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ORIGINAL RESEARCH PAPER

IDENTIFICATION OF METABOLITES ASSOCIATED WITH ANTIOXIDANT ACTIVITY IN ROBUSTA MOKA POT BREW AT DIFFERENT ROASTING DEGREES

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Abstract

This study aims to identify compounds associated with the antioxidant activity of Robusta moka pot coffee brewed at different roasting degrees using LC-HRMS and partial least squares regression (PLS-R) analysis. Robusta Basseang coffee was used as the raw material and roasted at three degrees (light, medium, and dark), followed by brewing using a moka pot. The brewed samples were analysed to determine the content of bioactive compounds (trigonelline, caffeine, chlorogenic acid, total phenolic content), untargeted profile compounds, and antioxidant activity (FRAP and DPPH assays). The results indicated that light-roasted coffee brew had the highest concentration of bioactive compounds and antioxidant activity. LC-HRMS analysis revealed metabolite profiles across roasting degrees, with several compounds detected exclusively at specific roasting degrees. The metabolites associated with antioxidant activity differed between the FRAP and DPPH assays. Chlorogenic acid, and 4-O-Feruloylshikimic acid were associated exclusively with DPPH assay, whereas 5-O-Feruloylshikimic acid, ferulic acid, caffeic acid, D-(−)-Quinic acid, and nicotinic acid associated exclusively with FRAP assay. This study provides insights into the interaction among roasting degree, metabolite composition, and antioxidant activity, and highlights the potential of metabolite profiling for coffee quality evaluation and roasting degree differentiation.

Keywords: moka pot; bioactive compounds; Robusta Basseang coffee; roasting degree; antioxidant activity

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Introduction

Coffee is one of the most widely consumed beverages worldwide, and it is well known for its distinctive flavour and aroma. In addition to these sensory attributes, coffee contains various bioactive compounds that provide health benefits, including chlorogenic acids, caffeine, and Maillard reaction products. These compounds are antioxidants that help protect the body from oxidative damage caused by free radicals (Herawati *et al*., 2019). The quantity of bioactive compounds in coffee is influenced by several factors, including coffee bean variety, postharvest processing methods, roasting degree, and brewing technique (Bastian *et al*., 2021; Kim *et al*., 2022; Yulianti *et al*., 2024). Yulianti *et al*. (2024) reported that 36 compounds with antioxidant potential were identified in Arabica coffee brew, and their quantities were influenced by postharvest processing. Furthermore, Anh-Dao *et al*. (2024) found that Robusta coffee contains higher degrees of caffeine and chlorogenic acids than Arabica coffee. However, information regarding other potential compounds contributing to antioxidant activity in Robusta coffee brew remains limited.

Roasting degree also plays a critical role in determining the quantity of bioactive compounds and the chemical profile of coffee brew. According to Kim *et al*. (2022), postharvest processing, roasting degree, and brewing method are correlated factors that influence the metabolite profile of brewed coffee. Coffee roasted at light, medium, and dark degrees exhibits variations in compound abundance, particularly chlorogenic acids, caffeic acid, trigonelline, and hydroxymethylfurfural (Freitas *et al*., 2023). Therefore, a more comprehensive investigation is required to understand the effect of roasting degrees on the metabolite profile of coffee.

In addition to roasting, the brewing method plays an important role in determining the composition of compounds in coffee brews. One of the most popular and useable brewing methods at home is the moka pot. This method utilizes high pressure generated by steam to extract compounds from ground coffee, producing a brew characterized by a rich mouthfeel, full body, and relatively high acidity (Santanatoglia *et al*., 2024). Furthermore, moka pot brew has been documented to possess the highest total phenolic and flavonoid concentrations, along with the most robust antioxidant capacity, as determined by DPPH and ABTS assays, in comparison to espresso, pod, and capsule brews (Gobbi *et al*., 2023). However, most studies have primarily focused on major bioactive compounds present in moka pot coffee brews. Therefore, more comprehensive research is needed to identify other bioactive compounds that contribute to antioxidant activity.

Advancements in food science and analytical technologies, Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) enables a more comprehensive identification of metabolite profiles associated with changes in chemical composition due to variations in roasting degree. When combined with multivariate analysis, compounds correlated with antioxidant activity can be effectively identified. Therefore, this study aims to investigate the effect of roasting degree on the metabolite profile and antioxidant activity of Robusta coffee brewed using a moka pot. In addition, this study seeks to identify compounds associated

with antioxidant activity using multivariate partial least squares regression (PLS-R) analysis. The findings of this study are expected to provide new insights into the relationship between roasting degree, chemical composition, and antioxidant activity in coffee brews, as well as contribute to the development of functional coffee products.

Materials and methods

Materials

The materials used in this study included Robusta Basseang coffee (Pinrang Regency, South Sulawesi, Indonesia, 2024 harvest), caffeine (Merck, KGaA, Germany), trigonelline hydrochloride (Sigma-Alderich, Switzerland), chlorogenic acid (Sigma-Alderich, Switzerland), methanol grade HPLC (Merck, KGaA, Germany), methanol grade LC-MS (Merck, KGaA, Germany), water grade LC-MS (Merck, KGaA, Germany),, formic acid (Merck, KGaA, Germany), Folin-Ciocalteu's phenol reagent (Merck, KGaA, Germany), sodium carbonate (Na2CO3) (Merck, KGaA, Germany), gallic acid (Sigma-Aldrich, China), ethanol (Merck, KGaA, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Merck, KGaA, Germany), TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) (Sigma-Alderich, Switzerland), FeCl3.6H2O (Merck, KGaA, Germany), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Alderich, Switzerland), Asam Acetat Glacial (Merck, KGaA, Germany), Sodium acetate trihydrate (Merck, KGaA, Germany), water for injection pirogen free (PT. Ikapharmindo Putramas, Indonesia), and aquadest (Water one, Indonesia).

Sample preparation

Coffee beans were roasted at three degrees: light (L 36.8), medium (L 33.5), and dark (L 31.5). Roasting was performed using a Gene Roaster (South Korea) with an initial temperature of 60 °C and a final temperature of 230 °C. Light roast beans were collected at minute 10, medium at minute 11, and dark at minute 12. Roasted beans were ground using a grinder at medium grind size. Brewing was conducted using a moka pot (Double Valve Mortca Poy, China) with a coffee : water ratio of 1 : 10. The brewing process lasted 5 minutes and 50 seconds.

Quantification of caffeine, trigonelline, and chlorogenic acid

Quantification was performed using a modified method from Yulianti et al. (2022) and Santanatoglia et al. (2024). UHPLC–UV–VIS (Thermo Fisher Scientific, Japan) equipped with a BDS Hypersil C18 column (150 × 4.6 mm) was used at 40 °C. The mobile phase consisted of 0.1% formic acid in methanol (A) and 0.1% formic acid in water (B) under isocratic elution at 40% A and 60% B, with a flow rate of 0.5 mL/min, injection volume of 10 µL, and total run time of 11 minutes. Caffeine and trigonelline were detected at 270 nm, whereas chlorogenic acid was measured at 325 nm. Quantification was performed using linear regression ($y = ax + b$) with calibration curves ranging from 5 to 150 ppm (Caffeine $R^2 = 0.99$, LOQ = 0.39 mg/100 mL, LOD : 0.13 mg/100 mL; Trigonelline $R^2 = 0.99$, LOQ = 1.32

mg/100 mL, LOD : 0.44 mg/100 mL; and chlorogenic acid R^2 = 0.99, LOQ = 4.69 mg/100 mL LOD : 1.55 mg/100 mL).

Total phenolic content

Total phenolic content was determined using a modified method from Shetty et al. (1995). A total of 20 µL of sample was mixed with 20 µL of 95% ethanol, 100 µL of water for injection, and 10 µL of 50% Folin reagent. After incubation for 5 minutes, 20 µL of 5% Na_2CO_3 was added. The mixture was incubated for 60 minutes, and absorbance was measured at 725 nm using a Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific, Japan). Quantification was based on a gallic acid calibration curve (10–100 ppm, R^2 = 0.99, LOQ = 2.50 mg/100 mL, LOD = 0.83 mg/100 mL).

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH radical scavenging activity was assessed using a modified method from Yulianti et al. (2024). A mixture of 80 µL acetate buffer (100 mM, pH 5.5), 80 µL ethanol, 40 µL DPPH (250 µM), and 10 µL sample was shaken for 1 minute and incubated for 30 minutes. Absorbance was measured at 517 nm using a Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific, Japan). Quantification was based on a Trolox calibration curve (20–120 ppm, R^2 = 0.99, LOQ = 3.58 mg/100 mL, LOD = 1.18 mg/100 mL).

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was measured using a modified method from Yulianti et al. (Yulianti et al., 2024). FRAP reagent was prepared from 2.5 mL TPTZ (10 mM in 40 mM HCl), 2.5 mL $FeCl_3 \cdot 6H_2O$ (20 mM), and 25 mL acetate buffer (0.3 mM, pH 3.6), incubated at 37 °C for 30 minutes. In a 96-well microplate, 200 µL FRAP reagent, 20 µL water, and 10 µL sample were combined, shaken for 1 minute, and incubated at 37 °C for 30 minutes. Absorbance was measured at 595 nm using a Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific, Japan). Quantification was conducted using a Trolox calibration curve (25–300 ppm, R^2 = 0.99, LOQ = 14.08 mg/100 mL, LOD = 4.65 mg/100 mL).

LC–HRMS based metabolite profiling

Metabolite profiling was performed according to Windarsih et al. (2022) using Liquid Chromatography (Thermo Fisher Scientific Vanquish UHPLC, Japan) coupled with Orbitrap high-resolution mass spectrometry (Thermo Fisher Scientific Q Exactive, Japan). Separation was achieved using an Accucore Phenyl–Hexyl column (100 mm × 2.1 mm × 2.6 µm). Mobile phases were LC–MS-grade water with 0.1% formic acid (A) and LC–MS-grade methanol with 0.1% formic acid (B) under gradient elution at 0.3 mL/min. Untargeted MS and dd-MS^2 acquisition were performed in both positive and negative ion modes at a scan range of 66.7-1000 m/z. Instrumental parameters followed standard operating conditions. Compounds were annotated when their mzCloud Best Match score exceeded 50.

Statistical analysis

Data were evaluated using One-way ANOVA followed by Duncan's post hoc test was performed ($p < 0.05$). LC–HRMS chemical compounds profile data were analyzed using Partial Least Squares-Discriminant Analysis (PLS-DA), and correlations between antioxidant-related compounds and antioxidant activities were assessed using Partial Least Squares-Regression (PLS-R). All analyses were carried out using XLSTAT Student 2025 software (Lumivero, USA).

Results and discussion

Bioactive compoundss of moka pot brewed robusta basseang coffee

The results demonstrated that higher roasting degrees led to a progressive reduction in trigonelline content (Table 1). Trigonelline is thermolabile; therefore, prolonged exposure to high roasting temperatures increases its degradation rate (Perrone *et al*., 2008). This compound may decompose into nicotinic acid and several volatile compounds, such as furans, which contribute to the characteristic aroma of coffee (Bastian et al., 2021). The degradation of trigonelline from light to medium roast occurred at a lower magnitude (Viencz *et al*., 2023). Caffeine content also exhibited a decreasing trend from light to dark roasts (Table 1). Although caffeine is more heat-stable than trigonelline, extended roasting may still induce partial degradation, resulting in a lower caffeine concentration (Duke *et al*., 2025). The caffeine concentration in moka pot brew was previously reported to be comparable to that of cold brew and higher than that of V60, French press, and Aeropress brews (Angeloni *et al*., 2019).

Table 1. Bioactive compounds in moka pot brewed robusta basseang coffee at different roasting degrees.

Roast Degree	Trigonelline (mg/100 mL)	Caffeine (mg/100 mL)	Chlorogenic Acid (mg/100 mL)	Total Phenolic Content (mg GAE/100 mL)
Light	12.33±0.05^{a}	79.13±0.17^{a}	100.23±0.25^{a}	229.10±9.99^{a}
Medium	11.42±0.20^{b}	73.07±0.74^{b}	95.21±3.07^{b}	185.94±1.81^{b}
Dark	11.03±0.19^{c}	64.11±1.55^{c}	79.81±1.51^{c}	170.15±4.95^{c}

Note: Values followed by the same letter are not significantly different ($p > 0.05$). Values are the mean and standard deviation of 3 replications.

Chlorogenic acid exhibited the most substantial decrease compared with both caffeine and trigonelline (Table 1). This reduction corresponded with a decline in total phenolic content. Chlorogenic acid is highly sensitive to heat, and roasting promotes its conversion into quinic acid, caffeic acid, and other degradation products that contribute to bitterness and astringency (Pan *et al*., 2023; Anh-Dao *et*

al., 2024). The observed decline aligns with previous studies reporting that darker roasting degrees reduce phenolic compounds such as chlorogenic acid (Yeager *et al*., 2022; Anh-Dao *et al*., 2024). Nevertheless, the total phenolic content of moka pot brew remained higher than that of espresso but lower than that of Neapolitan-style brews (Caporaso *et al*., 2014) and was also higher than that of pod and capsule brews (Gobbi *et al*., 2023). Overall, the findings indicate that the roasting degree markedly influences the bioactive profile of Robusta moka pot brews. Light roast preserved the highest concentration of bioactive constituents, whereas dark roast resulted in substantial degradation of all four analyzed components. These results suggest that light-roasted Robusta coffee brewed using a moka pot offers greater potential health benefits due to its higher concentrations of trigonelline, caffeine, chlorogenic acid, and total phenolic compounds compared with medium and dark roasts.

Antioxidant activity of moka pot brewed Robusta Basseang coffee

The antioxidant activity of Robusta Basseang coffee brewed using a moka pot decreased as the roasting degree became dark (Table 2). This decline was observed in both the DPPH and FRAP assays. According to Król et al. (Król *et al*., 2020), light-roasted coffee exhibits higher antioxidant activity than dark-roasted coffee. This reduction is attributed to the degradation of phenolic compounds and organic acids caused by high temperatures during roasting. Phenolic compounds such as chlorogenic acids undergo hydrolysis and decarboxylation, generating volatile derivatives (Lee *et al*., 2015). Throughout the roasting process, the covalent bonds within chlorogenic acids and their derivatives may undergo isomerization, epimerization, and lactonization (Hu *et al*., 2020). As roasting progresses to dark degrees with higher temperatures, both reducing capacity and free radical scavenging ability decrease (Sangpimpa *et al*., 2025).

Table 2. Antioxidant activity of moka pot brewed Robusta Basseang coffee at different roasting degrees.

Roast Degree	Antioxidant Activity	
	DPPH (mg TEAC/100 mL)	FRAP (mg TEAC/100 mL)
Light	271.90±14.98 [a]	497.51±14.79 [a]
Medium	206.43±8.37 [b]	466.76±8.83 [b]
Dark	172.81±1.45 [c]	358.28±1.95 [c]

Note: Values followed by the same letter are not significantly different ($p > 0.05$). Values are the mean and standard deviation of three replications.

Table 2 shows that FRAP values were higher than those of DPPH. This difference is related to the distinct mechanisms involved in each assay. FRAP measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}, whereas DPPH assesses hydrogen atom–donating capacity, which is more susceptible to degradation of active compounds (Wu *et al*., 2022). However, when compared to V60, Turkish, and pure brew extraction methods, the antioxidant activity of moka pot brews remains

higher (Santanatoglia *et al*., 2023). Based on roasting degrees, light-roasted brews exhibited the highest antioxidant activity, followed by medium and dark roasts.

Chemical compound profile of moka pot brewed Robusta Basseang coffee

The compound profile of moka pot brewed Robusta Basseang coffee roasted at different degrees was analyzed using LC-HRMS. The analysis successfully identified 92 compounds with mzCloud Best Match scores above 50 (Table 3). Based on PLS-DA modeling (Figure 1), the results obtained were Q^2(cum) of 0.953, R^2Y(cum) of 0.992, and R^2X(cum) of 0.690. These values indicate that the generated model is reliable and excellent, as both Q^2 and R^2 approached 1 (Worley *et al*., 2013; Worley and Powers, 2016).

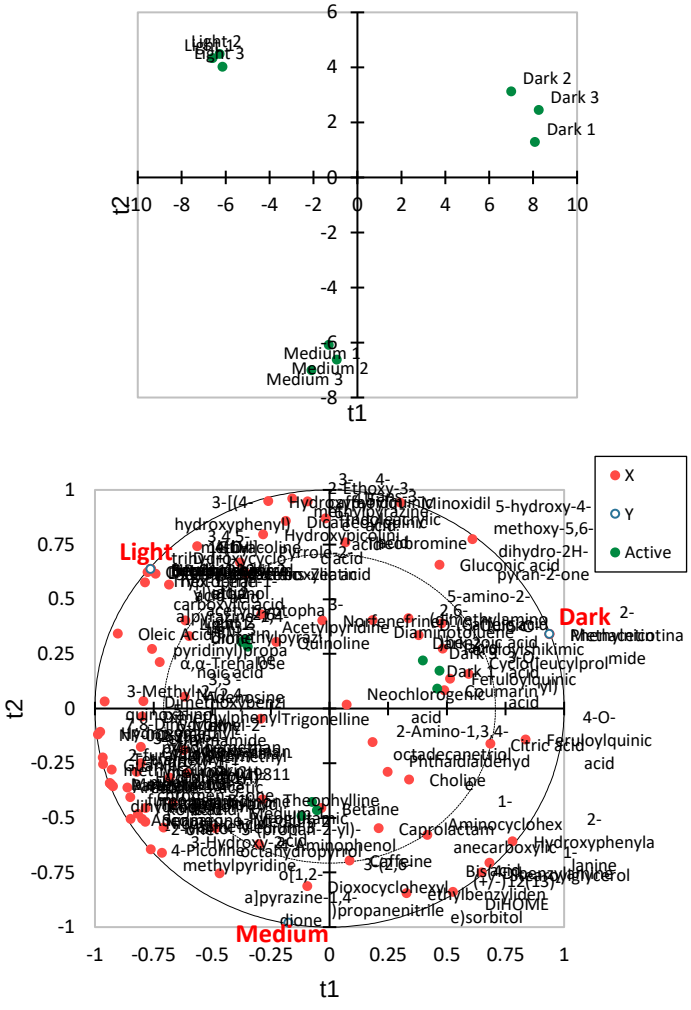

Figure 1. PLS-DA Modeling of Compounds identified in moka pot brewed Robusta Basseang coffee at different roasting degrees. a: Score plot; b: Loading plot.

The biplot (Figure 1a) demonstrates clear separation among roasting degrees. Additionally, the loading plot (Figure 1b) shows that the compound profiles of moka pot brews vary according to roasting degree. Several compounds were detected only in light roast and were absent in medium and dark roasts, whereas some compounds emerged in medium and dark roasts. These changes indicate that roasting induces substantial modification of coffee's compound composition due to heat exposure. The disappearance of certain compounds in light roast, the formation of new compounds in medium and dark roasts, and the stability of others collectively explain the distinct compositional patterns observed across roasting degrees. Both volatile and non-volatile compounds in brewed coffee are generated through Maillard reactions, pyrolysis, and Strecker degradation during roasting (Córdoba et al., 2021).

Table 4 shows that compounds disappearing or emerging at specific roasting degrees possess high VIP (Variable Influence of Projection) values, indicating their strong contribution to group discrimination. According to Galindo-Prieto et al. (2014), variables with VIP values greater than 1 exert substantial influence on class separation. Four compounds were identified exclusively in light-roasted brews: 4-O-feruloylshikimic acid and 3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, both phenolic acid derivatives that readily degrade during heating. Phenolic compounds and organic acids are thermolabile and undergo decarboxylation or fragmentation into simpler molecules at high temperatures (Lyrio et al., 2025). D-(+)-Tryptophan, an amino acid that serves as a substrate in Maillard reactions (Hu et al., 2021), was also detected only in light roast and is known to degrade during roasting (Martins & Gloria, 2010). Linoleic acid was similarly detected only in light roast. Roasting significantly alters fatty acid composition, influencing the aroma characteristics of coffee (Mitra et al., 2025).

Table 3. Profile compounds of moka pot brew Robusta Basseang at different roasting degrees (% relative).

Compunds	Formula	ESI	MS	RT [min]	Light	Medium	Dark
(+/-)12(13)-DiHOME	C18 H34 O4	$[M-H]^{-1}$	313.23828	15.537	nd	0.031	0.021
4-caffeoylquinic acid	C16 H18 O9	$[M+H]^{+1}$	355.10141	5.435	0.051	0.017	0.041
5-O-Feruloylshikimic acid	C16 H18 O9	$[M+H]^{+1}$	355.10193	9.689	2.554	2.494	2.723
4-O-Feruloylshikimic acid	C17 H20 O9	$[M+H]^{+1}$	369.11725	7.319	0.129	nd	nd
4-O-Feruloylquinic acid	C17 H20 O9	$[M+H]^{+1}$	369.11761	7.171	nd	0.433	0.969
1-(1H-Benzimidazol-2-yl)ethanol	C9 H10 N2 O	$[M+H]^{+1}$	163.08662	2.1	0.026	nd	nd
1-Amino cyclo hexanecarboxylic acid	C7 H13 N O2	$[M+H]^{+1}$	144.10158	0.99	0.228	0.246	0.241
1-Methylnicotinamide	C7 H8 N2 O	$[M+H]^{+1}$	137.07088	5.991	0.031	0.031	nd

1-Stearoylglycerol	C21 H42 O4	$[M+H]^{+1}$	359.31506	18.301	nd	0.032	0.029
2-Ethoxy-3-methylpyrazine	C7 H10 N2 O	$[M+H]^{+1}$	139.08656	3.906	0.163	nd	0.108
3-O-Feruloylquinic acid	C17 H20 O9	$[M-H]^{-1}$	367.10281	5.433	0.184	0.193	0.235
acetyltryptophan	C13 H14N2O3	$[M+H]^{+1}$	247.10776	7.917	0.009	0.008	0.008
2-Amino-1,3,4-octadecanetriol	C18 H39 NO3	$[M+H]^{+1}$	318.29926	13.531	1.172	1.266	1.296
2-Hydroxy phenylalanine	C9 H11 N O3	$[M+H]^{+1}$	182.08128	1.588	nd	0.086	0.090
2-Methylnicotinamide	C7 H8 N2 O	$[M+H]^{+1}$	137.07089	6	nd	nd	0.030
2-Pyridylacetic acid	C7 H7 N O2	$[M+H]^{+1}$	138.0549	3.914	0.039	nd	nd
2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	C9 H19 N O	$[M+H]^{+1}$	158.15372	12.129	0.008	0.008	nd
2,6-Diaminotoluene	C7 H10 N2	$[M+H]^{+1}$	123.0918	7.662	0.072	0.059	0.075
2,6-Dimethylpyrazine	C6 H8 N2	$[M+H]^{+1}$	109.07613	5.784	0.161	0.144	0.140
3-(2,6-Dioxocy clohexyl) propanenitrile	C9 H11 N O2	$[M+H]^{+1}$	166.08627	1.332	0.050	0.073	0.052
3-(3-pyridinyl) propanoic acid	C8 H9 N O2	$[M+H]^{+1}$	152.0703	6.269	0.045	0.031	0.023
3-(propan-2-yl)-octahydropyrrolo[1,2-a]pyrazine-1,4-dione	C10 H16N2 O2	$[M+H]^{+1}$	197.12825	5.883	0.098	0.132	0.079
3-[(4-hydroxyphenyl) methyl]-octahydropyrrolo[1,2-a]pyrazine-1,4-dione	C14 H16 N2 O3	$[M+H]^{+1}$	261.12329	5.629	0.017	nd	nd
3-Acetylpyridine	C7 H7 N O	$[M+H]^{+1}$	122.06024	4.986	0.019	0.016	0.017
3-ethyl-4-hydroxy-1-methyl-1,2-dihydroquinolin-2-one	C12 H13 N O2	$[M+H]^{+1}$	204.10161	2.247	0.070	0.070	nd
3-Hydroxy-2-methylpyridine	C6 H7 N O	$[M+H]^{+1}$	110.06008	1.008	0.546	0.634	0.415
3-Hydroxypyridine	C5 H5 N O	$[M+H]^{+1}$	96.04453	0.913	0.044	nd	0.025
3-Methyl-2-quinoxalinol	C9 H8 N2 O	$[M+H]^{+1}$	161.07101	9.794	0.046	0.043	0.038
3,3'-Dimethoxy benzidine	C14 H16N2 O2	$[M+H]^{+1}$	245.12845	10.611	0.015	0.014	0.013
3,4,5-tri hydroxycyclo hex-1-ene-1-carboxylic acid	C7 H10 O5	$[M+H]^{+1}$	173.04474	7.161	0.023	nd	nd
4-Aminophenol	C6 H7 N O	$[M+H]^{+1}$	110.06005	6.263	0.013	0.026	0.009
4-Picoline	C6 H7 N	$[M+H]^{+1}$	94.06528	1.006	0.059	0.069	0.041
4,5-Dicaffeoylquinic acid	C25 H24 O12	$[M-H]^{-1}$	515.11871	9.681	0.734	0.575	0.672

4,6-Dimethyl-2(1H)-pyrimidinone	C6 H8 N2 O	$[M+H]^{+1}$	125.07104	2.611	0.060	0.062	0.054
5-amino-2-(dimethylamino)benzoic acid	C9 H12 N2 O2	$[M+H]^{+1}$	181.09689	4.81	0.027	0.025	0.029
5-hydroxy-4-methoxy-5,6-dihydro-2H-pyran-2-one	C6 H8 O4	$[M+H]^{+1}$	145.04939	1.678	0.165	0.125	0.201
5-Hydroxymethyl-2-furaldehyde	C6 H6 O3	$[M+H]^{+1}$	127.03885	2.245	0.503	0.469	0.366
6-Hydroxypicolinic acid	C6 H5 N O3	$[M+H]^{+1}$	140.03421	5.106	0.026	0.022	0.024
6-Methyl-2-pyridinemethanol	C7 H9 N O	$[M+H]^{+1}$	124.07564	1.204	0.245	0.237	0.183
7-hydroxy-6-methoxy-2H-chromen-2-one	C10 H8 O4	$[M+H]^{+1}$	193.04971	7.79	0.026	0.026	0.016
7,8-Dihydroxy-4-methylcoumarin	C10 H8 O4	$[M+H]^{+1}$	193.0493	9.911	0.018	0.015	nd
Adenine	C5 H5 N5	$[M+H]^{+1}$	136.06201	0.953	0.046	0.058	nd
Adenosine	C10 H13N5 O4	$[M+H]^{+1}$	268.10379	1.122	0.092	0.085	0.071
Arecoline	C8 H13 N O2	$[M+H]^{+1}$	156.10199	4.909	0.021	0.022	nd
Betaine	C5 H11 N O2	$[M+H]^{+1}$	118.08627	0.851	0.135	0.159	0.140
Bis (4-ethyl benzyli dene) sorbitol	C24 H30 O6	$[M+H]^{+1}$	415.21109	14.612	0.021	0.032	0.026
Caffeic acid	C9 H8 O4	$[M+H]^{+1}$	179.08403	5.44	0.050	0.047	0.057
Caffeine	C8 H10 N4 O2	$[M+H]^{+1}$	195.08725	6.308	37.869	42.077	38.952
Caprolactam	C6 H11 N O	$[M+H]^{+1}$	114.09145	4.679	0.049	0.073	0.061
Chlorogenic acid	C16 H18 O9	$[M+H]^{+1}$	353.08746	5.483	6.445	4.386	4.239
Choline	C5 H13 N O	$[M+H]^{+1}$	104.107	0.826	0.690	0.781	0.784
Citric acid	C6 H8 O7	$[M-H]^{-1}$	191.01897	1.005	2.682	3.000	3.238
Coumarin	C9 H6 O2	$[M+H]^{+1}$	147.04404	10.915	0.012	0.012	0.014
Cyclo(leucylprolyl)	C11 H18N2 O2	$[M+H]^{+1}$	211.14394	7.925	0.218	0.220	0.232
Cyclo(phenylalanyl-prolyl)	C14 H16N2 O2	$[M+H]^{+1}$	245.12839	8.838	0.017	0.017	0.011
D-(-)-Fructose	C6 H12 O6	$[M-H]^{-1}$	179.05531	0.807	0.324	0.034	0.028
D-(-)-Quinic acid	C7 H12 O6	$[M-H]^{-1}$	191.05525	5.711	0.044	0.039	0.054
D-(+)-Tryptophan	C11 H12N2 O2	$[M+H]^{+1}$	205.09686	10.161	0.016	nd	nd
Dibenzylamine	C14 H15 N	$[M+H]^{+1}$	198.12767	7.803	nd	0.013	0.010
Ferulic acid	C10 H10 O4	$[M+H]^{+1}$	193.04985	7.216	0.010	0.011	0.008
Gluconic acid	C6 H12 O7	$[M-H]^{-1}$	195.15003	0.829	0.018	0.016	0.020
Guanine	C5 H5 N5 O	$[M+H]^{+1}$	152.05655	0.982	0.030	0.027	nd

Guvacoline	C7 H11 N O2	$[M+H]^{+1}$	142.08617	2.78	0.178	0.155	0.160
Hydrolyzed fumonisin B1	C22 H47 N O5	$[M+H]^{+1}$	406.35211	13.76	0.017	0.018	nd
Indole-3-acetic acid	C10 H9 N O2	$[M+H]^{+1}$	176.07072	4.427	0.036	0.037	0.027
Kojic acid	C6 H6 O4	$[M+H]^{+1}$	143.03375	3.097	0.066	0.069	0.051
L-Pyroglutamic acid	C5 H7 N O3	$[M+H]^{+1}$	130.04976	1.128	0.610	0.634	0.584
Linoleic acid	C18 H32 O2	$[M-H]^{-1}$	279.23291	17.999	0.017	nd	nd
Maltol	C6 H6 O3	$[M+H]^{+1}$	127.03896	3.473	0.468	0.511	0.378
Minoxidil	C9 H15 N5 O	$[M+H]^{+1}$	210.13484	1.338	0.024	nd	0.028
N-(2,4-Dimethylphenyl)formamide	C9 H11 N O	$[M+H]^{+1}$	150.09125	2.184	0.077	0.070	0.058
Neochlorogenic acid	C16 H18 O9	$[M-H]^{-1}$	353.08755	3.224	2.435	2.485	2.522
Nicotinic acid	C6 H5 N O2	$[M+H]^{+1}$	124.03925	1.055	0.183	0.199	0.136
Norfenefrine	C8 H11 N O2	$[M+H]^{+1}$	154.08641	7.962	0.040	0.038	0.040
Norharman	C11 H8 N2	$[M+H]^{+1}$	169.07574	6.338	0.047	0.046	0.019
NP-008993	C18 H34 O4	$[M-H]^{-1}$	313.23834	14.959	0.030	0.023	nd
NP-011220	C11 H18N2 O2	$[M+H]^{+1}$	211.14412	7.723	0.078	nd	nd
NP-019811	C6 H7 N O2	$[M+H]^{+1}$	126.05484	1.096	0.452	0.462	0.408
Oleic Acid	C18 H34 O2	$[M-H]^{-1}$	281.24829	18.344	0.030	0.024	0.021
Paracetamol	C8 H9 N O2	$[M+H]^{+1}$	152.07053	2.551	0.125	0.125	0.034
Phenacetin	C10 H13 N O2	$[M+H]^{+1}$	180.10213	8.959	nd	nd	0.009
Phthaldialdehyde	C8 H6 O2	$[M+H]^{+1}$	135.04385	5.434	0.190	0.211	0.208
Pyrrole-2-carboxylic acid	C5 H5 N O2	$[M+H]^{+1}$	112.03947	5.111	0.062	0.054	0.057
Quinoline	C9 H7 N	$[M+H]^{+1}$	130.06529	6.036	0.034	0.032	0.033
Scoparone	C11 H10 O4	$[M+H]^{+1}$	207.06473	11.199	0.029	0.033	0.017
Tetramethylpyrazine	C8 H12 N2	$[M+H]^{+1}$	137.10738	8.927	0.084	0.098	0.023
Theobromine	C7 H8 N4 O2	$[M+H]^{+1}$	181.07191	3.823	0.037	0.034	0.036
Theophylline	C7 H8 N4 O2	$[M+H]^{+1}$	181.07214	4.582	0.044	0.047	0.042
trans-3-Indoleacrylic acid	C11 H9 N O2	$[M+H]^{+1}$	188.07059	10.16	0.057	0.049	0.055
trans-Zeatin	C10 H13 N5 O	$[M+H]^{+1}$	220.119	1.158	0.487	0.430	0.451
Trigonelline	C7 H7 N O2	$[M+H]^{+1}$	138.05466	0.88	12.010	11.935	11.447
α,α-Trehalose	C12 H22 O11	$[M-H]^{-1}$	341.10852	0.789	0.066	0.051	0.040

Note : nd : not detected; Values are the mean of 3 replications.

Table 4. Discriminant compounds based on PLS-DA in moka pot brewed Robusta Basseang coffee at different roasting degrees.

Compounds	VIP 1	VIP 2
4-O-Feruloylshikimic acid	1.188	1.214
1-Methylnicotinamide	1.457	1.133
2-Hydroxyphenylalanine	1.251	1.191
2-Methylnicotinamide	1.461	1.133
3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid	1.187	1.213
Adenine	1.315	1.158
D-(+)-Tryptophan	1.187	1.213
Guanine	1.500	1.117
Linoleic acid	1.188	1.214

Compounds identified in medium-roasted brews were also found in both light and dark roasts. Adenine and guanine were detected in light and medium roasts (Table 3). These purine compounds are thermally labile and degrade under prolonged heating. Increasing temperatures promote the decomposition of purine nucleosides (Wang and You, 2015). Table 3 also shows that 2-hydroxyphenylalanine is one of the key discriminant compounds across roasting degrees, identified in medium and dark roasts. This compound 2-Hydroxyphenylalanine (o-tyrosine) is formed through partial oxidation of aromatic amino acids, particularly phenylalanine and tyrosine, via Strecker degradation or oxidative reactions during roasting (Hu *et al*., 2021; Sajapin *et al*., 2024). 1-Methylnicotinamide was detected in light and medium roasts, whereas 2-methylnicotinamide was identified in dark roast. N-methylnicotinamide is produced from the catabolism of nicotinamide (Dhuguru *et al*., 2023), which itself is formed from the degradation of trigonelline (Jeszka-Skowron *et al*., 2020). The presence of 2-methylnicotinamide in dark roast indicates isomerization of 1-methylnicotinamide, which may occur due to thermal cleavage of covalent bonds (Jeszka-Skowron *et al*., 2022). During medium roasting, Maillard and caramelization reactions accelerate (Duke *et al*., 2025), and this roasting degree is associated with the formation of the highest aroma compound concentrations (Caporaso *et al*., 2018).

Correlation of antioxidant potential compounds with antioxidant activity

A total of 20 compounds identified as antioxidants (Table 5) were evaluated for their association with antioxidant activity using PLS-R modelling (Figure 2). The model demonstrated reliable and excellent performance, as reflected by Q^2 cum values of 0.659, R^2Y cum of 0.919, and R^2X cum of 0.616 for the DPPH assay, and Q^2 cum of 0.923, R^2Y cum of 0.991, and R^2X cum of 0.657 for the FRAP assay. A PLS model with a Q^2 value above 0.5 is considered to possess strong predictive ability, while an R^2 value approaching 1 indicates robust descriptive quality (Triba *et al*., 2015). The modelling results revealed that 10 compounds contributed to the antioxidant activity measured by the DPPH assay, whereas 13 compounds contributed to the activity measured by the FRAP assay (Table 5). Compounds with significant contributions exhibited VIP (Variable Influence on Projection) values above 1. Galindo-Prieto *et al*. (2014) stated that variables with VIP values

greater than 1 are relevant to the response variable, whereas those with values below 0.5 are not considered relevant.

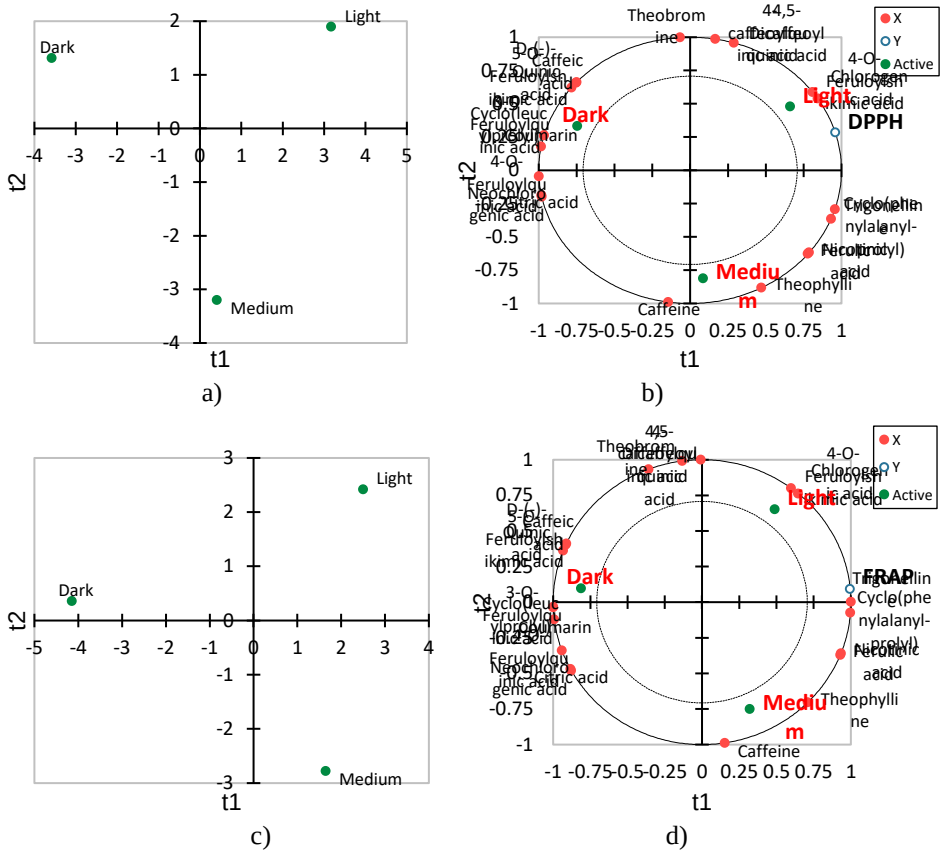

Figure 2. PLS-R modelling of antioxidant-potential compounds in moka pot brews of Robusta Basseang coffee at different roasting degrees. a: Score plot DPPH; b: Loading plot DPPH; c: Score plot FRAP; d: Loading plot FRAP.

Distinct sets of compounds were associated with the two antioxidant assays. Chlorogenic acid, and 4-O-Feruloylshikimic acid were associated exclusively with DPPH radical-scavenging capacity. The compounds 5-O-Feruloylshikimic acid, ferulic acid, caffeic acid, D-(−)-Quinic acid, and nicotinic acid contributed specifically to the reduction-based antioxidant capacity measured by the FRAP assay (Table 5). Chlorogenic acid (5-O-caffeoylquinic acid) is recognised as a strong antioxidant with activity comparable to that of ascorbic acid. Its mechanism involves single-electron transfer and proton transfer at the hydroxyl groups located at positions 3 and 4 (Haridas et al., 2023; Izu et al., 2024). The distinct antioxidant mechanisms of 4-O-Feruloylshikimic acid and 5-O-Feruloylshikimic acid arise from differences in their esterification sites. Zeng *et al*. (2012) reported that esterification at position 6 of shikimic acid enhances antioxidant activity more

effectively than esterification at positions 4 or 2 in caffeoylshikimic acids. Ferulic acid and caffeic acid function as major phenolic acids that can inhibit the formation of superoxide and other free radicals (Ceylan *et al*., 2025). The antioxidant activity of phenolic compounds is influenced by the number and position of hydroxyl groups on the aromatic ring as well as by the presence of double bonds in the chain C (Chen *et al*., 2020; Chen *et al*., 2024). Meanwhile, nicotinic acid possesses free electrons that can facilitate the reduction process (Kim *et al*., 2014; Ilkhani, 2016;).

Table 5. Compounds correlated with antioxidant activity.

Compounds	DPPH VIP(1)	VIP(2)	FRAP VIP(1)	VIP(2)	References
Neochlorogenic acid	1.367	1.314	1.141	1.138	(Moreira *et al*., 2005)
Citric acid	1.366	1.312	1.147	1.144	(Singh *et al*., 2022)
Coumarin	1.226	1.178	1.241	1.236	(Singh *et al*., 2020)
3-O-Feruloylquinic acid	1.178	1.143	1.258	1.252	(Moreira *et al*., 2005)
Cyclo(leucylprolyl)	1.167	1.125	1.240	1.234	(Deepak *et al*., 2020)
Cyclo(phenylalanyl-prolyl)	1.084	1.052	1.225	1.220	(Fuke *et al*., 2025)
Trigonelline	1.145	1.105	1.237	1.232	(Schouten *et al*., 2021)
4-O-Feruloylquinic acid	1.332	1.277	1.203	1.199	(Moreira *et al*., 2005)
Chlorogenic acid	1.319	1.288	0.887	0.891	(Moreira *et al*., 2005)
4-O-Feruloylshikimic acid	1.294	1.269	0.833	0.839	(Zeng *et al*., 2012)
5-O-Feruloylshikimic acid	0.788	0.807	1.111	1.108	(Zeng *et al*., 2012)
Ferulic acid	0.780	0.800	1.106	1.103	(Zeng *et al*., 2012)
Caffeic acid	0.736	0.767	1.084	1.082	(Sidoryk *et al*., 2018)
D-(-)-Quinic acid	0.728	0.760	1.080	1.077	(Benali *et al*., 2024)
Nicotinic acid	0.794	0.812	1.114	1.110	(Ilkhani, 2016)
4-caffeoylquinic acid	0.604	0.733	0.049	0.165	(Moreira *et al*., 2005)
4,5-Dicaffeoylquinic acid	0.753	0.844	0.106	0.191	(Moreira *et al*., 2005)
Caffeine	0.577	0.714	0.076	0.174	(Moreira *et al*., 2005)
Theobromine	0.302	0.539	0.336	0.366	(B.-G. Kim *et al*., 2013)
Theophylline	0.276	0.481	0.798	0.802	(B.-G. Kim *et al*., 2013)

Several additional phenolic constituents, including neochlorogenic acid and 3-O-feruloylquinic acid, also exhibited VIP values above 1 in both assays, which

indicates substantial contributions to total antioxidant activity. These compounds contain conjugated systems and carboxyl groups that stabilise radicals through electron delocalisation, thereby enhancing their antioxidant performance (Platzer *et al*., 2022). Phenolic compounds exhibit reducing properties because they contain reductones capable of donating hydrogen atoms that terminate free-radical chain reactions (Wu *et al*., 2022). Angeloni *et al*. (2019) identified chlorogenic acid as the main contributor to the antioxidant properties of coffee. This compound undergoes extensive degradation at dark roasting degrees, which leads to the formation of Maillard reaction products such as melanoidins. Although melanoidins retain antioxidant activity, their contribution is lower compared with native phenolic compounds (Bobková *et al*., 2020). Another compound that exhibits a positive correlation with antioxidant activity in both DPPH and FRAP assays is trigonelline. According to Schouten *et al*. (2021), trigonelline is a bioactive compound with potential health benefits, including antioxidant and antidiabetic properties, and it shows a positive correlation with both reducing capacity and radical scavenging activity. The overall antioxidant properties of roasted coffee therefore reflect a balance between the degradation of phenolic compounds, particularly chlorogenic acids, and the formation of new Maillard-derived compounds. The formation of these new compounds during roasting is difficult to control (Anh-Dao *et al*., 2024).

Conclusions

Roasting degree exerted a significant influence on the chemical composition and antioxidant activity of moka pot coffee brews. The light-roast brew exhibited the highest concentrations of bioactive compounds and antioxidant capacity compared with the other roasting degrees. In addition, the chemical profiles differed across roasting degrees, with four compounds detected exclusively in the light roast, three compounds identified in both the light and medium roasts, one compound identified in the medium and dark roasts, and one compound detected only in the dark roast. The findings also demonstrate that thermal processing induces structural modifications in chemical compounds, as indicated by the detection of 1-methylnicotinamide in the light and medium roasts, and 2-methylnicotinamide in the dark roast. Therefore, controlling the roasting degree is crucial for maintaining the functional quality and chemical profile of moka pot coffee, and may serve as a basis for developing quality standards and product innovations in coffee processing.

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Yulianti: conceived, methodology and performed experiments, wrote the manuscript, and funding. Idrawati Dian Utami, Lucky Prabowo Miftachul Alam, Dinar Suksmayu Saputri: performed experiments, and writing – Review & Editing. Yeyen Prestyaning Wanita, Aldicky Faizal Amri, Eko Heri Purwanto, Nendyo Adhi Wibowo, Mirwan Ardiansyah Karim, Fawzan Sigma Aurum : writing – Review & Editing.

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