fermented		Half Fermented		Non-Fermented		Pr > F**
				Mean* (ppb)	CV (%)	Mean* (ppb)	CV (%)	Mean* (ppb)	CV (%)	
Cyclotrisiloxane, hexamethyl-	000541-05-9	2.35	na.	1.58	20.91	2.57	115.20	0.83	96.25	0.53
Cyclotetrasiloxane, octamethyl-	000556-67-2	3.95	1007	2.48	36.30	0.73	87.67	2.00	56.35	0.13
Hexadecane, 2,6,11,15-tetramethyl-	000504-44-9	5.44	1080	0.50	87.18	0.00	0.00	0.00	0.00	0.08
Cyclopentasiloxane, decamethyl-	000541-02-6	7.86	1175	9.50	39.55	15.00	125.93	3.97	56.73	0.52
Dodecane	000112-40-3	8.58	1201	0.30	100.00	1.57	97.71	0.63	63.81	0.30
Pyrazine, methyl-	000109-08-0	10.51	1259	0.38	26.33	2.07	106.27	0.67	56.79	0.30
Pyrazine, ethyl-	013925-00-3	10.52	1323	0.00	0.00	1.07	100.24	0.00	0.00	0.13
2-Butanone, 3-hydroxy-	000513-86-0	11.06	1275	0.63	86.96	0.00	0.00	0.00	0.00	0.08
Pyrazine, 2,5-dimethyl-	000123-32-0	12.27	1312	0.57	36.95	3.40	104.53	1.10	39.63	0.28
Cyclohexasiloxane, dodecamethyl-	000540-97-6	13.29	1344	8.37	14.73	14.90	92.31	5.87	52.99	0.43
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	007216-56-0	13.81	1360	0.00	0.00	0.33	147.99	0.00	0.00	0.32
Pyrazine, 2-ethyl-6-methyl-	013925-03-6	14.28	1375	0.10	100.00	2.43	102.02	0.50	34.64	0.19
2-Nonanone	000821-55-6	14.36	1378	0.27	13.59	1.10	102.45	0.27	43.30	0.28
Nonanal	000124-19-6	14.47	1381	1.13	16.49	0.00	0.00	0.00	0.00	0.00
Pyrazine, trimethyl-	014667-55-1	14.88	1394	0.65	19.47	6.67	100.50	0.57	97.19	0.17
1-Methoxyadamantane	006221-74-5	15.30	1408	0.00	0.00	0.73	102.35	0.00	0.00	0.13
Octanoic acid, ethyl ester	000106-32-1	15.86	1426	0.00	0.00	1.90	126.43	0.00	0.00	0.23
Acetic acid	000064-19-7	16.12	1434	14.36	17.31	47.40	106.97	18.70	56.52	0.40
Furfural	000098-01-1	16.56	1448	0.39	22.79	2.67	102.03	3.07	50.66	0.23
Pyrazine, 2-ethyl-3,5-dimethyl-	013925-07-0	16.66	1452	0.00	0.00	0.00	0.00	0.37	31.49	0.00
2,3-Dimethyl-5-ethylpyrazine	015707-34-3	16.67	1452	0.00	0.00	3.23	98.40	0.00	0.00	0.12
.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	1000132-13-0	16.97	1462	0.16	25.84	0.77	94.36	0.00	0.00	0.14
Pyrazine, tetramethyl-	001124-11-4	17.10	1466	2.08	13.09	2.03	101.07	0.13	43.30	0.16
Pyrazine, 3,5-diethyl-2-methyl-	018138-05-1	17.65	1484	0.12	17.40	0.53	88.61	0.20	100.00	0.27
Ethanone, 1-(2-furanyl)-	001192-62-7	17.82	1489	0.23	31.84	0.87	113.25	0.27	43.30	0.37
Benzaldehyde	000100-52-7	18.26	1504	0.90	20.54	0.58	42.81	0.57	50.94	0.25
2,3,5-Trimethyl-6-ethylpyrazine	017398-16-2	18.29	1505	0.00	0.00	3.83	98.87	0.00	0.00	0.12

Compounds	CAS No.	RT	LRI	Fully Fermented		Half Fermented		Non-Fermented		Pr > F**
				Mean* (ppb)	CV (%)	Mean* (ppb)	CV (%)	Mean* (ppb)	CV (%)	
Cycloheptasiloxane, tetradecamethyl-	000107-50-6	18.68	1518	1.40	29.97	2.97	71.05	1.10	39.63	0.23
2-Furanmethanol, acetate	000623-17-6	18.91	1526	0.00	0.00	1.40	98.97	0.17	69.28	0.14
Propanoic acid	000079-09-4	19.00	1529	0.27	23.90	0.97	110.61	0.00	0.00	0.22
1,6-Octadien-3-ol, 3,7-dimethyl-	000078-70-6	19.41	1542	0.29	18.46	1.70	97.19	0.47	44.61	0.23
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]-	000475-20-7	19.57	1548	0.00	0.00	0.77	94.36	0.13	86.60	0.14
2-Furancarboxaldehyde, 5-methyl-	000620-02-0	19.85	1557	0.00	0.00	0.00	0.00	1.53	43.42	0.00
Propanoic acid, 2-methyl-	000079-31-2	19.90	1559	2.27	20.77	0.00	0.00	0.00	0.00	0.00
Butyrolactone	000096-48-0	21.32	1607	0.82	15.20	0.00	0.00	2.70	41.74	0.01
2-Furanmethanol	000098-00-0	22.65	1653	0.62	22.53	6.17	103.31	3.27	43.54	0.27
Naphthalene	000091-20-3	24.41	1715	0.00	0.00	0.00	0.00	0.23	89.21	0.09
Benzeneacetic acid, methyl ester	000101-41-7	25.22	1745	0.00	0.00	0.73	102.35	0.00	0.00	0.13
Benzeneacetic acid, ethyl ester	000101-97-3	25.95	1772	0.00	0.00	0.93	89.86	0.00	0.00	0.09
2-Butenoic acid, 3-methyl-	000541-47-9	26.33	1786	0.00	0.00	3.73	101.52	0.63	50.76	0.16
Acetic acid, 2-phenylethyl ester	000103-45-7	26.74	1801	0.00	0.00	4.00	124.90	0.00	0.00	0.23
3-Methyl-3-butenoic acid	001617-31-8	26.84	1804	0.00	0.00	0.00	0.00	0.63	102.73	0.14
1H-Pyrrole, 1-(2-furanylmethyl)-	001438-94-4	27.06	1812	0.00	0.00	1.10	102.45	0.00	0.00	0.13
1,2-Cyclopentanedione, 3-methyl-	000765-70-8	27.15	1816	0.00	0.00	0.83	110.85	0.23	24.74	0.22
Hexanoic acid	000142-62-1	27.71	1837	0.00	0.00	0.00	0.00	0.33	91.65	0.10
Phenol, 2-methoxy-	000090-05-1	27.91	1845	0.00	0.00	1.73	108.29	0.00	0.00	0.16
N-(3-Methylbutyl)acetamide	013434-12-3	28.36	1862	0.00	0.00	0.47	98.97	0.00	0.00	0.12
Benzyl Alcohol	000100-51-6	28.44	1865	0.10	100.00	0.70	111.57	0.23	24.74	0.31
Phenylethyl Alcohol	000060-12-8	29.35	1899	1.65	18.67	20.37	97.43	3.70	40.00	0.17
Benzeneacetaldehyde, .alpha.-ethylidene-	004411-89-6	29.67	1911	0.34	16.62	0.83	100.64	0.07	86.60	0.23
Maltol	000118-71-8	30.67	1950	0.00	0.00	0.83	110.85	0.10	100.00	0.20
Ethanone, 1-(1H-pyrrol-2-yl)-	001072-83-9	30.78	1955	0.71	24.99	3.63	102.03	1.00	43.59	0.26
1H-Pyrrole-2-carboxaldehyde	001003-29-8	32.04	2005	0.18	28.59	4.43	101.24	0.97	39.16	0.18

Compounds	CAS No.	RT	LRI	Fully Fermented		Half Fermented		Non-Fermented		Pr > F**
				Mean* (ppb)	CV (%)	Mean* (ppb)	CV (%)	Mean* (ppb)	CV (%)	
5-Methyl-2-phenyl-2-hexenal	021834-92-4	33.30	2065	0.32	22.08	0.00	0.00	0.00	0.00	0.00
Phenol	000108-95-2	31.67	1990	0.00	0.00	0.43	113.84	0.10	0.00	0.23
2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-	052126-90-6	32.22	2014	0.00	0.00	0.00	0.00	0.37	95.78	0.11
Octanoic Acid	000124-07-2	33.15	2058	0.07	86.60	0.13	114.56	0.00	0.00	0.30
Phenol, 4-methyl-	000106-44-5	33.53	2077	0.00	0.00	0.43	113.84	0.00	0.00	0.18
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	0011192-58-1	33.95	2097	0.00	0.00	0.83	100.64	0.13	43.30	0.16
Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	005912-86-7	35.44	2160	0.00	0.00	0.00	0.00	0.10	100.00	0.13
2-Methoxy-4-vinylphenol	007786-61-0	36.10	2188	0.00	0.00	0.17	124.90	0.00	0.00	0.23
3-Pyridinol	000109-00-2	40.44	2406	0.00	0.00	0.77	106.23	0.13	43.30	0.18
Indole	000120-72-9	40.73	2425	0.00	0.00	0.37	103.25	0.00	0.00	0.14
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	000067-47-0	41.70	2487	0.00	0.00	0.00	0.00	0.40	43.30	0.00
1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-	000058-08-2	40.94	2438	0.00	0.00	0.77	140.68	0.00	0.00	0.29

Notes: CAS indicates the chemical abstract service number of the compounds; RT = retention time; LRI = linear retention index; data mean of 3 replications; CV = coefficient of variation in percentage; Pr > F** data in bold indicate significant differences ($p<0.05$) among treatments

4. Conclusions

The fermentation duration influences the difference in the chemical and sensory properties of single-origin chocolate. The effect is prominent for protein, fats, and carbohydrates, where full fermentation of cocoa leads to the highest fat, highest protein, and lowest carbohydrate contents for cocoa beans and nibs. Nonfermented chocolate, half-fermented chocolate, and fully fermented chocolate can be distinguished based on their different volatile compositions. The fully fermented cocoa beans were indeed higher in aldehyde, ester, and alcohol, while the half-fermented beans could be clustered due to their pyrazine, furans, and acids. The nonfermented samples were distinguished because of their lower concentrations of volatiles. An investigation of the chocolate products by their aroma profiles revealed that the aromas, such as nutty, cocoa, sour, and dried fruits, were higher than those of the other samples, where nutty notes dominated the aroma of all the samples. Compared with half-fermented chocolate, fully fermented chocolate has the highest intensity of cocoa notes. This research highlights the potential for further investigation into the impact of fermentation duration on cocoa quality, particularly by exploring different cocoa varieties or applying alternative methods under controlled conditions. Such an approach offers a practical and efficient strategy for farmers to enhance the quality of their cocoa without significantly extending processing time. By optimizing fermentation practices, the resulting improvement in quality could increase the market value of single-origin cocoa. This, in turn, may provide economic benefits if farmers are able to secure premium prices for higher-quality beans.

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Author Contributions

W.S. conceived and designed the experiments, wrote and revise the paper; N.W. performed the chemical and sensory analysis; A.S. contributed materials, processed the cocoa and chocolates, and reviewed the paper; D.M. concepting the research, contributed reagents/analysis tools; N.E. analyzed and interpret the GC–MS data.

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Institutional Review Board Statement

This research was conducted in accordance with the approved protocols HEALTH RESEARCH ETHICS COMMITTEE STATE POLYTECHNIC OF HEALTH MALANG (Reg. No. :008/KEPK-POLKESMA/2021).

Data Availability Statement

"invalid"

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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