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Chemical fingerprint of robusta coffee beans from various local clones in Tanggamus, Lampung Province, Indonesia

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Abstract. *Yani A, Murhadi, Subeki, Utomo TP. 2025. Chemical fingerprint of robusta coffee beans from various local clones in Tanggamus, Lampung Province, Indonesia. Biodiversitas 26: 2222-2234.* This research was conducted to analyze the chemical composition of dry-processed green coffee beans from several local robusta clones, namely Randu Alas, Kasio, Komari, Kopi Hijau, and Kopi Kuning. The study was held from May to August 2024 in Way Harong Village, Lampung Province, Indonesia, with analysis done in two laboratories. The analysis included defect values, coffee quality, moisture content, yield, and density based on the Indonesian National Standard (SNI). The study was designed using a Completely Randomized Design with five treatments and three replications. Chemical fingerprints were identified using UPLC-MS/MS and MassLynx software. The results showed defect values of 14.15-29.15 (grades 2-3), yields of 19.30-21.16% (highest in Kopi Hijau and Komari), moisture content of 7.79-11.34% (lowest in Kopi Kuning), and density of 601.66-605.66 g/cm³. The chemical compositions were consistent up to Rt 7.38 minutes but varied beyond this point, with 18 compounds in Randu Alas, 20 in Kasio, 19 in Komari, 15 in Kopi Hijau, and 18 in Kopi Kuning. Overall, 10 major compounds were identified, including caffeine, chlorogenic acid, 4-aminobenzoic acid, trans-zeatin, umbelliferone, hymecromone, cynarine, (3aR,4R,5R,7aR)-4-(4-Hydroxy-3-methoxyphenyl)-1-oxo-7-[(2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-1,3,3a,4,5,7a-hexahydro-2-benzofuran-5-carboxylic acid, benzyl 2-methyl-4-(4-nitrophenyl)-6-oxo-1,4,5,6-tetrahydro-3-pyridine carboxylate and Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate.

Keywords: Chemical fingerprint, data interpretation, physical analysis, robusta coffee clones

Abbreviations: UPLC-MS/MS: Ultra Performance Liquid Chromatography Mass Spectrophotometer/MS

INTRODUCTION

Coffee is one of the agricultural commodities that play a significant role in the economics of many countries (Bicho et al. 2013; Kim et al. 2022; Khemira et al. 2023). Among many coffee varieties, robusta coffee (*Coffea canephora* Pierre ex A.Froehner) is a notable one, known for its high productivity, disease resistance, and ability to thrive in low-altitude areas. As a result, robusta coffee is often cultivated in tropical and subtropical countries such as Brazil, Vietnam, Colombia, and Indonesia (Uganda Coffee Development Authority 2019; Freitas et al. 2024). In Indonesia, the Tanggamus District in Lampung Province has a significant potential as a robusta coffee-producing region, with various local clones or varieties of robusta coffee that have developed over the years. Each clone has a unique characteristic that influences the quality and flavor of the coffee produced.

To gain a deeper understanding of Tanggamus robusta coffee, studying the chemical composition patterns of green beans from various local robusta clones in Tanggamus is essential. Robusta coffee has a more bitter taste, slightly acidic notes, and contains a higher caffeine level compared to Arabica (Duque and Blair 2022). Coffee beans contain a variety of chemical compounds that affect their characteristics, including carbohydrates, proteins, fats, minerals, caffeine, trigonelline, aliphatic acids (carboxylic acids), chlorogenic acids, glycosides, and volatile components (Zanin et al. 2016; Herawati et al. 2019). For instance, the bitter taste in coffee is due to the presence of alkaloids and phenolic compounds in the beverage (Herawati et al. 2019). Furthermore, there are several factors that influence the chemical components and quality of coffee, such as plant varieties, growing conditions, processing methods, and coffee bean roasting techniques (Mills et al. 2013; Toledo et al. 2016; Núñez et al. 2020; Núñez et al. 2021a). Therefore, proper research is important to determine the overall quality and flavor of coffee.

As a modern omics approach, fingerprinting-a key aspect of metabolomics-systematically explores complex biological phenomena through untargeted analysis. In coffee research, it maps the chemical composition of coffee beans and links it to physical properties like defect rate, density, and moisture content. They are associated with metabolic compounds, such as caffeine and chlorogenic acid, that influence coffee's quality and flavor (Peterson et al. 2008; Stilo et al. 2021).

Fingerprinting provides both quantitative and qualitative profiles of the chemical compounds in coffee, making it essential for identifying coffee varieties and determining

their quality. Moreover, chemical composition fingerprint analysis can help coffee producers ensure the quality, authenticity, and consistency of their products, as well as make recommendations to maximize the potential flavor and aroma of the coffee. Additionally, fingerprint analysis can assist in developing new coffee varieties with distinct flavor profiles (Núñez et al. 2020).

Coffee chemical composition fingerprinting can be conducted using chromatography and spectroscopy techniques. These techniques can separate, identify, and measure the contents of compounds in coffee (Núñez et al. 2021b). Recently, various analytical methodologies have been developed to determine the characterization and authenticity of coffee (Toci et al. 2016). Some examples are the chemometric analysis of 1H-NMR for *C. arabica* cultivated under different conditions (Hatumura et al. 2018) and the use of an "electric nose" to detect volatile compounds (Marek et al. 2020). Meanwhile, fingerprinting using HPLC-FLD produces a chemical composition to distinguish the original country of the coffee beans, either arabica or robusta variety, and the level of coffee roasting (Núñez et al. 2021b).

The coffee bean samples used in this study were authenticated based on morphological characteristics by local experts, as well as by consulting reference books on coffee species and varieties. This study aimed to identify the specific chemical composition fingerprint of each local robusta coffee clone from Tanggamus. By analyzing the chemical profile of unroasted (green) coffee beans, this research intended to provide a deeper understanding of the unique characteristics of each clone.

MATERIALS AND METHODS

Study area and time of research

The research was conducted in Way Harong Village, Air Naningan Sub-district, Tanggamus District, Lampung, Indonesia (Figure 1), at the Research Center for Agroindustry Laboratory of National Research and Innovation Agency (BRIN) and the Forensic Laboratory Center of Indonesian Criminal Investigation Agency from May to August 2024.

Materials and equipment

Materials used for this research were robusta coffee beans from various local clones (Randu Alas, Kasio, Komari, Kopi Hijau, and Kopi Kuning) (Figure 2) obtained from Way Harong Village, Air Naningan Sub-district, Tanggamus District, Lampung, Indonesia, technical ethanol 96%, methanol, formic acid, acetonitrile, and 0.05% water injection. The research equipment included harvesting tools such as harvesting baskets and sacks, buckets, drying racks, huller, scales, grinder, and the UPLC-MS/MS equipment (Table 1).

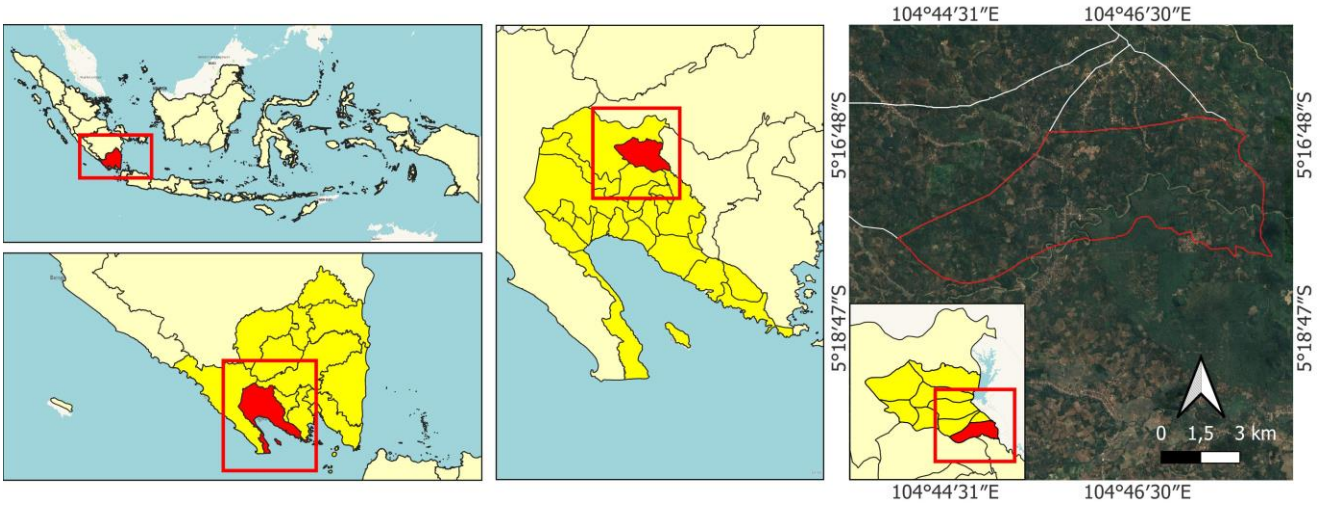


Figure 1. Map of research locations in Way Harong Village, Air Naningan Sub-district, Tanggamus, Lampung, Indonesia

Figure 2. Local clones of robusta coffee in Tanggamus District, Lampung, Indonesia. A. Randu Alas; B. Kasio; C. Komari; D. Kopi Hijau; E. Kopi Kuning

Data analysis

The study was designed using a Completely Randomized Design (CRD) with five treatments and three replications. The treatments were (G1) Randu Alas, (G2) Kasio, (G3) Komari, (G4) Kopi Hijau, and (G5) Kopi Kuning, all processed separately using a natural/dry method. All data were analyzed using Analysis of Variance (ANOVA); if the treatment significantly affected the observed variables, further analysis would be performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. The chemical fingerprints of the local robusta coffee clones from Lampung were then analyzed descriptively and presented in tables.

Post-harvest processing

Dry post-harvest process of robusta coffee

The coffee cherries were harvested selectively by picking the red ones (without stripping). They were then sorted, dried in parchment form on drying racks by the sunlight for 14-15 days, and hulled to obtain green beans. The post-harvest processing of the coffee was carried out according to the general standard operating procedures (SOP). The flowchart for post-harvest processing of robusta coffee using the dry method can be seen in Figure 3.

Observation procedure

Defect values and quality

The physical characteristics of the green coffee beans were assessed based on the defect values found in them, referring to the Indonesian National Standard SNI 01-2907-2008. A 300 g sample of coffee beans was weighed, sorted, and cleaned of defective beans and contaminants. The sample was then placed in a cup. Next, the defect values were calculated according to the defect scoring form, and the quality was classified based on the defect scoring system. The total defect values and quality classifications of robusta green coffee beans based on SNI 01-2907-2008 are quality 1: max 11; quality 2: 12-25; quality 3: 26-44; quality 4a: 45-60; quality 4b: 61-80: quality 5: 81-150; and quality 6: 151-225 (Badan Standardisasi Nasional 2019).

Yield (SNI 01-2907-2008)

To calculate the yield, 10 kg of freshly picked red coffee cherries were prepared. Then, using an analytical balance, the weight of the processed green coffee beans derived from the 10 kg of red cherries was measured. The yield was calculated as follows:

Yield = (Weight of green coffee beans × 100%) / Weight of fresh coffee cherries

Moisture Content (SNI 01-2907-2008)

Five g of robusta green coffee beans sample was weighed and placed into a dish. The dish was then placed in an oven pre-heated to 105°C to dry for 16 hours, with its cover left open and placed nearby. It was then placed in a desiccator and cooled to room temperature. Finally, it was weighed, and the water content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{(m_1 - m_2)}{(m_1 - m_o)} \times 100\%$$

Where:

m_0: Weight of the dish and cover (g)

m_1: Weight of the dish, cover, and coffee sample before drying (g)

m_2: Weight of the dish, cover, and coffee sample after drying (g)

Density (Bizimungu et al. 2022)

First, a 100 mL measuring cylinder was prepared, and 10 mg of sorted green coffee beans was weighed and added into it. Then, 50 mL of water was added to the green coffee beans. The change in the water height was then noted, and the density was calculated as follows:

Density (ρ) (g/cm^3) = Mass (m) (g) / Volume (V) (cm^3)

Table 1. UPLC-MS/MS instrument specifications

Instrument	Specification	Detail
LC system	ACQUISITION UPLC H-Class system (Waters, USA)	UPLC (Ultra Performance Liquid Chromatography)
LC column	ACQUISITION UPLC BEH C18 (2.1 × 100 mm, 1.7 µm: Waters, USA)	UPLC Column BEH (Ethylene Bridge Hybrid)
Mass spectrometer	Xevo G2-S Q-ToF (Waters, USA)	Quadro pole time-of-flight mass spectrometry

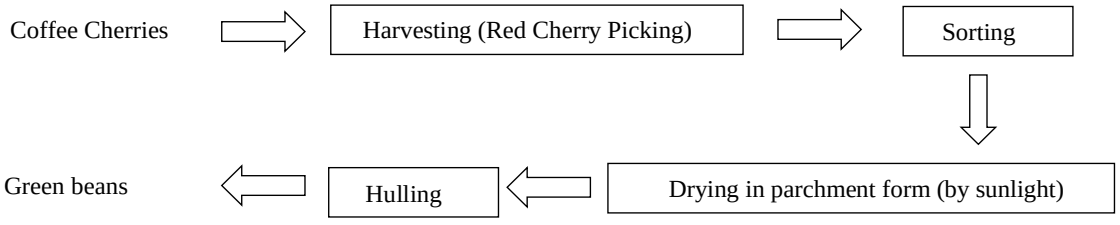


Figure 3. Flowchart of post-harvest processing of robusta coffee in dry method

Extraction and chemical composition analysis using the Ultra Performance Liquid Chromatography Mass Spectrophotometer/ MS (UPLC- MS/MS)

Coffee beans were ground to fine powders in a laboratory mill. About 1.0 g of the powdered sample was extracted with 10 mL of methanol:water (80:20, v/v). The extraction was conducted with ultrasound for 30 min at 40°C after vibrating for 2 min. After extraction, the samples were centrifuged for 10 minutes at 4,000 rpm. The supernatant was collected and filtered using 0.22 µm PTFE syringe filters and then placed in amber vials for UPLC-MS/MS analysis.

The chemical profiling was performed using an Ultra Performance Liquid Chromatography system with a Tandem Mass Spectrometry (UPLC-MS/MS) device (Waters Xevo TQD, Milford, USA). The mobile phases for the chromatographic separation were (A) water and 0.1% formic acid and (B) acetonitrile and 0.1% formic acid. The separation was performed at 40°C using an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 µm) column (Table 1). The gradient elution program was solvent A (95-0% A, 0-15 min), solvent B (5-100% B, 0-15 min), and A (95% A, 15-23 min) (Table 2).

The flow run rate was 0.3 mL/min, and 5 µL was injected in each analysis; the total run time was 23 min. Detection by mass spectrometry was performed on positive electrospray ionization (ESI+) mode under multiple response monitoring (MRM). Capillary voltage was set at 3.0 kV, source temperature at 150°C, and desolvation temperature at 450°C. Nitrogen was employed as desolvation gas and cone gas at flow rates of 800 L/h and 50 L/h, respectively. Chromatograms containing polar compounds appeared first, followed by compounds with lower polarity. The scanned results were then read by the Q-Tof-MS detector, which generated chromatogram peaks.

The MassLynx 4.1 software (Waters, Milford, USA) was used for data acquisition and processing. Then, the predicted compounds could be interpreted with the help of ChemSpider and PubChem websites. The extraction procedure and analytical method were adapted and modified based on previous studies by Chawla and Ranjan (2016), Mehari et al. (2016), Chen et al. (2016), and Alhaidrai et al. (2023).

RESULTS AND DISCUSSION

Defect values and quality

The analysis results of the defect values and quality of green coffee beans of some local robusta coffee clones from Tanggamus processed using the dry method are shown in Table 3. The Kopi Hijau clone had the lowest defect value (14.15) and is classified as Quality 2, indicating better quality than others. For the coffee industry, this means more consistent raw materials and better product standards. For consumers, it offers cleaner taste, better aroma, and a more enjoyable coffee experience. The Indonesian National Standard (SNI 01-2907-2008) states that coffee beans to be categorized as Quality 2 (Grade 2) must have a moisture content of ≤12.5%, a maximum of 11 to 25 complete defects per 300 g sample, and be free of contaminants like mold, foreign matter, or insect damage. These requirements indicate a high-quality product, but not the highest grade.

This lower defect value may be attributed to better post-harvest handling practices or inherent genetic traits of the Kopi Hijau clone that reduce its susceptibility to physical damage or pest infestation. Furthermore, clones with fewer defects are often associated with improved chemical stability and preservation of desirable flavor compounds, reinforcing the importance of selecting high-quality clones for specialty coffee production.

Physical characteristics

The physical characteristics, namely yield, moisture content, and density, of green coffee beans of some local Robusta coffee clones from Tanggamus processed using the dry method are summarized in Table 4.

Table 2. Solvent ratios used based on the gradient elution

Time (minute)	Mixture A (%)	Mixture B (%)
0.00	95.0	5.0
2.00	75.0	25.0
3.00	75.0	25.0
14.00	0.0	100.0
15.00	0.0	100.0
19.00	95.0	5.0
23.00	95.0	5.0

Table 3. Defect values and quality of green coffee beans of five Tanggamus local Robusta coffee clones processed using the dry method

Clone	Total defect value	Quality
Randu Alas	18.50	2
Kasio	23.65	2
Komari	29.15	3
Kopi Hijau	14.15	2
Kopi Kuning	16.50	2

Table 4. The physical properties of green coffee beans of five Tanggamus local robusta coffee clones processed using the dry method

Coffee clone	Yield (%)	Moisture content (%)	Density (g/cm^3)
Randu Alas	20.12 ± 0.427 ^{b}	7.79 ± 0.005 ^{c}	600.66 ± 1.155 ^{a}
Kasio	19.75 ± 0.387 ^{ab}	11.34 ± 0.056 ^{d}	601.66 ± 1.528 ^{a}
Komari	21.05 ± 0.068 ^{c}	9.16 ± 0.046 ^{c}	602.00 ± 2.646 ^{a}
Kopi Hijau	21.16 ± 0.399 ^{c}	10.07 ± 0.036 ^{b}	602.66 ± 1.155 ^{a}
Kopi Kuning	19.30 ± 0.170 ^{a}	9.31 ± 0.036 ^{a}	605.66 ± 3.786 ^{a}

Note: a-d: Different superscript letters within the same column indicate significant differences between the mean values of the coffee clones ($p<0.05$) while same letters indicate no significant difference

Results showed that the yields from all clones in this study ranged from 19.30% to 21.16%. The highest yields were recorded for the Kopi Hijau and Komari clones, while the lowest yield was recorded for the Kopi Kuning clone. The results of Duncan's multiple range test analysis showed a significant difference in yield among the analyzed robusta coffee clones, indicating that the clones did not produce similar yields.

The moisture content ranged from 7.79% to 11.34%, with the highest percentage observed in the Kasio clone and the lowest in the Kopi Kuning clone. The results of Duncan's multiple range test analysis also showed significant differences in moisture content among the analyzed clones.

Meanwhile, the densities of the observed green beans ranged from 601.66 to 605.66 g/cm³, with no significant differences based on the Duncan's multiple range test analysis, unlike the yield and moisture content. Coffee beans typically have a bulk density of approximately 600-750 kg/m³ (Dutra et al. 2001; Clark and Landolt 2017; Niwagaba and Kipkoechsitienei 2019; Yusibani et al. 2023). These variations indicate genetic differences and physiological responses among the clones, affecting yield efficiency and drying behavior. Meanwhile, the relatively uniform bean density suggests consistent bean structure, which supports even roasting and maintains quality.

Chemical composition analysis using UPLC-MS/MS

The chromatograms of each local robusta coffee clone from Tanggamus processed using the dry method are presented in Figure 4. The highest peak corresponds to caffeine, with a retention time (Rt) range of 4.14-4.18 minutes.

The chromatogram patterns of all five clones showed similarities in the initial retention time up to 7.38 minutes. Within this interval, the similar patterns indicated that the detected compounds-such as caffeine, chlorogenic acid, and other volatile compounds-were common or stable across all clones. Caffeine, which is stable and commonly found in all coffee types, is likely to appear at the first peak. Chlorogenic acid, frequently found in green coffee beans, exhibits similar peaks in all clones as well. Other volatile compounds, such as hormones or simpler molecules, are also detected in the early retention times.

The similarity in chromatogram patterns reflects the stability of primary chemical compounds among the coffee clones, which is likely influenced by genetic similarities. Moreover, the minimal processing in the dry method does not significantly alter the structure of these compounds, resulting in consistent chromatogram patterns across the clones.

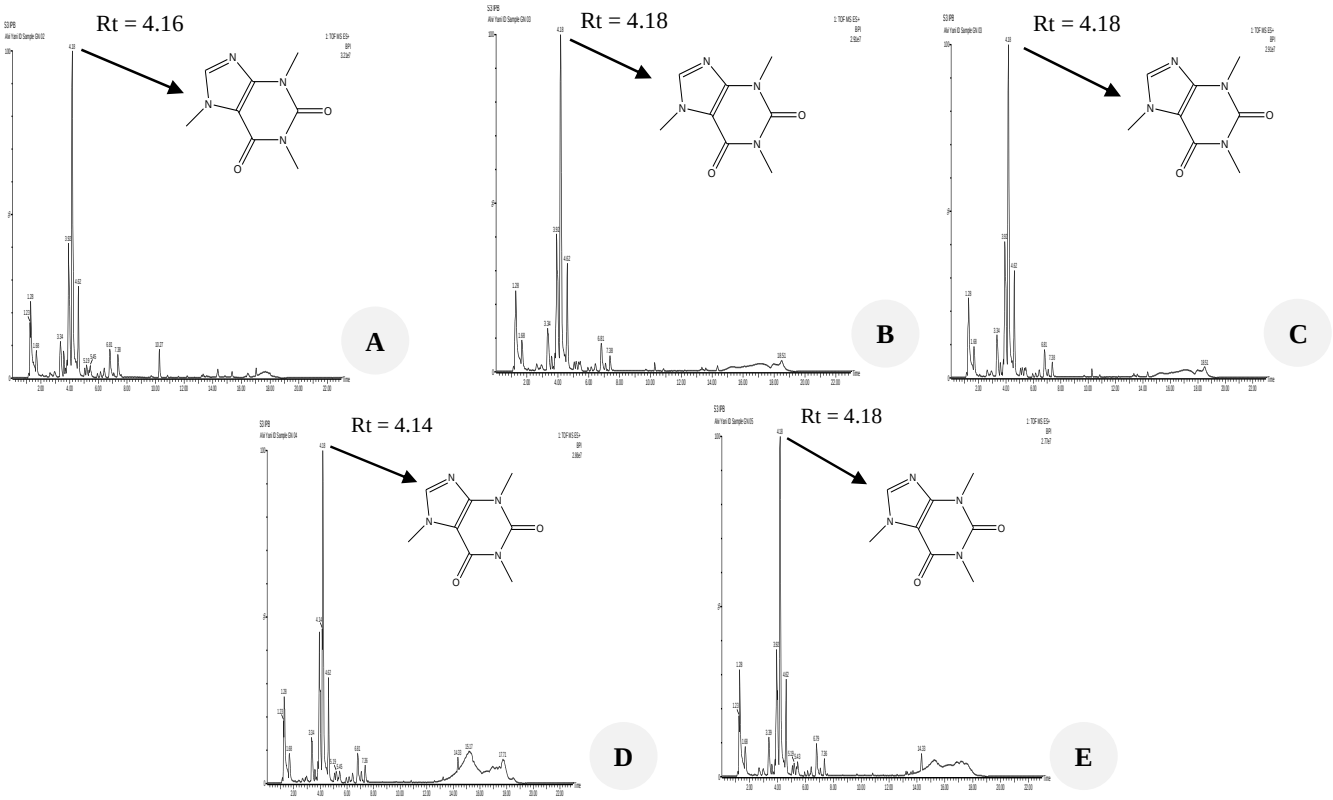


Figure 4. UPLC-MS/MS chromatograms of robusta coffee beans from five local clones in Tanggamus, Lampung. A. Randu Alas; B. Kasio; C. Komari; D. Kopi Hijau; E. Kopi Kuning-processed using the semi-washed method, showing the presence of key marker compounds including caffeine (Rt = 4.14 - 4.18 min), chlorogenic acid (Rt = 3.39-3.92 min), and other chemical compounds after Rt 17.0 min, with distinct retention times and fragmentation patterns that reflect the chemical diversity and potential biomarkers for clone differentiation and quality assessment

In this study, after the retention time (Rt) of 7.38 minutes, variations in chemical compounds began to appear among the tested robusta coffee clones, although the differences were not highly significant. This indicated differences in the chemical composition between the clones, but the differences were likely insufficient to cause drastic changes in the coffee's flavor or aroma. This observation is consistent with the chromatogram data shown in Figure 4, where divergence in peak intensity and retention time becomes more noticeable in the later stages, suggesting the presence of unique secondary metabolites specific to certain clones.

The chemical variations observed after Rt 7.38 minutes may be attributed to several factors, such as: (i) clone genetics, in which each clone has distinct secondary metabolic mechanisms, resulting in variations in compounds like phenolic acids, flavonoids, or volatile compounds; (ii) microenvironment during processing, in which differences in natural microbes or environmental conditions during the drying process can influence the formation of unique compounds in each clone; and (iii) degradation of primary compounds, in which some clones may be more prone to the degradation of compounds like chlorogenic acid, leading to the formation of derivatives or new compounds (Yuan et al. 2012).

The chromatograms presented in Figure 4 showed that the Kopi Hijau clone exhibited a unique pattern between Rt 12 and 14 minutes. This pattern suggested the presence of distinct chemical components or higher concentrations of specific compounds in this clone compared to others. Such a unique pattern can serve as a characteristic marker of this clone. The differences in chromatogram patterns among these robusta coffee clones highlight that, despite belonging to the same species, there are significant variations in their chemical compositions. These variations may influence the flavor, aroma, and other attributes of the coffee produced. Understanding these differences is crucial for selecting clones that meet consumer preferences or market demands.

The generated chromatograms were further analyzed using the MassLynx 4.1 software to identify and predict the molecular formulas of each compound. Each chromatogram peak represented a single compound. Molecular formula prediction was performed based on the measured mass values in the spectrum, subtracting the mass of one hydrogen atom (1.0078), as hydrogen atoms were added during separation by ESI (+) ionization. The results of the chemical interpretation using the UPLC-MS/MS instrument of each local robusta coffee clone used in this study are presented in Tables 5 to 9.

A total of 18 chemical compounds were identified in the Randu Alas clone, 20 compounds in the Kasio clone, 19 compounds in the Komari clone, 15 compounds in the Kopi Hijau clone, and 18 compounds in the Kopi Kuning clone. Based on the chromatogram results (Figure 4) and correlating them with Tables 5 to 9, a synergy was observed, indicating both similarities and differences in the chemical compounds found in each clone. These findings demonstrated the chemical composition diversity present in dry-processed local robusta coffee clones.

Table 5. The chemical compounds of the extracted Randu Alas green coffee bean clone processed using the dry method as identified by UPLC-MS/MS

Rt (min)	m/z	Formula	Compound names
1.23	381.0797	$C_9H_{20}N_2O_{12}S$	Unknown
1.28	138.0515	$C_7H_7NO_2$	4-Aminobenzoic acid
1.72	220.1205	$C_{10}H_{13}N_5O$	trans-Zeatin
3.39	355.1034	$C_{16}H_{18}O_9$	Chlorogenic acid
3.92	163.0381	$C_9H_6O_3$	Umbelliferone
4.16	195.088	$C_8H_{10}N_4O_2$	Caffeine
4.62	177.0539	$C_{10}H_8O_3$	Hymecromone
5.21	499.1262	$C_{25}H_{22}O_{11}$	(3aR,4R,5R,7aR)-4-(4-Hydroxy-3-methoxyphenyl)-1-oxo-7-[2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-1,3, 3a,5,7 a-hexahydro-2-benzofuran-5-carboxylic acid
6.81	367.1296	$C_{16}H_{14}N_8O_3$	1-Methyl- N- [7-(3-methyl-1,2,4-oxadiazol-5-yl) [1,2,4] triazolo[4,3-a] pyridin-3- yl] methyl -6-oxo-1,6-dihydro-3-pyridazinecarboxamide
7.12	516.249	$C_{26}H_{29}N_3O_5$	Varioxepine A
7.38	351.1335	$C_{20}H_{18}N_2O_4$	Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-cearboxylate
10.29	274.2758	$C_{12}H_{31}N_7$	2-[(4S)-4-Amino-5-({3-[(3-aminopropyl)amino]propyl}amino)pentyl]guanidine
12.97	281.2971	$C_{18}H_{36}N_2$	N, N'-Dicyclohexyl-1,6-hexanediamine
13.28	307.3111	$C_{20}H_{38}N_2$	2-Heptadecylimidazole
14.37	485.3751	$C_{30}H_{48}N_2O_3$	4-Acetyl-5-[4-(dimethyl amino) phenyl]-1-hexadecyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one
15.36	513.4027	$C_{32}H_{52}N_2O_3$	(3β)-N-(2-Aminoethyl)-3-hydroxy-11-oxoolean-12-en-30-amide
17.03	471.3948	$C_{26}H_{46}N_8$	N~2~, N~2~-Dibutyl-N~4~-[4-(diethylamino) phenyl]-N~6~-[3-(dimethyl amino) propyl]-1,3,5-triazine-2,4,6-triamine
18.00	499.4273	$C_{28}H_{50}N_8$	N~2~-Butyl-N~4~-[4-(diethylamino) butyl]-N~6~-[4-(diethylamino)-2-methylphenyl]-N~2~-ethyl-1,3,5-triazine-2,4,6-triamine

Table 6. The chemical compounds of the extracted Kasio green coffee bean clone processed using the dry method as identified by UPLC-MS/MS

Rt (min)	m/z	Formula	Compound names
1.28	138.0525	$C_7H_7NO_2$	4-Aminobenzoic acid
1.68	220.1207	$C_{10}H_{13}N_5O$	trans-Zeatin
2.95	120.0756	$C_3H_9N_3O_2$	Guanidine acetate
3.59	520.1943	$C_{20}H_{25}N_9O_8$	4-(2Z)-2-[(2,4-Dinitrophenyl) hydrazono]-3-[4-(4-morpholinyl)-5-nitro-1H-imidazol-1-yl] propyl} morpholine
3.92	355.1051	$C_{16}H_{18}O_9$	Chlorogenic acid
3.92	163.0385	$C_9H_6O_3$	Umbelliferone
4.18	195.0878	$C_8H_{10}N_4O_2$	Caffeine
4.62	369.1191	$C_{17}H_{20}O_9$	Methyl chlorogenate
4.62	177.0544	$C_{10}H_8O_3$	Hymecromone
5.19	517.1321	$C_{25}H_{24}O_{12}$	Cynarine
5.19	499.1216	$C_{25}H_{22}O_{11}$	4,4-Bis(6,8-dihydroxy-3-methyl-1-oxo-1H-isochromen-7-yl)-2-methoxybutanoic acid
5.94	531.1497	$C_{26}H_{26}O_{12}$	Methyl (1R,3R,4S,5R)-3,4-bis[2E)-3-(3,4-dihydroxyphenyl)-2-propenoyl] oxy-1,5-dihydroxycyclohexanecarboxylate
6.15	513.1368	$C_{26}H_{24}O_{11}$	9-(1,3-Benzodioxol-5-yl)-4-[(2S,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl) tetrahydro-2-furanyl] oxy-6,7-dimethoxynaphtho[2,3-c] furan-1(3H)-one
6.42	531.1495	$C_{22}H_{22}N_6O_{10}$	3-(2-Nitrobenzyl)-7-(2,3,5-tri-O-acetyl-D-ribofuranosyl)-3,7-dihydro-4H-imidazo[4,5-d][1,2,3]triazin-4-one
6.81	367.1281	$C_{20}H_{18}N_2O_5$	Benzyl 4-(1,3-benzodioxol-5-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate
7.38	351.1348	$C_{20}H_{18}N_2O_4$	Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate
10.27	274.2747	$C_{16}H_{35}NO_2$	Lauryldiethanolamine
14.35	485	$C_{30}H_{48}N_2O_3$	4-Acetyl-5-[4-(dimethylamino) phenyl]-1-hexadecyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one
15.34	513.4041	$C_{32}H_{52}N_2O_3$	(3β)-N-(2-Aminoethyl)-3-hydroxy-11-oxoolean-12-en-30-amide
17.03	471.3947	$C_{30}H_{50}N_2O_2$	Petrosin-A

Table 7. The chemical compounds of the extracted Komari green coffee bean clone processed using the dry method as identified by UPLC-MS/MS

Rt (min)	m/z	Formula	Compound names
1.28	138.0517	$C_7H_7NO_2$	4-Aminobenzoic acid
1.68	220.1204	$C_{10}H_{13}N_5O$	trans-Zeatin
2.64	383.1252	$C_{25}H_{18}O_4$	4,4′-(9H-Fluorene-9,9-diyl)di(1,2-benzenediol)
2.95	120.0759	$C_3H_9N_3O_2$	Guanidine acetate
3.59	520.1909	$C_{20}H_{25}N_9O_8$	4-(2Z)-2-[2,4-Dinitrophenyl) hydrazono]-3-[4-(4-morpholinyl)-5-nitro-1H-imidazol-1-yl] propyl} morpholine
3.83	188.0696	$C_{11}H_9NO_2$	3-Amino-2-naphthoic acid
3.92	355.1022	$C_{16}H_{18}O_9$	Chlorogenic acid
3.92	163.0367	$C_9H_6O_3$	Umbelliferone
4.18	195.0884	$C_8H_{10}N_4O_2$	Caffeine
4.62	369.1165	$C_{17}H_{20}O_9$	Methyl chlorogenate
4.62	177.0537	$C_{10}H_8O_3$	Hymecromone
5.06	517.1354	$C_{25}H_{24}O_{12}$	Cynarine
5.19	499.1245	$C_{25}H_{22}O_{11}$	3aR,4R,5R,7aR)-4-(4-Hydroxy-3-methoxyphenyl)-1-oxo-7-[(2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-1,3a,4,5,7a-hexahydro-2-benzofuran-5-carboxylic acid
6.44	531.1492	$C_{26}H_{26}O_{12}$	Methyl (1R,3R,4S,5R)-3,4-bis[2E)-3-(3,4-dihydroxyphenyl)-2-propenoyl] oxy-1,5-dihydroxycyclo-hexanecarboxylate
6.81	367.1300	$C_{20}H_{18}N_2O_5$	Benzyl 2-methyl-4-(4-nitrophenyl)-6-oxo-1,4,5,6-tetrahydro-3-pyridinecarboxylate
7.38	351.1342	$C_{20}H_{18}N_2O_4$	Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate
10.27	274.2755	$C_{11}H_9NO_3C_{12}$	Unknown
14.33	485.3746	$C_{26}H_{44}N_8O$	N-[2-(Diethylamino) ethyl]-N-[4-(diethylamino)-2-methylphenyl]-6-(2,6-dimethyl-4-morpholinyl)-1,3,5-triazine-2,4-diamine
18.51	758.2206	$C_{50}H_{32}N_3O_3Cl$	Unknown

This chemical variability is likely influenced by genetic factors, growing conditions, as well as differences in agricultural practices and processing methods, consistent with the findings of Kiwuka et al. (2021). The genetic variation among robusta coffee clones, influenced by geographical distribution and diverse climatic conditions, also contributes to the diversity of chemical compounds in the coffee beans.

Based on Tables 5 to 9, the patterns of chemical compounds (fingerprints) found the same chemical compounds in all five clones up to the retention time of 7.38 minutes. Such consistency in chemical composition agrees with earlier studies on the principal chemical profile of robusta coffee (Huang et al. 2023; Nguyen et al. 2024). The dominant chemical compounds that are commonly found within this Rt range are shown in Table 10.

After 7.38 minutes, significant changes were observed in the chemical compound profiles identified (Tables 5-9). Some clones exhibited the presence of compounds with larger and more complex structures, such as Vicenistatin, Petrosin-A, as well as several unidentified compounds (Unknown).

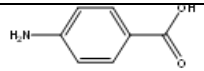
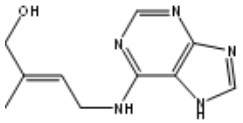
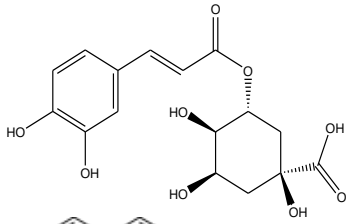
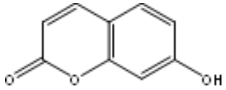
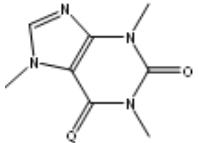
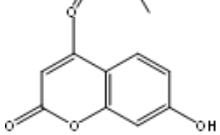
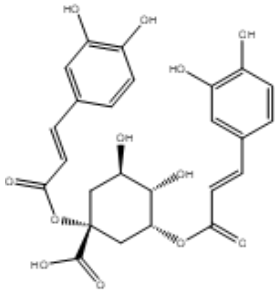
Table 8. The chemical compounds of the extracted Kopi Hijau green coffee bean clone processed using the dry method as identified by UPLC-MS/MS

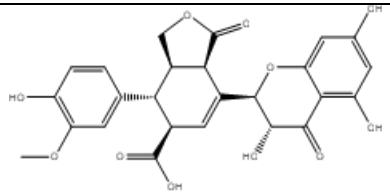
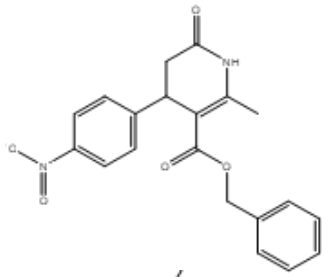
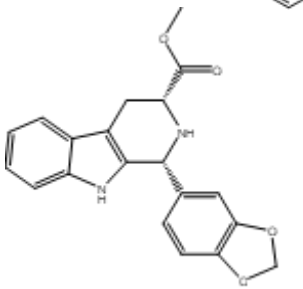
Rt (min)	m/z	Formula	Compound names
1.23	381.0811	$C_9H_{16}N_8O_5S_2$	Unknown
1.28	138.0517	$C_7H_7NO_2$	4-Aminobenzoic acid
1.68	220.1202	$C_{10}H_{13}N_5O$	trans-Zeatin
3.92	355.1030	$C_{16}H_{18}O_9$	Chlorogenic acid
3.92	163.0368	$C_9H_6O_3$	Umbelliferone
4.14	195.0880	$C_8H_{10}N_4O_2$	Caffeine
4.62	177.0564	$C_{10}H_8O_3$	Hymecromone
5.19	499.1237	$C_{25}H_{22}O_{11}$	3aR,4R,5R,7aR)-4-(4-Hydroxy-3-methoxyphenyl)-1-oxo-7-[(2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-1,3a,4,5,7a-hexahydro-2-benzofuran-5-carboxylic acid
5.45	517.1330	$C_{25}H_{24}O_{12}$	Cynarine
6.81	367.1323	$C_{20}H_{18}N_2O_5$	Benzyl 2-methyl-4-(4-nitrophenyl)-6-oxo-1,4,5,6-tetrahydro-3-pyridinecarboxylate
7.36	351.1341	$C_{20}H_{18}N_2O_4$	Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate
14.33	485.3755	$C_{30}H_{48}N_2O_3$	4-Acetyl-5-[4-(dimethylamino) phenyl]-1-hexadecyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one
15.17	758.2225	$C_{55}H_{32}N_OCl$	Unknown
16.95	906.2571	$C_{59}H_{40}N_3O_3SCl$	Unknown
17.71	758.2191	$C_{46}H_{36}N_5S_2Cl$	Unknown

Table 9. The chemical compounds of the extracted Kopi Kuning green coffee bean clone processed using the dry method as identified by UPLC-MS/MS

Rt (min)	m/z	Formula	Compound names
1.23	381.0812	$C_9H_{16}N_8O_5S_2$	Unknown
1.28	138.0525	$C_7H_7NO_2$	4-Aminobenzoic acid
1.68	220.1212	$C_{10}H_{13}N_5O$	trans-Zeatin
2.69	383.1241	$C_{20}H_{18}N_2O_6$	Z-Phg-OSu
2.97	120.0758	$C_3H_9N_3O_2$	Guanidine acetate
3.39	355.1025	$C_{16}H_{18}O_9$	Chlorogenic acid
3.59	520.1953	$C_{24}H_{29}N_3O_{10}$	4-(D-Galactopyranosyloxy)-3-nitrobenzyl 4-phenyl-1-piperazinecarboxylate
3.92	163.0375	$C_9H_6O_3$	Umbelliferone
4.18	195.0873	$C_8H_{10}N_4O_2$	Caffeine
4.62	177.0537	$C_{10}H_8O_3$	Hymecromone
5.19	499.1245	$C_{25}H_{22}O_{11}$	3aR,4R,5R,7aR)-4-(4-Hydroxy-3-methoxyphenyl)-1-oxo-7-[(2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-1, 3, 3a,4,5 ,7a-hexahydro-2-benzofuran-5-carboxylic acid
5.43	517.1354	$C_{25}H_{24}O_{12}$	Cynarine
6.42	531.1504	$C_{26}H_{26}O_{12}$	Methyl (1R,3R,4S,5R)-3,4-bis[2E)-3-(3,4-dihydroxyphenyl)-2-propenoyl] oxy-1,5-dihydroxycyclohexanecarboxylate
6.79	367.1285	$C_{20}H_{18}N_2O_5$	Benzyl 2-methyl-4-(4-nitrophenyl)-6-oxo-1,4,5,6-tetrahydro-3-pyridinecarboxylate
7.36	351.135	$C_{20}H_{18}N_2O_4$	Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate
13.21	501.371	$C_{30}H_{48}N_2O_4$	Vicenistatin
14.33	485.3722	$C_{26}H_{44}N_8O$	N-(4-Butoxyphenyl)-N-[4-(diethylamino) butyl]-6-(4-methyl-1-piperazinyl)-1,3,5-triazine-2,4-diamine
15.27	832.2418	$C_{61}H_{34}NOCl$	Unknown

Table 10. The dominant chemical compounds and their structures as identified in the green bean extracts of five local robusta coffee clones from Tanggamus processed using the dry method

Compound names	Rt (min)	Chemical structure	Description	Polarity
4-Aminobenzoic acid	1.28		It serves as a precursor in the biosynthesis of several biological compounds, such as antibiotics, and plays a role in metabolic processes in plants.	Polar, due to the presence of the amino (-NH_2) and carboxyl (-COOH) groups, which can interact with polar solvents.
trans-Zeatin	1.68-1.72		An active form of cytokinin, involved in cell division and tissue elongation in plants, and helps in plant growth and aging processes.	Non-polar, due to its aromatic nature and long hydrocarbon chain.
Chlorogenic acid	3.39-3.92		A phenolic compound that contributes to the acidic taste of coffee, acts as an antioxidant, and contributes to coffee's flavor stability and health properties (Huang et al. 2023; Nguyen et al. 2024).	Polar, as it contains hydroxyl (-OH) groups that can interact with polar solvents.
Umbelliferone	3.92		A phenolic compound with antioxidant and antibacterial activities.	Polar, due to the presence of hydroxyl (-OH) groups that increase its polarity
Caffeine	4.14-4.18		An alkaloid that stimulates the central nervous system, increasing alertness and contributing to the bitter taste of coffee.	Non-polar. Despite the presence of an amine group (-NH_2), the purine ring structure is hydrophobic, making caffeine predominantly non-polar.
Hymecromone	4.62		An aromatic compound with anti-inflammatory, antimicrobial, and antioxidant properties.	Non-polar. Despite the carbonyl group, the aromatic structure makes it more non-polar.
Cynarine	5.06-5.45 (except for the Randu Alas clone)		An organic compound belonging to the class of phenolic esters. Structurally, cynarine consists of caffeoylquinic acid (caffeic acid bound to quinic acid). It has antioxidant and anti-inflammatory properties.	Polar, as it contains hydroxyl (-OH) and carboxyl (-COOH) groups that form hydrogen bonds, making it soluble in polar solvents such as water and methanol (de Falco et al. 2015).

(3aR,4R,5R,7aR)-4-(4-Hydroxy-3-methoxyphenyl)-1-oxo-7-[(2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-1,3,3a,4,5,7a-hexahydro-2-benzofuran-5-carboxylic acid	5.19-5.21 (except for the Kasio clone)		A complex phenolic compound containing benzofuran, carboxyl, hydroxyl-methoxyphenyl, and chrome groups, exhibiting potential antioxidant properties. It also has potential as an antioxidant, antimicrobial, and anti-inflammatory agent.	Highly polar, due to the presence of multiple hydroxyl (-OH), carboxyl (-COOH), and carbonyl (C=O) groups, which increase its solubility in polar solvents (Chen et al. 2015).
Benzyl 2-methyl-4-(4-nitrophenyl)-6-oxo-1,4,5,6-tetrahydro-3-pyridine carboxylate	6.79-6.81 (except for the Randu Alas clone)		A pyridine derivative ester with a benzyl group and a nitrophenyl substituent, providing electrophilic properties to the molecule. It may have applications as a synthetic precursor for pharmaceuticals or other functional chemicals. The nitro and benzyl groups suggest potential biological activities, such as antimicrobial or anticancer properties.	Polarity: Moderately polar, due to the carbonyl (C=O) and nitro (-NO_2) groups, but its polarity is reduced by the non-polar benzyl group (Evitachem 2024).
Methyl (1R,3R)-1-(1,3-benzodioxol-5-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate	7.38		A β-carboline derivative, which is a heterocyclic alkaloid. The β-carboline group consists of an indole core fused with a pyridine ring. It exhibits anticancer properties due to the cytotoxic activity of β-carboline derivatives against cancer cells, as well as antimicrobial and neuroprotective properties, attributed to its ability to inhibit the enzyme monoamine oxidase (MAO).	Semi-polar, as the combination of ester and benzodioxol groups suggests that this compound has both polar and non-polar characteristics. The carbonyl and dioxolane groups enhance solubility in polar solvents, while the aromatic part increases its affinity for non-polar solvents. It is soluble in moderately polar organic solvents such as methanol or ethanol (Piechowska et al. 2019)

These compounds are generally non-polar and have larger molecular sizes, requiring longer elution times from the chromatographic column. This characteristic is associated with the structure of the compounds, which includes large aromatic rings, long hydrocarbon chains, or other hydrophobic functional groups that reduce their solubility in polar solvents. Consequently, these compounds appear at higher retention times in the chromatogram.

According to Gallardo-Ignacio et al. (2023), polar and non-polar compounds play different yet complementary roles in determining coffee quality. Therefore, the presence of both types of compounds is a crucial aspect of the chemical characterization of coffee beans.

In addition, the unknown compounds found in some clones warrant additional investigation using modern mass spectrometric methods, as they may play a major part in flavor, aroma, or even bioactivities in health. That complexity contributes to the increasing perception that, in addition to key components such as caffeine and chlorogenic acid, the fine profile of minor and late-eluting compounds might markedly influence finding the sensory and functional properties of robusta coffee (Freitas et al. 2024).

Discussion

Green coffee beans of several local robusta coffee clones in Tanggamus, Lampung Province, Indonesia, were chemically fingerprinted, and the results showed both clear differences and similarities in their chemical profiles. Major chemicals including caffeine, chlorogenic acid, 4-aminobenzoic acid, and hymecromone were present in all clones, which was in line with findings from earlier research that highlighted their contributions to the sensory qualities and health advantages of coffee (Zanin et al. 2016; Herawati et al. 2019). These common substances point to a steady metabolic profile that is probably impacted by robusta coffee's genetic heritage (Cheserek et al. 2022).

Caffeine, which is well-known for its stimulating qualities, is seen in all samples at almost the same retention times. Chlogenic acid's persistent presence as a potent antioxidant suggests that it plays a substantial influence in the overall chemical makeup of robusta coffee beans (Duque and Blair 2021). This general stability demonstrates the clones' genetic conservation even in a variety of agronomic and environmental settings (Howard 2011; Kiwuka et al. 2021).

Despite the shared primary metabolites, variations beyond the retention time (Rt) of 7.38 minutes highlight the chemical diversity among the clones. For instance, the distinct peaks observed in the Kopi Hijau and Komari clones indicate the presence of unique secondary metabolites, which could result in flavor and aroma differences (Núñez et al. 2021a). These variations are typically ascribed to environmental differences and the particular metabolic pathways of each clone (Toledo et al. 2016).

The differences in number of identified compounds among the various clones indicated the variation of metabolic activity between clones. For instance, the Kopi Hijau and Komari clones had much richer and complicated chemical profiles, which reflected their increased capability for biosynthesis. These variations may be important to determine the sensory (nutritional) and functional (antioxidant, antidiabetic, etc.) performance of each clone and represent important information for future coffee breeding programs (Freitas et al. 2024).

At the same time, physical characteristics like yield, moisture content, and density also showed notable correlations with the chemical profiles. The higher yield observed in the Komari and Kopi Hijau clones corresponded to their richer and more complex chemical compositions, as evidenced by the number of identified compounds. Likewise, moisture and density variation could affect the retention and transformation of some chemical compounds during post-harvest processing (Khemira et al. 2023).

This finding indicates that physical traits may serve as accessible proxies for deeper chemical quality, especially in practical or field-based quality assessments. The ability to predict chemical richness or stability based on simple physical measurements could greatly benefit farmers, processors, and breeders in targeting high-quality clones without the need for expensive instrumentation. Therefore, certain physical characteristics may be used as markers of chemical quality. For example, clones that are denser and have less moisture tend to have more concentrated and stable chemical profiles. This connection emphasizes how crucial it is to combine chemical and physical tests when evaluating the quality of coffee beans (de Melo Pereira et al. 2019; Stilo et al. 2021).

Such integrations are particularly valuable for small-holder systems, where precision tools may not always be available. Moreover, the correlation between physical and chemical traits opens possibilities for marker-assisted selection in breeding programs, where phenotypic cues can reliably reflect underlying metabolomic potential.

These results are in line with newly published metabolomics research, integrating data on how genetic and environmental variables influence chemical composition and physical quality. For instance, good processing practices can optimize moisture content and improve coffee bean quality (Adnan et al. 2017; Freitas et al. 2024), and the diversity of local robusta coffee clones across various post-harvest processing methods reveals that local genetic variation yields distinct physical attributes (Analianasari et al. 2023). Thus, the chemical fingerprinting data presented here not only affirm the uniqueness of local robusta clones but also support their potential in developing differentiated coffee products with traceable origin and targeted functional benefits.

Given the chemical diversity among Tanggamus robusta clones in this study, this finding has essential implications not only for the breeding of clones but also for producing coffees with specific flavors and quality attributes (Pereira et al. 2019; Duque and Blair 2022). Future research should continue to explore the sensory relevance of these chemical differences and the ways genetic and environmental factors interact to shape the expression of unique metabolites. Thus, knowledge of the relationship between specific chemical fingerprints and physical properties will provide important implications for coffee quality optimization, as well as leading to the definition of robust markers for coffee authenticity and traceability (Stilo et al. 2021).

In conclusion, the five robusta coffee clones exhibited a similar chemical composition pattern up to a retention time (Rt) of 7.38 minutes. Beyond this point, variations in the chemical composition patterns were observed among the clones. Specifically, the Randu Alas clone contained 18 compounds, Kasio 20 compounds, Komari 19 compounds, Kopi Hijau 15 compounds, and Kopi Kuning 18 compounds. Overall, 10 major compounds were identified across these robusta coffee clones. The Kopi Hijau clone stood out with the lowest defect value (14.15) and the best quality (Grade 2), indicating superior physical characteristics and consistency. This should translate to cleaner taste, better aroma, and enhanced coffee experience for consumers. Its high yield and stable chemical composition would make it a reliable raw material for coffee manufacturers. Despite differences in chemical profiles among clones after Rt 7.38 minutes, these variations would minimally affect flavor, suggesting that Kopi Hijau's quality and consistency mark it as a promising option for high-standard coffee products.

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