BIODIVERSITAS
Volume 25, Number 7, July 2024
Pages: 3264-3276

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250748

Biochemical composition and volatile profile analysis of three varieties of *Coffea arabica* and their correlation with the microclimate of Mount Tangkuban Perahu, West Java, Indonesia

ROSY ANNAZA FIRNABILLAH, ERI MUSTARI, ERLY MARWANI♥

School of Life Sciences and Technology, Institut Teknologi Bandung. Jl. Ganesha 10, Bandung 40132, West Java, Indonesia. Tel.: +62-22-2511575, Fax.: +62-22-2534107, ♥email: erly@itb.ac.id

Manuscript received: 25 December 2023. Revision accepted: 25 July 2024.

Abstract. *Firnabillah RA, Mustari E, Marwani E. 2024. Biochemical composition and volatile profile analysis of three varieties of* Coffea arabica *and their correlation with the microclimate of Mount Tangkuban Perahu, West Java, Indonesia. Biodiversitas 25: 3264-3276.* The quality of coffee is determined by various biochemical compounds, including chlorogenic acid, caffeine, trigonelline, sucrose, and oil, as well as the volatile metabolite profile in coffee beans. Genetic factors and environmental conditions at the coffee cultivation site influence the composition of these compounds. This study aimed to analyze the biochemical composition and volatile profile of various coffee samples, including Ateng, Tim-Tim, and Sigarar Utang, and investigate their correlation with microclimate factors. Different analytical methods were used to examine the green beans, including High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) for caffeine, chlorogenic acids, sucrose, and trigonelline, Soxhlet methods for oil content determination, and Gas Chromatography-Mass Spectrometry (GC-MS) for volatile compound analysis. The results showed distinct biochemical components and metabolite profiles among the three arabica coffee varieties. The Ateng variety had the highest concentrations of chlorogenic acid, while the Tim-Tim variety had the lowest content of these compounds. Trigonelline and caffeine content was similar across all varieties. Sucrose content was highest in the Sigarar Utang variety, while oil content was higher in the Ateng and Tim-Tim varieties compared to Sigarar Utang. Principal Component Analysis (PCA) showed significant metabolite variation among the three varieties, with PC1 and PC2 accounting for 69.2% of the variance. Furthermore, Partial Least Squares-Discriminant Analysis (PLS-DA) successfully distinguished the three varieties and identified potential biomarker compounds for classification. Additionally, the study discovered that the environmental factor, specifically light intensity affected the sucrose and oil compounds in arabica green beans coffee. The study concluded that the three varieties' biochemical composition and volatile metabolites differed. Certain compounds were found to correlate with measured microclimatic factor.

Keywords: Arabica varieties, biochemical component, GC-MS, HPLC-MS, metabolite profile

INTRODUCTION

Coffee is one of the most consumed beverages worldwide because of its unique taste, potential health benefits, and the influence of social and historical factors (Ayelign and Sabally 2013). More than sixty different species of coffee can be made as coffee drinks. However, only three (*Coffea arabica* L., *Coffea canephora* Pierre ex A.Froehner, and *Coffea liberica* W.Bull) have significant commercial value worldwide as drinks (Tsegay et al. 2020). *Coffea arabica* remains the most popular among the three varieties because of its rich taste and moderate bitterness, making it a favorite among many consumers (Cheng et al. 2016).

Arabica in Indonesia is cultivated in several provinces, including Aceh, North Sumatra, Bengkulu, Lampung, Central Java, East Java, South Sulawesi, East Nusa Tenggara, and West Java (BPS 2022). In West Java, internationally recognized popular Arabica coffees include Masigit Mountain, Cilutung Mountain, Halu Mountain, Cikuray Mountain, Gede Cianjur Mountain, and Patuha Mountain (Humas Jabar 2022). The superior Arabica coffee varieties spread throughout Ateng, Sigarar Utang, and Tim-Tim (Dani et al. 2019). Three varieties are widely planted on the slopes of Mount Tangkuban Perahu. However, research on these three varieties of biochemical composition and volatile metabolites is still limited. These varieties can be developed to increase the availability of single-origin coffees from local coffee varieties. Single-origin coffees, which come from a specific coffee variety and growing region without a mixture of other coffee varieties, are increasingly in demand in the coffee industry (Knysak 2017).

The three Ateng, Tim-Tim, and Sigarar Utang suspected different qualities depending on many factors, including physical properties such as density, size, color, and defects. The processing methods include wet, dry, semi-wash, and fermentation varieties. Moreover, altitude, shade, climate, and chemical compounds depend on high-quality coffee (Leonel and Philippe 2007; Barbosa et al. 2012; Assa et al. 2021). The chemical compound content accumulated in coffee beans influences the quality of coffee and is often associated with its taste and aroma. Several biochemical compounds, including caffeine, chlorogenic acid, trigonelline, oils, and sucrose, contribute to the taste profile of coffee (Heo et al. 2020), and volatile compounds are fragrant components found in specific parts of certain plants. In the case of green coffee beans, these compounds are responsible for the

aroma profile (Czerny et al. 1999; Mehari et al. 2019).

The content of biochemical compounds varies among coffee varieties (Mengistu et al. 2020). Happyana et al. (2021) reported that caffeine, asam chlorogenic acid, trigonelline, and sucrose content varied among three varieties: Ateng, Buhun, and Sigarar Utang. Interestingly, Mazzafera and Silvarolla (2010) reported that the caffeine content of most Arabica varieties is similar at around 1%. Cheserek et al. (2021) reported that the Ruiru and Batian varieties of *C. arabica* do not differ significantly, but both differ significantly from the SL28 variety. Furthermore, the chlorogenic acid content of the Ruiru and SL28 varieties is similar but different from that of the Batian varieties. Additionally, the oil content in three arabica varieties, including Batian and SL28, does not differ but differs from that of Ruiru.

Moreover, the profile of volatile metabolites in coffee beans that are believed to affect coffee taste. Akiyama et al. (2008) analyzed volatile aroma compounds extracted from Arabica coffee in Ethiopia, Tanzania, and Guatemala in three countries. The study revealed notable differences in the aroma profiles between Ethiopian Sidama coffee and Tanzanian and Guatemalan coffee. Specifically, the compound 4-(4-hydroxyphenyl)-2-butanone was identified as the distinctive aroma characteristic of freshly brewed Ethiopian Sidama coffee. Quantitative analysis demonstrated that lightly roasted Ethiopian coffee exhibited a higher content of these compounds. In a separate study by Amanpour and Selli (2016), the volatile profiles of Turkish coffee and French Press coffee showed similarities.

Several recent studies have reported the influence of microclimate on the chemical composition of green coffee beans. Mengistu et al. (2020) assessed the biochemical components of coffee cultivated in various locations throughout Ethiopia. Bertrand et al. (2012) demonstrated the direct impact of climatic factors, particularly average air temperature, on the sensory profile and combination of volatile compounds in green coffee. Putri et al. (2017) demonstrated that each region in Indonesia has unique compounds that can serve as discriminant markers of coffee authenticity. Joët et al. (2010) evaluated the influence of environmental factors, wet processing, and their interactions on the biochemical composition of Arabica green beans. Castro-Moretti et al. (2023) assessed the impact of genotype, environment, and processing on Arabica coffee's chemical composition and sensory quality.

Therefore, a reliable identification method is needed to determine the volatile compounds that play a role in the flavor quality of the Ateng, Tim-Tim, and Sigarar Utang varieties of arabica coffee grown on the slopes of Mount Tangkuban Perahu, West Java. Research conducted by Ongo et al. (2020) has demonstrated that a metabolomic approach can differentiate the metabolite compositions between arabica and robusta coffee species consumed by Asian palm civets and those that are not, taking into consideration the geographical location and coffee processing. Comprehensive analysis of metabolites in biological tissue can be performed using a metabolomic approach (Saputri et al. 2020). Metabolomics is an omics science that can comprehensively analyze low-molecular-weight metabolites in biological samples, including coffee beans. Metabolomics identifies and quantifies metabolites in complex matrices, thereby discovering discriminant metabolites that contribute to the taste and quality of coffee (Braga and Adamec 2018).

This study employs a metabolomics approach to distinguish between Ateng, Tim-Tim, and Sigarar Utang Arabica, which are then correlated with microclimate coffee varieties grown on the slopes of Mount Tangkuban Perahu, thought to have different microclimates.

MATERIALS AND METHODS

Microclimate measurement

Microclimate variables were measured during the coffee plant's flowering to fruit maturation period, which is about three months, from Mei 2022 to July 2022. The microclimate variables measured were air temperature, humidity, and light intensity using an Onset U12-012 data logger. Rainfall data was obtained from the nearest climatology station to Lembang, which is located in West Bandung, West Java, Indonesia (https://shorturl.at/dTk7h).

Samples collection and preparation

The arabica coffee samples from the Ateng, Tim-Tim, and Sigarar Utang varieties were obtained from coffee farmers at Cikole Village, Lembang Sub-district, West Bandung District, West Java, Indonesia around Mount Tangkuban Perahu (Figure 1). The coffee cherries were harvested at the beginning of August 2022. The post-harvest coffee process was carried out by the farmers which includes several steps, i.e. (i) harvesting the coffee cherries, (ii) peeling the coffee cherries, (iii) washing until clean, (iv) drying in direct sunlight, (v) peeling the dry coffee beans with the skin still on, (vi) sorting the best quality Arabica coffee beans, which are then packed in plastic bags and stored in a freezer at -20°C before further used. The green beans coffee samples of three different varieties were then ground into a powder with a 200 µm size using the Cypruz GR006 coffee grinder. The samples were then dried in an oven until the samples weight were constant. Finally, the samples were stored in a dark, airtight bottle at -21°C to reduce evaporation before further used. The samples from each variety were prepared in triplicate.

Biochemical analysis

Biochemical analysis was conducted at the laboratory of Natural Product Analysis Institut Teknologi Bandung for analysis of caffeine, chlorogenic acid, sucrose, and oil. On the other hand, the analysis of trigonelline was carried out at the Jember Polytechnic Laboratory, East Java. Analysis of biochemicals component such as caffeine, chlorogenic acid, trigonelline, sucrose, and oil were carried out in two stages which include, stage (i) sample extraction and stage (ii) analysis of caffeine, chlorogenic acid, and sucrose by using HPLC, trigonelline analysis by using LC-MS, and oil analysis by using Soxhlet. The standard solutions of caffeine and chlorogenic acid were purchased from Sigma-Aldrich, United States, while sucrose was purchased from Himedia, India and trigonelline was purchased from USP Reference Standards, United States.

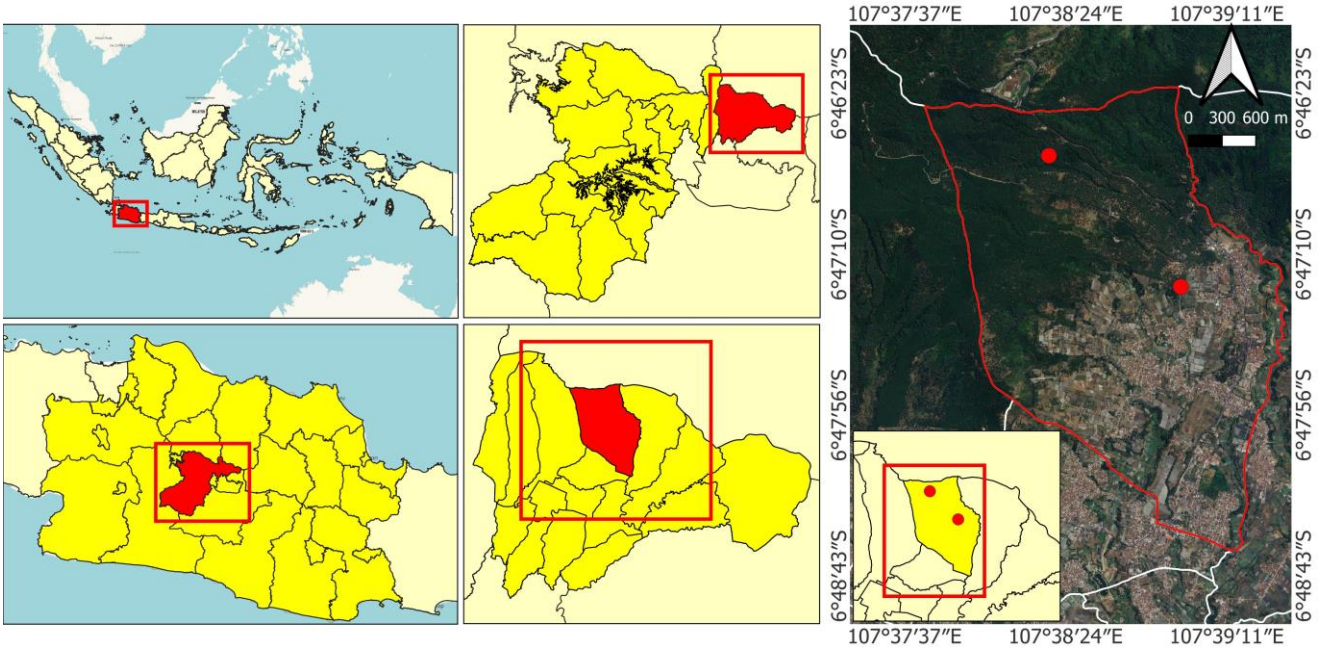


Figure 1. Location of sampling in Cikole Village, Lembang Sub-district, West Bandung District, West Java, Indonesia. Note: location point 1 (107°38'19.187" E, 6°46'40.89" S) and location point 2 (107°38'57.484" E, 6°47'19.039" S)

Extraction of CGA and caffeine

One gram of finely ground dried arabica coffee beans was placed into separate centrifuge tubes. The compound chlorogenic acid was dissolved in 30 mL of ethyl acetate, while caffeine was dissolved in 30 mL of methanol: water (6:4 v/v) (Ceylan et al. 2020). The samples were then subjected to ultrasonication for 10 minutes at a temperature of 65°C, followed by centrifugation at 3500 rpm for 3 minutes. This procedure was repeated three times, and the supernatant was collected after each centrifugation cycle. The supernatant was then evaporated using a rotary evaporator to obtain a crude extract. Subsequently, the samples were filtered using a 0.22 µm PTFE (polytetrafluoroethylene) syringe filter with a diameter of 13 mm. The filtered samples were stored in a refrigerator at a temperature of -21°C for further use in subsequent steps.

Extraction of trigonelline

One gram of finely ground dried arabica coffee beans was weighed and placed into separate 100 mL Erlenmeyer flasks. The samples were then dissolved in 50 mL of distilled water (Perrone et al. 2008). The solution was heated using a hotplate stirrer for 20 minutes at a temperature of 100°C. Afterward, the solution was filtered using Whatman No.1 filter paper, and the filtrate was further filtered using a syringe filter with a size of 0.45 µm and a diameter of 13 mm. The filtered samples were stored in a refrigerator at a temperature of -21°C for further use in subsequent steps.

Extraction of sucrose

One gram of finely ground dried arabica coffee beans was weighed and transferred into separate Erlenmeyer flasks. The samples were then dissolved in 40 mL of distilled water. The flasks were heated using a hot plate and stirred with a magnetic stirrer at a speed of 250 rpm and a temperature of 60°C for 90 minutes (Constantino et al. 2020). Afterward, the samples were filtered using Whatman No.1 filter paper. Dilution was performed by adding deionized sterile water until the total volume reached 40 ml. The resulting diluted extraction was then filtered using a 0.22 µm PTFE syringe filter with a diameter of 13 mm. The filtered samples were stored in a refrigerator at a temperature of -21°C for further use in subsequent steps.

Extraction of oil

Five g of finely ground dried arabica coffee beans were weighed and placed into a Soxhlet thimble. The Soxhlet thimble was then inserted into the Soxhlet extraction apparatus. Extraction was performed using 100 mL of n-hexane solvent for 8 hours (Efthymiopoulos 2018). The Soxhlet extraction yield was evaporated using a rotary evaporator and dried in an oven at 105°C. Subsequently, the sample was cooled in a desiccator and weighed. The drying and weighing process was repeated at 30-minute intervals until a constant sample weight was obtained.

Analysis of CGA and caffeine extraction

The analysis of caffeine and chlorogenic acid in the sample was performed using an HPLC instrument with the specifications of a Shimadzu Prominence Type 20A, equipped with an LC 20 pump and a UV detector set at a wavelength of 272 nm for caffeine and 324 nm for chlorogenic acid. A volume of 10 µL of the sample was injected into the HPLC system. The column used to analyze caffeine and chlorogenic acid was a Shimpack C18 column. Isocratic elution was carried out using distilled water (phase A) and methanol (phase B) with a ratio of 50:50 v/v at a flow rate of 1 mL/min for caffeine, and distilled water (phase A) and methanol (phase B) with a ratio of 75:25 v/v at a flow rate of 1 mL/min for chlorogenic acid.

Analysis of trigonelline

The analysis of trigonelline was performed using an LCMS instrument, Shimadzu brand, located at Politeknik Jember, equipped with an LC diode array pump and an MS detector. Each sample volume of 10 µL was injected into the LCMS system. Subsequently, each chromatographic process was detected by recording the MS spectrum with UV λ 266 nm using a Water C18 column. Isocratic elution was performed using distilled water (phase A) and methanol (phase B) with a ratio of 75:25 v/v and 2% formic acid at a flow rate of 1 mL/min (Campa et al. 2004).

Analysis of sucrose

The analysis of sucrose was carried out using an HPLC instrument with the specifications of a Shimadzu Prominence Type 20A, equipped with an LC 20 pump and a RID-10A (Refractive Index Detector). A volume of 20 µL of the sample extract was injected into the HPLC system using a shim-pack SCR-101C column (7.9 mmØ x 30 cm). Isocratic elution was performed using distilled water as the mobile phase at a 1 mL/min flow rate.

Analysis of oil

The analysis of oils was performed based on the method described by Efthymiopoulos (2018) using the following equation:

$$\text{Oil}\,(\%) = 100 \times \frac{W1}{W2}$$

Where:

W1 : Weight of extracted oils (g)

W2 : Dried coffee bean sample (g)

Volatile metabolites analysis

The volatile metabolite analysis was conducted by the Flavor Analysis Laboratory of the Center for Rice Plant Research, West Java. The analysis of volatile metabolites was conducted in two stages: (i) sample extraction and (ii) sample analysis using GC-MS.

Extraction and analysis of volatile metabolites

The extraction of coffee bean samples was conducted with slight modifications to the method described by Putri et al. (2017). Finely ground dried arabica coffee beans, weighing 1.5 grams each, were dissolved in a mixture of 30 mL of methanol: chloroform: distilled water (5:2:2 v/v). The solution was then sonicated for 30 minutes at a temperature of 30°C. Subsequently, centrifugation was carried out at a speed of 5,000 rpm for 10 minutes. The resulting supernatant was filtered using Whatman No.1 filter paper. The supernatant from each sample was evaporated using a rotary evaporator to obtain a crude extract. The analysis of volatile metabolite in the crude extract coffee samples was conducted using an Agilent 7890A GCMS instrument equipped with an Agilent 5975C XL EI/CI MS (Electron Ionization/Chemical Ionization Mass Spectrometry), a splitless injector at 250°C, and an HP-5MS column (30 m x 250 µm x 0.25 µm). Helium was used as the carrier gas at a 0.8 mL/min flow rate. The column temperature started at 60°C for 2 minutes, followed by a 5°C/min ramp until reaching 240°C for 7 minutes. The interface temperature was 250°C, with a mass scan range of 29-550 amu. The MS source temperature was set to 230°C, and the MS Quad temperature was set to 150°C. The analysis utilized the NIST14 library for compound identification. The GCMS analysis results consisted of spectra matched with compounds in the NIST14 library. The data included m/z values, retention times, and intensities, which were wed in a CSV (Comma Separated Value) format (Procida et al. 2020).

Statistical analysis

Statistical analysis of biochemical compounds was conducted using ANOVA (Analysis of Variance). Meanwhile, the relationship between biochemical compounds and microclimate factors was analyzed using Pearson correlation in SPSS 21 software. The analysis of volatile metabolites was conducted using MetaboAnalyst 5.0 software, which included PCA (Principal Component Analysis), PLS-DA (Partial Least Squares-Discriminant Analysis) with VIP (Variable Importance in Projection), and hierarchical clustering. Furthermore, Pearson correlation analysis examined the relationship between 10 discriminatory metabolites and microclimate.

RESULTS AND DISCUSSION

Biochemical composition of coffee varieties

The biochemical composition of Ateng, Tim-Tim, and Sigarar Utang green coffee beans varies (Table 1). The analysis of chlorogenic acid compounds in the three varieties yielded different results. The Ateng variety has the highest chlorogenic acid content at 6.08%, while the Tim-Tim variety has the lowest at 5.337%. The caffeine and trigonelline content in the Ateng, Tim-Tim, and Sigarar Utang varieties did not differ significantly. However, sucrose content showed variations. The Sigarar Utang variety had the highest sucrose content at 7.275%, and the Tim-Tim variety had the lowest concentration at 5.503%. The oil content was highest at 18.2% and 18.1% in the Ateng and Tim-Tim varieties, respectively. In contrast, the Sigarar Utang variety has the lowest oil content at around 16%, compared to the other two varieties.

Chlorogenic Acid (CGA) content influences the formation of the pigments, taste, flavor of roasted coffee, acidity and influences cup quality and brew preference (Farah and Donangelo 2006). CGA was present in arabica coffee at levels ranging from 4% to 8.4% (Ky et al. 2001; Budiastra et al. 2020; Mengistu et al. 2020). According to the report by Happyana et al. (2021), there was significant variation in the biochemical composition of the Ateng, Sigarar Utang, and Buhun coffee varieties that were collected from Mount Wayang, east Java. The Ateng variety has the highest chlorogenic acid content compared to the Sigarar Utang and Buhun varieties. These findings are similar to this research, where the Ateng variety also exhibited higher chlorogenic acid content than the Tim-Tim and Sigarar Utang varieties (Table 1).

Table 1. The biochemical compound content of Ateng, Tim-Tim, and Sigarar Utang varieties of Arabica coffee from the Cikole region (0.05>P)

Sample	Chlorogenic acid (%)±SD	Caffeine (%)±SD	Trigonelline (%)±SD	Sucrose (%)±SD	Oil (%)±SD
Ateng	$6.08^{a} \pm 0.04$	$1.963^{a} \pm 0.12$	$0.244^{a} \pm 0.03$	$5.687^{a} \pm 0,16$	$18.2^{a} \pm 0.12$
Tim-Tim	$5.337^{c} \pm 0.05$	$1.727^{a} \pm 0.09$	$0.224^{a} \pm 0.02$	$5.503^{a} \pm 0.24$	$18.1^{b} \pm 0.14$
Sigar Utang	$5.57^{b} \pm 0.07$	$1.817^{a} \pm 0.25$	$0.243^{a} \pm 0.02$	$7.275^{b} \pm 0.67$	$16.2^{c} \pm 0.18$

Note: Within a column, letter differences in values indicate significant mean differences with p-value <0.05

The caffeine contributes to the bitterness, strength, body, and cup quality of coffee (Awwad et al. 2021). In general, the higher the caffeine content, the more bitter the taste and the lower the cup quality (Ky et al. 2001; Silvarolla et al. 2004). It was reported that caffeine was present in the range from 0.6% to 1.9% (Franca et al. 2005; Belay et al. 2008). The results of the present study generally show relatively higher caffeine content. As indicated in Table 1, the caffeine content does not differ significantly in the three varieties. It has been suggested that the three varieties may produce a similar amount of caffeine. Mazzafera and Silvarolla (2010) reported that the caffeine content of most Arabica varieties is similar at around 1%.

Trigonelline acts as a precursor to aroma, contributing to the flavor profile of brewed coffee and forming important aroma compounds such as mainly niacin, pyridines, and some pyrroles (Seninde and Chambers 2020). The trigonelline content in the present study (0.224%) was lower than that reported by Cheserek et al. (2021) and Mengistu et al. (2020) for Arabica coffee, 0.93% to 1.5%. As indicated in Table 1, the trigonelline content did not differ significantly within the three varieties.

Sucrose is an important component that affects the overall quality of the cup by influencing the coffee's aroma characteristics during the roasting process (Ky et al. 2001). Joët et al. (2010) reported that sucrose levels in arabica coffee increased up to the ripening stage of the berries and continued to accumulate throughout fruit development. The results of the present study are generally in line with the findings of other researchers, who reported that the sucrose content of coffee varieties ranges from 0.88% to 8.2%, depending on the coffee species (Cheserek et al. 2021). As indicated in Table 1, this difference was likely due to variations in varieties and the environmental conditions of coffee plants in producing sucrose compounds.

Furthermore, coffee oil contains fat-soluble vitamins, which provide additional nutritional value and contribute to the richness of the final flavor (Oestreich-Janzen 2010). The results of the present study showed relatively greater oil content compared to the findings of Gimase et al. (2014) which the oil content in arabica coffee ranged from 12.5% to 18.4%. As indicated in Table 1, the oil content in arabica coffee samples also varies among the Ateng, Tim-Tim, and Sigarar Utang varieties. The differences in biochemical content are due to variations in varieties in biochemical production and the environmental conditions of coffee plant growth (Gichimu et al. 2014).

Volatile metabolite profile

There were differences in the total compounds of the Ateng, Sigarar Utang, and Tim-Tim varieties (Table 2). The Ateng variety has 121 compounds, the Tim-Tim variety has 145, and the Sigarar Utang variety has 143 including acetol, alcohol, oxime, cycloalkane, alkene, heterocyclic, ketone, diketone, ester, alkyne, amide, carboxylic acid, indole, aldehyde, hydrocarbon, lactone, benzonitrile, nitrile, pyrazine, pyran, phenol, carboxylic acid, indole, aldehyde, lactone, benzene, N-methyl pyrrolidone, guaiacol, aniline, hydroxy-ketone, sulfide, thiazole, thiophenol, triazine, phthalate, furan ether phenol, benzoic acid, tetralin, alkaloid, aminopyridine, and fatty acids. The most abundant group of compounds found in the three varieties are alkaloids, such as caffeine (Table 2).

As indicated on Table 2, phenolic compounds such as pyridines, guaiacol, and pyrazines were identified. These compounds are known for their bitter, burnt, roasted, and astringent characteristics (Heo et al. 2020; Gancarz et al. 2022). Thus, the Ateng and Sigarar Utang varieties are thought to have a burnt taste. Pyridine is produced through the degradation of trigonelline during roasting via the Maillard reaction. A higher degree of roasting is usually associated with a higher pyridine content (De Maria et al. 1996). Main phenolic compounds such as guaiacol and 4-ethyl guaiacol were also identified in the Ateng, Tim-Tim, and Sigarar Utang varieties. Guaiacol is associated with smoke, sweet, and medicinal characteristics (Flavornet in Heo et al. 2020) and can evoke a burning sensation even at very low concentrations (Arctander 1994). 4-ethyl guaiacol is associated with spice and clove flavors (Heo et al. 2020). Pyrazines are crucial in coffee beans as an aroma compound, contributing to the nutty, chocolatey, caramel, and spicy notes. Its function is primarily olfactory. Pyrazines are formed by the Maillard reaction between carbohydrates and amino acids during coffee roasting (Angeloni et al. 2021; Zakidou et al. 2021). These compounds contribute to the characteristic roasted, nutty, and earthy aromas associated with coffee, as well as the unique aromatic profile of each coffee bean or blend (Mortzfeld et al. 2020; Angeloni et al. 2021; Zakidou et al. 2021).

Table 2. The results of the compound analysis using gas chromatography-mass spectrometry

Retention time (min)	Compound name	The presence of the compound			Group
		Ateng	Tim-Tim	Sigarar Utang	
3.69	Acetylcarbinol	+	+	+	Acetol
4.02	2-Ethoxyacetic acid	+	+	+	Acetol
4.97	1-Butanol	-	+	-	Alcohol
5.96	1,2-Ethanediol	+	-	-	Alcohol
4.96	Oxime-, methoxy-phenyl-	+	-	+	Oxime
5.63	Cyclobutane, 1,2-dimethylene-	-	+	+	Sikloalkana
5.75	3-Methyl-3-vinyl-1-cyclopropene	-	-	+	Alkene
5.94	1,2-Ethanediol	+	+	+	Alcohol
6.31	Pyridine	+	-	+	Phenolic
6.86	4-Penten-2-one	-	-	+	Ketones
6.96	1,6-Heptadiyne	-	+	+	Alkyne
7.41	2-Butanone, 4-hydroxy-3-methyl-	+	-	-	Ketones
7.41	1,4-Butanedione	-	+	+	Diketones
7.90	Isobutanol	+	-	-	Alcohol
7.89	Methyl acetate	-	+	+	Ester
8.31	1-Propanol	+	+	+	Alcohol
8.62	Ethyl glycolate	+	+	+	Ester
8.77	Thiazolidine, 3-methyl-	-	+	+	Heterocyclic
9.64	Isopropyl acetate	-	+	+	Ester
9.77	Benzyl alcohol	-	+	+	Alcohol
9.83	Methyl glycolate	-	+	+	Ester
9.64	Isopropyl acetate	+	-	-	Ester
10.30	1-Heptyn-4-ol	-	-	+	Alkyne
10.80	2-Butoxyethanol	-	+	+	Glycol ether
9.78	Benzyl alcohol	+	-	-	Alcohol
9.84	Methyl glycolate	+	-	-	Ester
10.29	1-Heptyn-4-ol	+	-	-	Alkyne
11.42	Acetic acid	+	+	+	Carboxylic acid
11.58	Methyl isobutyrate	+	+	+	Ester
12.49	1-Penten-3-ol	-	+	+	Alcohol alkenyl
12.63	3-Furanmethanol	-	+	+	Alcohol heterocyclic
12.82	Formic acid	-	+	+	Carboxylic acid
12.91	2-Phenylindole	+	+	+	Indole
13.06	Benzaldehyde	-	+	+	Aldehyde
13.52	Propanoic acid	-	+	+	Carboxylic acid
13.70	2,3-Butanediol, [S-(R*,R*)]-	-	+	+	Alcohol
1402	N.aphthalene, 2,6-di-tert-butyl-	+	+	+	Hidrokarbon aromatic
14.33	Furfural, 5-methyl-	+	+	+	Aldehyde
14.49	2(1H)-Pyrazinone	+	+	+	Heterocyclic
14.58	2,3-Butanediol	+	+	+	Alcohol
14.90	2-Propanol, 1,3-dimethoxy-	+	+	+	Alcohol
15.29	Methyl benzoate	+	+	+	Ester
15.54	Butyrolactone	+	+	+	Lakton
15.68	Isobutyl formate	+	+	+	Ester
15.84	Benzeneacetaldehyde	+	+	+	Aldehyde
15.89	Benzaldehyde, 2-methyl-	+	+	+	Aldehyde.
16.07	Benzene, (ethenyloxy)-	-	+	+	Benzene (ethenyloxy)
16.42	2-Furanmethanol	+	+	+	Alcohol
16.65	Isovaleric acid	+	+	+	Carboxylic acid
16.89	1-Methylpyrrolidinone	+	+	+	N-methyl pyrrolidone (NMP)
16.96	m-Methoxybenzontrile	+	-	+	Benzonitrile
17.23	2,5-Furandione, 3-methyl-	+	+	+	Citraconic anhydrides
17.54	Disparlure	-	-	-	Feromone
17.86	3-Furancarboxylic acid	+	-	+	Ester
17.61	1-Penten-3-one, 2-methyl-	-	-	-	Alkene
17.73	3-Furancarboxylic acid	-	-	+	Carboxylic acid
18.03	Naphthalene	+	+	+	Hidrokarbon aromatic
18.16	Benzonitrile	+	+	+	Nitrile
18.25	2(5H)-Furanone	+	+	+	Organic compound
18.57	Methyl 2-phenylacetate	+	+	+	Ester
18.81	1,2-Cyclopentanedione	+	-	-	Organic compound
18.80	3-Methyl-5-pyrazolone	-	+	+	Pyrazolone
18.86	1,2-Cyclopentanedione	-	+	+	Diketones

18.98	Methyl nicotinate	+	+	+	Ester
19.30	2,4-Diphenylthiazole	+	+	+	Heterocyclic aromatic
19.40	3-Methylcrotonic acid	+	-	+	Carboxylic acid.
19.48	2-Cyclohexen-1-ol	+	+	+	Alcohol.
19.44	Cyanamide, dimethyl-	-	+	-	Amina
19.89	1-Methyl-2-phenylindole	+	-	-	Heteroosiklik aromatic
20.12	Cyclotene	+	+	+	Sikloalkene aromatic
20.52	Naphthalene, 2-methyl-	-	+	+	Hidrokarbon aromatic
20.72	o-Guaiacol	+	+	+	Guaiacol
21.00	5-Methoxyindanone	-	+	+	Organic compound
21.12	Benzyl alcohol	+	+	+	Alcohol
21.19	4-Methyl-5H-furan-2-one	-	+	+	Lakton
21.47	Tetrahydrothiazole	+	+	+	Heterocyclic-amina
21.62	Naphthalene, 7-butyl-1-hexyl-	-	+	-	Aromatic-hidrokarbon
21.86	Phenylethyl alcohol	-	+	+	Alcohol
22.72	2,4-Diaminophenol	-	+	+	Amina aromatic
21.86	Phenylethyl alcohol	-	-	-	Alcohol
22.90	1,2,3-Benzenetriol	-	-	+	Phenol
23.11	2,5-Furandicarboxaldehyde	+	+	+	Aldehyde
23.21	Pyrazine, 2-methoxy-6-methyl-	+	+	+	Pyrazine
23.41	2H-Pyran-2,6(3H)-dione	+	+	+	Piran
23.64	Cyclododecane	+	+	+	Hidrokarbon-aromatic
23.71	Phenol	+	+	+	Phenol
24.21	4-Ethylguaiacol	+	+	+	Guaiacol
24.35	Furaneol	+	+	+	Heterocyclic
24.44	2-Pyrrolidinone	+	+	+	Lakton
24.65	Maltol	+	-	+	Piran
24.76	1,3-Butadiene, 1-[(1-methyl ethyl)thio]-, (E)-	+	-	-	Alkene
25.22	p-Cresol	-	+	+	Phenol
24.75	1,3-Butadiene, 1-[(1-methyl ethyl)thio]-, (E)-	-	-	+	Alkene
25.28	2(3H)-Furanone, dihydro-4-hydroxy-	+	+	+	Lakton
25.41	1-Phenyl-1,2-butanediol	-	-	-	Alcohol
25.55	Butyl maleate	-	+	+	Ester
25.58	2(3H)-Furanone, 5-heptyldihydro-	+	+	+	Lactone
25.93	2,5-Piperazinedione	+	+	+	Organic compound
26.22	Pyrazine, 2-methoxy-3-(1-methyl ethyl)-	-	-	+	Pyrazine
26.35	2-Formylpyrrole	-	+	+	Piroles
26.66	4,5-Diamino-2-pyrimidinol	+	+	+	Pyrimidine
26.69	2-Acetylcyclopentanone	+	+	+	Ketones
26.82	3-Hydroxydihydro-2(3H)-furanone	+	+	+	Lakton
27.06	2-Ethylhexenal	+	+	+	Alkene
27.32	2-Methoxy-4-vinyl phenol	+	+	+	Phenol
27.45	Docosane	+	+	+	Alkane
27.48	Thiophene, 2-nitro-	+	-	+	Hydrocarbon
27.82	Methyl palmitate	+	+	+	Ester
28.00	Mepivacaine	+	+	+	Amida
28.25	Isopropyl palmitate	+	+	+	Ester
28.34	1,3,5-Benzenetriol	+	+	+	Phenol
28.51	2-Thiophenemethanol	+	+	-	Alcohol
28.67	Phenol, 2,6-dimethoxy-	+	+	+	Phenol
28.68	Pyranone	+	+	+	Pyrone
28.81	2-Ethylcyclohexanone	-	+	+	Cyclohexanone
28.87	1-[(Allyloxy)methyl]naphthalene	+	-	+	Hydrocarbon aromatic
29.11	5-Hydroxymaltol	+	+	+	Hidroxy-ketones
29.18	Diphenyl sulfide	+	+	+	Sulfide
29.24	5-Hydroxymaltol	+	+	+	Hidroxy-ketones
29.50	2,4-Di-tert-butyl-phenol	+	+	+	Phenol
29.67	γ-Nonalactone	-	+	+	Fatty acid
29.76	Isopropylmethylnitrosamine	+	+	+	Hydrocarbon
30.12	Methyl 2-thienyl acetate	+	-	+	Piran
30.33	3-Ethoxyaniline	+	-	+	Aniline
30.46	Diethyl phthalate	+	+	+	Phthalate ester
30.66	Cyclotetradecane	+	-	+	Sikloalkana
30.72	Cyclohexadecane	-	+	+	Alkane
30.81	Benzofuran, 2,3-dihydro-	+	+	+	Heterocyclic

30.97	Cyclobutanone, 2-tert-butyl-	+	+	+	Ketones
31.17	N-Benzylphthalimide	+	+	+	Amida
31.27	2-Furoylhydrazide	+	-	+	Heterocyclic
31.45	Methyl stearate	+	+	+	Ester
31.54	Caprylic acid	-	+	-	Caprylate
31.62	Benzoic acid	+	+	+	Carboxylic acid aromatic
31.79	Methyl 6-octadecenoate	+	+	+	Ester
32.16	3-Phenoxybenzyl alcohol	+	-	-	Alcohol
31.93	4-Isothiazolecarboxamide	-	-	+	Isothiazole
32.15	3-Phenoxybenzyl alcohol	-	-	+	Alcohol
32.25	2(3H)-Furanone, dihydro-5-propyl-	+	+	+	Ester
32.31	Thiazole, 2,2'-thiobis-	+	-	+	Thiazole
32.41	Diisopropyl phthalate	+	-	+	Ftalat
32.49	Lauric acid	+	+	+	Saturated fatty acids
32.60	5-Hydroxymethylfurfural	+	+	+	Furan
32.93	3-Methoxythiophenol	+	+	+	Thiophenol
33.00	6-t-Butylamino-[1,3,5]triazine-2,4-diol	+	+	+	Triazine
33.34	Butyl isobutyl phthalate	+	+	+	Ftalat
33.41	6-Nitrophthalide	-	-	+	Phthalide
33.61	Vanillin	+	+	+	Aldehyde phenolic
33.73	Benzeneacetic acid	+	+	+	Carboxylic acid aromatic
33.80	9,10-Dihydrophenanthrene	+	+	+	Hidrokarbon aromatic
33.92	1-(2-Furyl)-1,2-ethanediol	+	-	+	Furan
33.99	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	+	+	+	Ester
34.14	Benzene, [(1-methylpropyl)thio]-	+	+	+	Sulfide aromatic
34.23	Benzene, 1,4-diethoxy-	+	+	+	Eter aromatic
34.37	Catechol	+	+	+	Phenol
34.55	Phenol, 3-butoxy-	+	+	+	Eter phenolic
34.82	Phenol, 4-proxy-	+	+	+	Eter phenolic
35.21	Phenol, 2-ethoxy-	-	+	+	Eter phenol
35.32	2-Isopropoxyphenol	+	+	+	Phenol
35.59	3(2H)-Pyridazinone, 6-methyl-	+	+	+	Pyridazine
35.78	Dibutyl phthalate	+	+	+	Ftalat
35.90	Benzoic acid, 3,5-dimethyl-	-	+	-	Asam benzoate
36.00	Myristic acid	+	+	+	Fatty acid
36.34	2-Amino-3-hydroxy pyridine	-	+	+	Aminopyridine
36.44	3-Methyl-1-tetralone	+	+	+	Ketones
36.60	2,6-Dimethyltetralin	+	+	+	Tetralin
36.91	3-Thiophenecarboxaldehyde	-	+	+	Aldehyde
39.02	Palmitic acid	+	+	+	Fatty acid
41.17	Phenol, 4-(heptyl oxy)-	+	+	+	Eter phenolic
42.61	2-(Methylselanyl)-1-benzothiophene-3-carbaldehyde	-	+	-	-
43.29	Stearic acid	-	+	+	Saturated fatty acids
44.16	Caffeine	+	+	+	Alkaloid
44.41	Diisooctyl phthalate	+	-	-	Ftalat
45.58	Linoelaidic acid	+	+	+	Fatty acid trans
47.19	4-Butoxybutanol	+	+	+	Alcohol
49.94	Enanthic acid	+	-	-	Saturated fatty acids

Note: +: any and -: not detected

Principal Component Analysis (PCA)

The data set extracted from the GCMS was evaluated with multivariate data analysis for classifying the green coffee beans according to their varieties. At the beginning of the analysis, PCA was applied. PCA can be used as a basis for analysis as the total PC1 and PC2 values are close to 70% (Jolliffe 2002). The best group separation on the score plot of the PCA model was obtained by PC1 (41.2%) and PC2 (28%) combined explaining 69.2% (Figure 2). The results of the volatile metabolites in the Ateng, Tim-Tim, and Sigarar Utang varieties were positioned differently. The Ateng variety was positioned in Quadrant 2. the Sigarar Utang variety was positioned in Quadrant 3, and the Tim-Tim variety was positioned in Quadrant 4. This score plot indicated the diversity of volatile metabolites in three varieties. This is due to the genetic factors unique to each Arabica coffee variety and the complex environmental conditions in which they are grown (Gichimu et al. 2014; Gimase et al. 2014). In this research, we correlate the effect of microclimatic data, including light intensity, temperature, humidity, and rainfall, with the content of discriminant volatile metabolites, which will be discussed later.

Partial Least Square-Discriminant Analysis (PLS-DA)

The PLS-DA results show that the Score plot model can sharply separate samples and classify them based on the

Ateng, Tim-Tim, and Sigarar Utang varieties (Figure 3). The score plot is generated by combining 38.3% of the data from Component 1 and 30.3% from Component 2.

Further investigation was conducted to identify the discriminating compounds that play a role in grouping arabica coffee varieties Ateng, Tim-Tim, and Sigarar Utang. Based on a VIP value >1.5, Ten of the candidate biomarker compounds were obtained. These ten compounds include 5-Hydroxymaltol, 2(1H)-Pyrazinone, 2-Phenylindole, Docosane, γ-Nonalactone, 3-Thiophenecarboxaldehyde, Methyl glycolate, Formic acid, 1,6-Heptadiyne, and Mepivacaine (Figure 3). The investigation successfully identified ten discriminating compounds with VIP values >1.5 in grouping Arabica coffee varieties Ateng, Tim-Tim, and Sigarar Utang. These compounds can potentially serve as biomarkers to differentiate between the three Arabica coffee varieties.

In addition, these ten compounds are potential biomarkers commonly found in coffee plants (Papandreou et al. 2019; Ongo et al. 2020; Farag et al. 2022; Silva et al. 2022; Castro-Moretti et al. 2023). The following groups are included: pyran, pyrazine, fatty acid, thiophene, glycolate, formate, alkyne, and piperidine. Three of the ten compounds are known to play a role in taste and aroma: 5-Hydroxymaltol, γ-Nonalactone, and formic acid, of which the three compounds were found highest in the Tim-Tim variety. The group of pyrans known as 5-Hydroxymaltol has been found to affect the quality and taste of apples (Demirci et al. 2018). γ-Nonalactone is an oil with fatty esters contributing to a coconut-like, sweet, fatty, fruity taste (PubChem 2023). Additionally, formic acid contributes to the pungent aroma of formyl vinegar, while its taste is acetic, astringent, fruity, mustardy, and bready, with a nuance of pyruvic acid (PubChem 2023).

Hierarchical clustering

The hierarchical clustering analysis clearly classified the green bean coffee metabolomes according to their coffee varieties (Figure 4). The hierarchical clustering analysis showed that the Ateng and Tim-Tim samples were in the same cluster, while the Sigarar Utang variety was in a different cluster. Therefore, based on the metabolome profiles in the hierarchical clustering analysis, the arabica coffee varieties Ateng and Tim-Tim possessed a closer relationship compared to the Sigarar Utang coffee. This is indicated by the proximity of the clades between the Ateng and Tim-Tim samples, while the Sigarar Utang variety is in a separate clade from the other samples. The hierarchical clustering results prove that the arabica coffee varieties Ateng, Tim-Tim, and Sigarar Utang differ in their metabolite compound content.

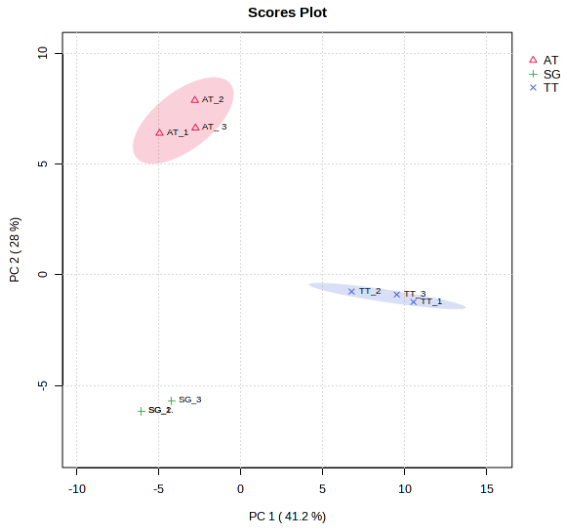
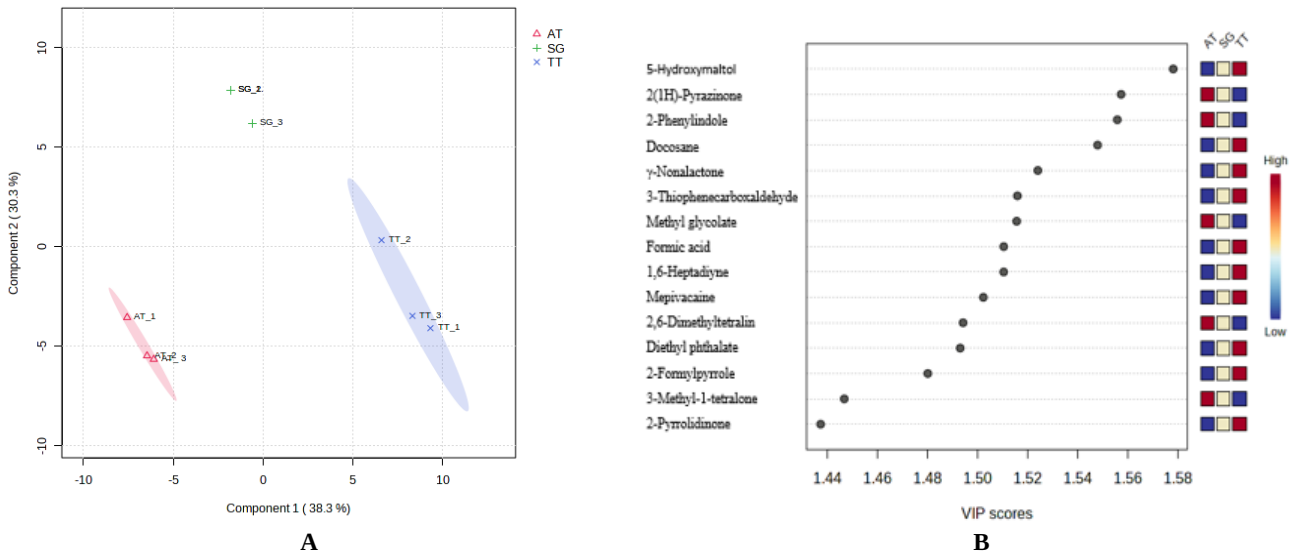


Figure 2. 2D Score plot PCA of sample coffee (AT: Ateng; TT: Tim-Tim; and SG: Sigarar Utang)



A **B**

Figure 3. A. The score plot; B. VIP score of PLSDA from the coffee sample (AT: Ateng; TT: Tim-Tim; and SG: Sigarar Utang)

It has been reported that the Ateng variety is the result of natural crossbreeding between Tim-Tim and Caturra varieties (DitjenPKH 2005), while the Tim-Tim variety is the result of natural crossbreeding between arabica coffee varieties Typica and robusta. On the other hand, the Sigarar Utang variety results from natural crossbreeding between Blawan Pasumah and Katimor (Dani et al. 2019). Based on the distance in the dendrogram, the Ateng and Tim-Tim varieties are indicated to have a closer lineal relationship compared to the Sigarar Utang variety (Figure 4). Additionally, the Sigarar Utang variety is indicated to have a closer lineal relationship with the Ateng variety as it originated from Katimor. Moreover, this diversity is believed to be related to microclimatic factors (Table 3). Previous research has shown that microclimatic factors like temperature, humidity, rainfall, and altitude correlate with metabolites in robusta coffee beans in West Java (Awaliyah et al. 2022).

The differences in biochemical composition among the three Arabica coffee varieties were influenced by the varieties themselves and are believed to be affected by the locations where the coffee plants are grown (Tabel 3). The Pearson correlation coefficient test results indicated correlations between the biochemical components and microclimate factors, such as light intensity, temperature, humidity, and rainfall (Table 4).

Correlation of biochemical components with microclimates

The microclimate data available in this study were light intensity, temperature, humidity, and rainfall (Table 3).

The study found that sucrose content was very strongly positively correlated with light intensity (r = 0.720). Meanwhile, the oil content was strongly negatively correlated with light intensity (r = -0.702). While other compounds did not show a strong correlation with any microclimate. This was consistent with research showing that coffee grown in an open environment had higher total sugar content than coffee grown in a shaded environment (Alves et al. 2018). According to Araújo et al. (2016), growing coffee in a shaded environment alters microclimatic conditions, resulting in lower light intensity, increased air humidity, and reduced water evaporation over a longer time. Mariño et al. (2016) noted that this increase in shading can lead to an increase in pathogenic microorganisms and pest attacks, which can ultimately damage the coffee cherries. These microorganisms produce enzymes that degrade the coffee cherries' mucus, causing fermentation. This fermentation process triggers physicochemical changes, such as reduced water content, lower simple sugar content, and the formation of aroma and flavor precursors (Koutouleas et al. 2022). On the other hand, the study found that oil content was very strongly negatively correlated with light intensity (r = -0.702). These results were consistent with the research of Odeny et al. (2016), which showed that the shaded coffee plants recorded higher mean oil content compared to coffee grown under full sun. This phenomenon is caused by the effects of higher altitude and humidity, as well as lower light intensity, on the process of coffee fruit ripening. These environmental conditions support the development and accumulation of higher oil compounds in coffee beans (Villarreal et al. 2014; Martins et al. 2020; Cassamo et al. 2022).

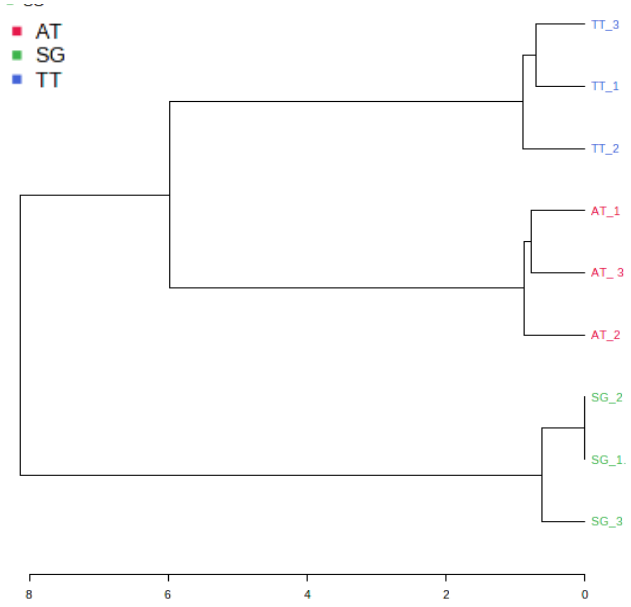


Figure 4. Hierarchical clustering from the coffee sample (AT: Ateng, TT: Tim-Tim, dan SG: Sigarar Utang)

Table 3. Monthly averages of microclimate data study sites

Sample	Month	Light intensity	Temperature	Humidity	Rainfall
Ateng	Mei	2097	22	82	147
	Juni	2713	19	80	151
	Juli	2377	18	78	99
Timtim	Mei	2097	22	82	147
	Juni	2713	19	80	151
	Juli	2377	18	78	99
Sigarar	Mei	3767	22	82	147
Utang	Juni	3692	21	80	151
	Juli	2377	21	78	99

Table 4. Pearson correlation coefficients between biochemical components and microclimatic factors (light intensity, temperature, humidity, rainfall)

Biochemical components	Light intensity (r)	Temperature (r)	Humidity (r)	Rainfall (r)
Caffeine	0.108 [a]	-0.017 [a]	-0.188 [a]	-0.063 [a]
Chlorogenic acid	-0.154 [a]	-0.029 [a]	0.083 [a]	-0.017 [a]
Trigonelline	0.094 [a]	0.382 [b]	0.310 [b]	-0.039 [a]
Sucrose	0.720 [d]	0.563 [c]	-0.220 [b]	-0.045 [a]
Oil	-0.702 [d]	-0.485 [c]	0 [a]	0 [a]

Note: $p<0.05$. The Pearson correlation between the biochemical composition and microclimate factors was determined using three replications of coffee samples. r = Pearson's linear-correlation coefficients. The correlation values were categorized as follows: 0.00-0.199: Very low correlated data (a); 0.20-0.399: Low correlated data (b); 0.40-0.599: Moderately strong correlated data (c); 0.60-0.799: Strongly correlated data (d); 0.80-1.00: Very strongly correlated data (e). The closer the correlation value is to 1 or -1, the closer the relationship, whereas values closer to 0 indicate a weaker relationship (Pearson 1901)

Table 5. Pearson correlation between biomarker candidates with microclimate

Compounds	Light intensity (r)	Temperature (r)	Humidity (r)	Rainfall (r)
5-Hydroxymaltol	-0.017 [a]	-0.108 [a]	0.063 [a]	-0.009 [a]
2(1H)-Pyrazinone	0.041 [a]	0.367 [b]	0.322 [b]	-0.045 [a]
2-Phenylindole	0.333 [b]	0.373 [b]	0.309 [b]	-0.043 [a]
Docosane	-0.036 [a]	-0.045 [a]	-0.019 [a]	0.003 [a]
γ-Nonalactone	0.178 [a]	0.147 [a]	0.046 [a]	-0.006 [a]
3-Thiophenecarboxaldehyde	0.461 [c]	0.369 [b]	0.034 [a]	-0.005 [a]
Methyl glycolate	0.457 [c]	0.367 [b]	0.190 [a]	-0.026 [a]
Formic acid	-0.054 [a]	-0.019 [a]	0.085 [a]	-0.012 [a]
1,6-Heptadiyne	0.437 [c]	0.309 [b]	0.080 [a]	-0.011 [a]
Mepivacaine	0.371 b	0.277[b]	0.109 [a]	-0.015 [a]

Note: p <0.05, The Pearson correlation between the biochemical composition and microclimate factors was determined using three replications of coffee samples. r = Pearson's linear-correlation coefficients. The correlation values were categorized as follows: 0.00-0.199: Very low correlated (a); 0.20-0.399: Low correlated data (b); 0.40-0.599: Moderately strong correlated data (c); 0.60-0.799: Strongly correlated data (d); 0.80-1.00: Very strongly correlated data (e). The closer the correlation value is to 1 or -1, the closer the relationship, whereas values closer to 0 indicate a weaker relationship (Pearson 1901)

Correlation of discriminant compound with microclimate

Correlation analysis shows that ten volatile compounds in arabica coffee green beans correlate with microclimate factors such as light intensity, temperature, humidity, and rainfall (Table 5).

Overall, three volatile compounds showed moderate positive correlations with light intensity. The 3-Thiophenecarboxaldehyde (r = 0.461), methyl glycolate (r = 0.457), and 1,6-Heptadiyne (r = 0.535) exhibited moderate positive correlations with light intensity (Table 5). While the other compounds did not show a strong correlation with any microclimate. This differs from a literature review by Ahmed et al. (2021), which found that microclimate factors like altitude correlate with some volatile metabolites such as terpenes (Bertrand et al. 2012; Sridevi and Giridhar 2014). Phenolic compounds, conversely, have a negative correlation with light intensity but do not correlate with temperature (Delaroza et al. 2017; Tolessa et al. 2017; Dos Santos Scholz et al. 2018). There is limited research on the relationships between 3-thiophene carboxaldehyde, Methyl glycolate, and 1,6-Heptadiyne, and microclimate factors. Geographic location is believed to be a significant factor that influences these differences. This includes variations in the nutritional conditions of coffee plants, microorganisms that inhabit the surrounding areas, and complex environmental conditions. Apart from that, differences in cultivation practices in each location can also play a role in shaping the characteristics and quality of the coffee produced (Arifin and Devnita 2008; Araújo et al. 2016; Martins et al. 2020; Anhar et al. 2021)

In conclusion, the biochemical composition of the Ateng, Tim-Tim, and Sigarar Utang varieties of Arabica coffee differs significantly in the chlorogenic acid, sucrose, and oil. At the same time, there are no significant differences in the caffeine and trigonelline compounds. The metabolite profiles of the Ateng, Tim-Tim, and Sigarar Utang Arabica green bean coffee varieties exhibited a relatively high level of diversity, with a diversity index of 69.2%. It appears that the concentrations of sucrose and oil are related to local microclimate factors, specifically light intensity, where sucrose shows a positive correlation and oil shows a negative correlation with light intensity. The compounds 3-Thiophenecarboxaldehyde, Methyl glycolate, and 1,6-Heptadiyne show a positive Moderately strong correlation with light intensity. The remaining seven compounds did not show any correlation with the environmental factors examined.

ACKNOWLEDGEMENTS

This work was financially supported by the Research, Community Services, and Innovation Program of Institut Teknologi Bandung, Indonesia, grant number 61/IT1.C11/SK-TA/2023.

REFERENCES

Ahmed S, Brinkley S, Smith E, Sela A, Theisen M, Thibodeau C, Warne T, Anderson E, Van Dusen N, Giuliano P, Ionescu KE, Cash SB. 2021. Climate change and coffee quality: Systematic review on the effects of environmental and management variation on secondary metabolites and sensory attributes of *Coffea arabica* and *Coffea canephora*. Front Plant Sci 12: 708013. DOI: 10.3389/fpls.2021.708013.

Akiyama M, Murakami K, Hirano Y, Ikeda M, Iwatsuki K, Wada A, Tokuno K, Onishi M, Iwabuchi H. 2008. Characterization of headspace aroma compounds of freshly brewed Arabica coffees and studies on a characteristic aroma compound of Ethiopian coffee. J Food Sci 73 (5): C335-C346. DOI: 10.1111/j.1750-3841.2008.00752.x.

Alves AL, Pessoa MS, De Souza PEN, Partelli FL, Moscon PS, da Silva EC, Guimarães AO, Muniz EP, Pinheiro PF, Borém FM, Morais PC. 2018. Influence of environmental and microclimate factors on the coffee beans quality (*C. canephora*): Correlation between chemical analysis and stable free radicals. Agric Sci 9 (9): 1173-1187. DOI: 10.4236/as.2018.99082.

Amanpour A, Selli S. 2016. Differentiation of volatile profiles and odor activity values of Turkish coffee and French press coffee. J Food Process Preserv 40 (5): 1116-1124. DOI: 10.1111/jfpp.12692.

Angeloni S, Mustafa AM, Abouelenein D, Alessandroni L, Acquaticci L, Nzekoue FK, Petrelli R, Sagratini G, Vittori S, Torregiani E, Caprioli G. 2021. Characterization of the aroma profile and main key odorants of Espresso coffee. Molecules 26 (13): 3856. DOI: 10.3390/molecules26133856.

Anhar A, Abubakar Y, Widayat HP, Muslih AM, Romano, Baihaqi A. 2021. Altitude, shading, and management intensity effect on Arabica

coffee yields in Aceh, Indonesia. Open Agric 6: 254-262. DOI: 10.1515/opag-2021-0220.
Arctander S. 1994. Perfume and Flavor Chemicals (Aroma Chemicals): Volume I. Allured Publishing Corporation, USA.
Araújo AV, Partelli FL, Oliosi G, Pezzopane JRM. 2016. Microclimate, development and productivity of robusta coffee shaded by rubber trees and at full sun. Rev Ciênc Agron 47 (4): 700-709. DOI: 10.5935/1806-6690.20160084.
Arifin M, Devnita R. 2008. Micromorphological characterization of some volcanic soil in West Java. Indones J Geosci 3 (4): 195-203. DOI: 10.17014/ijog.vol3no4.20082.
Assa A, Loppies JE, Amalia AN, Indriana D, Mamang URR, Ariyanti M, Winaldi A. 2021. Chemical compounds and sensory characteristics of Arabica coffee (*Coffea arabica*) as a novel specialty coffee from Sinjai regency, Indonesia. Food Res 5 (2): 107-112. DOI: 10.26656/Fr.2017.5(S2).001.
Awaliyah S, Widiyanto SNB, Maulani RR, Hidayat A, Husyari UD, Syamsudin TS, Marwani E. 2022. Correlation of microclimate of west Java on caffeine and chlorogenic acid in *Coffea canephora* var. *robusta*. J Biol Sci Technol Manag 4 (1): 54-60. DOI: 10.5614/3bio.2022.4.1.7.
Awwad S, Issa R, Alnsour L, Albals D, Al-Momani I. 2021. Quantification of caffeine and chlorogenic acid in green and roasted coffee samples using HPLC-DAD and evaluation of the effect of degree of roasting on their content. Molecules 26 (24): 7502. DOI: 10.3390/Molecules26247502.
Ayelign A, Sabally K. 2013. Determination of Chlorogenic Acids (CGA) in coffee beans using HPLC. Am J Res Commun 1 (2): 78-91.
Barbosa JN, Borém FM, Cirillo MÂ, Malta MR, Alvarenga AA, Alves HMR. 2012. Coffee quality and its interactions with environmental factors in Minas Gerais, Brazil. J Agric Sci 4 (5): 181-190. DOI: 10.5539/jas.v4n5p181.
Belay A, Ture K, Redi M, Asfaw A. 2008. Measurement of caffeine in coffee beans with UV/Vis spectrometer. Food Chem 108 (1): 310-315. DOI: 10.1016/j.foodchem.2007.10.024.
Bertrand B, Boulanger R, Dussert S, Ribeyre F, Berthiot L, Descroix F, Joët T. 2012. Climatic factors directly impact the volatile organic compound fingerprint in green Arabica coffee bean as well as coffee beverage quality. Food Chem 135 (4): 2575-2583. DOI: 10.1016/j.foodchem.2012.06.060.
Braga CP, Adamec J. 2018. Metabolome analysis. In: Ranganathan S, Gribskov M, Nakai K, Schönbach C (eds). Encyclopedia of Bioinformatics and Computational Biology. Elsevier, Amsterdam, Netherlands. DOI: 10.1016/B978-0-12-809633-8.20134-9.
Budiastra IW, Sutrisno M, Widyotomo S, Ayu PC. 2020. Determination of trigonelline and Chlorogenic Acid (CGA) concentration in intact coffee beans by NIR spectroscopy. Agric Eng Intl 22 (1): 162-168.
BPS (Badan Pusat Statistik Indonesia). 2022. Statistik Perkebunan Unggulan Nasional 2020-2022. Direktorat Jenderal Perkebunan Kementerian Pertanian Republik Indonesia, Jakarta. [Indonesian]
Campa C, Ballester JF, Doulbeau S, Dussert S, Hamon S, Noirot M. Trigonelline and sucrose diversity in wild *Coffea* species. Food Chem 88: 39-43. DOI: 10.1016/j.foodchem.2004.01.020.
Castro-Moretti FR, Cocuron J-C, Castillo-Gonzalez H, Escudero-Leyva E, Chaverri P, Guerreiro-Filho O, Slot JC, Alonso AP. 2023. A metabolomic platform to identify and quantify polyphenols in coffee and related species using liquid chromatography mass spectrometry. Front Plant Sci 13: 1057645. DOI: 10.3389/fpls.2022.1057645.
Cassamo CT, Mangueze AVJ, Leitão AE, Pais IP, Moreira R, Campa C, Chiulele R, Reis FO, Marques I, Scotti-Campos P, Lidon FC, Partelli FL, Ribeiro-Barros AI, Ramalho JC. 2022. Shade and altitude implications on the physical and chemical attributes of green coffee beans from Gorongosa Mountain, Mozambique. Agronomy 12 (10): 2540. DOI: 10.3390/agronomy12102540.
Ceylan R, Zengin G, Guler GO, Aktumsek A. 2020. Bioactive constituents of *Lathyrus czeczottianus* and ethyl acetate and water extracts and their biological activities: An endemic plant to Turkey. S Afr J Bot 143: 306-311. DOI: 10.1016/j.sajb.2020.11.023.
Cheng B, Furtado A, Smyth HE, Henry RJ. 2016. Influence of genotype and environment on coffee quality. Trends Food Sci Technol 57: 20-30. DOI: 10.1016/j.tifs.2016.09.003.
Cheserek JJ, Ngugi K, Muthomi JW, Omondi CO, Ezekiel KN. 2021. Green bean biochemical attributes of arabusta coffee hybrids from Kenya using HP-LC and soxhlet extraction methods. Aust J Crop Sci 15 (2): 201-208. DOI: 10.21475/Ajcs.21.15.02.P2581.
Constantino LV, Zeffa DM, Koltun A, Urbano MR, Sanzovo AWS, Nixdorf SL. 2020. Extraction of soluble sugars from green coffee beans using hot water and quantification by a chromatographic method without an organic solvent. Acta Chromatogr 32 (4): 242-246. DOI: 10.1556/1326.2020.00704.
Czerny M, Mayer F, Grosch W. 1999 Sensory study on the character impact odorants of roasted Arabica coffee. J Agric Food Chem 47 (2): 695-699. DOI: 10.1021/jf980759i.
Dani D, Rokhmah D, Pranowo D. 2019. Identifikasi awal perbedaan karakter morfofisiologi antar empat kultivar kopi Arabika. Jurnal Tanaman Industri dan Penyegar 6 (3): 119. DOI: 10.21082/jtidp.v6n3.2019.p119-126. [Indonesian]
Delaroza F, Rakocevic M, Malta GB, Sanchez PM, Bruns RE, Scarminio IS. 2017. Factorial design effects of plant density, pattern and light availability on the caffeine, chlorogenic acids, lipids, reducing sugars and ash contents of *Coffea arabica* L. beans and leaves. Anal Methods 9 (24): 3612-3618. DOI: 10.1039/C7AY00721C.
De Maria CAB, Trugo LC, Neto FRA, Moreira RFA, Alviano CS. 1996. Composition of green coffee water-soluble fractions and identification of volatiles formed during roasting. Food Chem 55 (3): 203-207. DOI: 10.1016/0308-8146(95)00104-2.
Demirci MA, Ipek Y, Gul F, Ozen T, Demirtas I. 2018. Extraction, isolation of heat-resistance phenolic compounds, antioxidant properties, characterization and purification of 5-hydroxymaltol from Turkish apple pulps. Food Chem 269: 111-117. DOI: 10.1016/j.foodchem.2018.06.147.
Dos Santos Scholz MB, Kitzberger CSG, Durand N, Rakocevic M. 2018. From the field to coffee cup: impact of planting design on chlorogenic acid isomers and other compounds in coffee beans and sensory attributes of coffee beverage. Eur Food Res Technol 244: 1793-1802. DOI: 10.1007/s00217-018-3091-7.
Efthymiopoulos I, Hellier P, Ladommatos N, Kay A, Mills-Lamptey B. 2018. Integrated strategies for water removal and lipid extraction from coffee industry residues. Sustain Energy Technol Assessments 29: 26-35. DOI: 10.1016/j.seta.2018.06.016.
Farah A, Donangelo CM. 2006. Phenolic compounds in coffee. Mini-review. Braz J Plant Physiol 18 (1): 1-15. DOI: 10.1590/S1677-04202006000100003.
Farag MA, Zayed A, Sallam IE, Abdelwareth A, Wessjohann LA. 2022. Metabolomics-based approach for coffee beverage improvement in the context of processing, brewing methods, and quality attributes. Foods 11 (6): 864. DOI: 10.3390/foods11060864.
Franca AS, Mendonça JCF, Oliveira SD. 2005. Composition of green and roasted coffees of different cup qualities. LWT-Food Sci Technol 38 (7): 709-715. DOI: 10.1016/j.lwt.2004.08.014.
Gancarz M, Dobrzański Jr B, Malaga-Toboła U, Tabor S, Combrzyński M, Ćwikła D, Strobel WR, Oniszczuk A, Karami H, Darvishi Y, Żytek A, Rusinek R. 2022. Impact of coffee bean roasting on the content of pyridines determined by analysis of volatile organic compounds. Molecules 2: 1559. DOI: 10.3390/molecules27051559.
Gichimu BM, Gichuru EK, Mamati GE, Nyende AB. 2014. Biochemical composition within *Coffee arabica* cv. *ruiru* 11 and its relationship with cup quality. J Food Res 3 (3): 31-44. DOI: 10.5539/Jfr.V3n3p31.
Gimase JM, Thagana WM, Kirubi DT, Gichuru EK, Kathurima CW. 2014. Beverage quality and biochemical attributes of arabusta coffee (C. *arabica* L. x C. *canephora* Pierre) and their parental genotypes. Afr J Food Sci 8 (9): 456-464. DOI: 10.5897/Ajfs2014.1132.
Happyana N, Pratiwi A, Hakim EH. 2021. Metabolite profiles of the green beans of Indonesian Arabica coffee varieties. Intl J Food Sci 2021: 5782578. DOI: 10.1155/2021/5782578.
Heo J, Adhikari K, Choi KS, Lee J. 2020 Analysis of caffeine, chlorogenic acid, trigonelline, and volatile compounds in cold brew coffee using high-performance liquid chromatography and solid-phase microextraction gas chromatography-mass spectrometry. Foods 9 (12): 1746. DOI: 10.3390/foods9121746.
Joët T, Laffargue A, Descroix F, Doulbeau S, Bertrand B, de Kochko A, Dussert S. 2010. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. Food Chem 118 (3): 693-701. DOI: 10.1016/j.foodchem.2009.05.048.
Jolliffe IT, Cadima J. 2016. Principal component analysis: A review and recent developments. Philos Trans A Math Phys Eng Sci 374: 20150202. DOI: 10.1098/rsta.2015.0202.
Humas Jabar. 2022. 10 Kopi Terbaik Jabar Tampil di Ajang World of Coffee Italia. Kota Bandung: Jabarprov. https://jabarprov.go.id/berita/

10-kopi-terbaik-jabar-tampil-di-ajang-world-of-coffee-italia-6022. [Indonesian]
Knysak D. 2017. Volatile compounds profiles in unroasted *Coffea arabica* and *Coffea canephora* beans from different countries. Food Sci Technol 37: 444-448. DOI: 10.1590/1678-457X.19216.
Koutouleas A, Sarzynski T, Bordeaux M, Bosselmann AS, Campa C, Etienne H, Turreira-García N, Rigal C, Vaast P, Ramalho JC, Marraccini P, Ræbild A. 2022. Shaded-coffee: A nature-based strategy for coffee production under climate change: A review. Front Sustain Food Syst 6: 877476. DOI: 10.3389/fsufs.2022.877476.
Ky C-L, Louarn J, Dussert S, Guyot B, Hamon S, Noirot M. 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. Food Chem 75 (2): 223-230. DOI: 10.1016/S0308-8146(01)00204-7.
Leonel L-E, Philippe V. 2007. Effects of altitude, shade, yield and fertilization on coffee quality (*Coffea arabica* L. var. *caturra*) produced in agroforestry systems of the Northern Central Zones of Nicaragua. In: 2nd International Symposium on Multi-Strata Agroforestry Systems with Perennial Crops. Catie, Turrialba, Costa Rica, 17-21 September 2007. DOI: 10.13140/RG.2.1.4689.1289.
Mariño YA, Pérez M-E, Gallardo F, Trifilio M, Cruz M, Bayman P. 2016. Sun vs. shade affects infestation, total population and sex ratio of the coffee berry borer (*Hypothenemus hampei*) in Puerto Rico. Agric Ecosyst Environ 222: 258-266. DOI: 10.1016/j.agee.2015.12.031.
Martins PMM, Batista NN, da Cruz Pedrozo Miguel MG, Simão JBP, Soares JR, Schwan RF. 2020. Coffee growing altitude influences the microbiota, chemical compounds and the quality of fermented coffees. Food Res Intl 129: 108872. DOI: 10.1016/j.foodres.2019.108872.
Mazzafera P, Silvarolla B. 2010. Caffeine content variation in single green Arabica coffee seeds. Seed Sci Res 20 (3): 163-167. DOI: 10.1017/S0960258510000140.
Mehari B, Redi-Abshiro M, Chandravanshi BS, Combrinck S, McCrindle R, Atlabachew M. 2019. GC-MS profiling of fatty acids in green coffee (*Coffea arabica* L.) beans and chemometric modelling for tracing geographical origins from Ethiopia. J Sci Food Agric 99 (8): 3811-3823. DOI: 10.1002/jsfa.9603.
Mengistu MW, Workie MA, Mohammed AS. 2020. Biochemical compounds of Arabica coffee (*Coffea arabica* L.) varieties grown in northwestern highlands of Ethiopia. Cogent Food Agric 6 (1): 1741319. DOI: 10.1080/23311932.2020.1741319.
Mortzfeld FB, Hashem C, Vranková K, Winkler M, Rudroff F. 2020. Pyrazines: Synthesis and industrial application of these valuable flavor and fragrance compounds. Biotechnol J 15 (11): 2000064. DOI: 10.1002/biot.202000064.
Odeny DA, Chemining'wa GN, Shibairo, Khaturima CW. 2016. Biochemical components of shaded coffee under different management levels. Adv J Food Sci Technol 12 (9): 519-526. DOI: 10.19026/ajfst.12.3063.
Ongo EA, Montevecchi G, Antonelli A, Sberveglieri V, Sevilla F. 2020. Metabolomics fingerprint of Philippine coffee by spme-gc-ms for geographical and varietal classification. Food Res Intl 134: 109227. DOI: 10.1016/J.Foodres.2020.109227.
Oestreich-Janzen S. 2010. Chemistry of Coffee. CAFEA GmbH, Elsevier Ltd., Hamburg. DOI: 10.1016/B978-008045382-8.00708-5.
PubChem. https://pubchem.ncbi.nlm.nih.gov/. [9 January 2024]
Papandreou C, Hernández-Alonso P, Bulló M et al. 2019. Plasma metabolites associated with coffee consumption: A metabolomic approach within the PREDIMED study. Nutrients 11: 1032. DOI: 10.3390/nu11051032.
Pearson K. 1901. On lines and planes of closest fit to systems of points in space. Phil Mag 2: 559-572. DOI: 10.1080/14786440109462720.
Perrone D, Donangelo CM, Farah A. 2008. Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatograph-mass spectrometry. Food Chem 110 (4): 1030-1035. DOI: 10.1016/j.foodchem.2008.03.012.PMid:26047298.
Putri SP, Nusantara FJP, Putri SE. 2017. Aplikasi pendekatan metabolomik untuk ilmu pangan dan mikro-biologi. Bunga Rampai Forum Peneliti Muda Indonesia 2017. [Indonesian]
Procida G, Lagazio C, Cateni F, Zacchigna M. 2020. Characterization of Arabica and Robusta volatile coffees composition by reverse carrier gas headspace gas chromatography–mass spectrometry based on a statistical approach. Food Sci Biotechnol 29 (10): 1319-1330. DOI: 10.1007/s10068-020-00779-7.
Saputri M, Lioe HN, Wijaya CH. 2020. Pemetaan karakteristik kimia biji kopi Arabika Gayo dan Robusta Gayo. Jurnal Teknologi dan Industri Pangan 31 (1): 76-85. DOI: 10.6066/jtip.2020.31.1.76. [Indonesian]
Seninde DR, Chambers E. 2020. Coffee flavor: A review. Beverages 6 (3): 44. DOI: 10.3390/Beverages6030044.
Silvarolla MB, Mazzafera P, Fazuoli LC. 2004. Plant biochemistry: A naturally decaffeinated Arabica coffee. Nature 429 (6994): 826. DOI: 10.1038/429826a.
Silva ACR, Garrett R, Rezende CM, Meckelmann SW. 2022. Lipid characterization of Arabica and Robusta coffee beans by liquid chromatography-ion mobility-mass spectrometry. J Food Compos Anal 111: 104587. DOI: 10.1016/j.jfca.2022.104587.
Sridevi V, Giridhar P. 2014. Changes in caffeine content during fruit development in *Coffea canephora* P. ex. Fr. grown at different elevations. J Biol Earth Sci 4 (2): B168-B175.
Tolessa K, D'heer J, Duchateau L, Boeckx P. 2017. Influence of growing altitude, shade and harvest period on quality and biochemical composition of Ethiopian specialty coffee. J Sci Food Agric 97: 2849-2857. DOI: 10.1002/jsfa.8114.
Tsegay G, Redi-Abshiro M, Chandravanshi BS, Ele E, Mohammed AM, Mamo H. 2020. Effect of altitude of coffee plants on the composition of fatty acids of green coffee beans. BMC Chem 14 (1): 36. DOI: 10.1186/s13065-020-00688-0.
Villarreal PD, Baena CLM, Posada SHE. 2014. Análisis de lípidos y ácidos grasos en café verde de líneas avanzadas de Coffea Arabica cultivadas en Colombia. https://biblioteca.cenicafe.org/handle/10778/520. [Spanish] [8 January 2024]
Zakidou P, Plati F, Matsakidou A, Varka E-M, Blekas G, Paraskevopoulou A. 2021. Single origin coffee aroma: From optimized flavor protocols and coffee customization to instrumental volatile characterization and chemometrics. Molecules 26: 4609. DOI: 10.3390/molecules26154609.