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# Roasting-Dependent Changes in Antioxidant Activity and Metabolite Profiles of *Coffea liberica*: Unraveling Bioactive Compounds and Their Functional Properties

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SPECIAL ISSUE ARTICLE

# Roasting-Dependent Changes in Antioxidant Activity and Metabolite Profiles of *Coffea liberica*: Unraveling Bioactive Compounds and Their Functional Properties

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## ABSTRACT

*Coffea liberica*, despite its high antioxidant activity reported in the green bean, has had limited research in the literature on changes in metabolite profiling across different roasting levels. Therefore, the objective of the present study was to evaluate the antioxidant activity and metabolite profiling of the green beans and various roasting levels of *Coffea liberica* brew using multiple antioxidant assays namely (total phenolic content, total flavonoid content, 1,1-Diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), and hydrogen peroxide scavenging activity) and liquid chromatography mass spectrometry coupled with quadrupole time-of-flight (LC/MS-QTOF), respectively. Notably, light-roasted coffee brew retained the highest antioxidant activity across most antioxidant assays. A total of 95 significant metabolites were identified, encompassing different classes of compounds such as phenolic acids, flavonoids, amino acids, alkaloids, heterocyclics, organic acids, and others. Chlorogenic acid derivatives, flavonoids, and alkaloids were the significant key metabolites ($p < 0.05$) that discriminated *Coffea liberica* brew across different roasting conditions. These findings demonstrate that light-roasted coffee brew has the potential to

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enhance the formation and alteration of bioactive compounds that may provide health benefits, thereby highlighting it as a potential source of natural food that is beneficial to health.

**Keywords:** Antioxidant activity, *Coffea liberica*, LC/MS-QTOF, Metabolite profiling, Roasting levels

## Introduction

Coffee is a widely consumed beverage known for its sensory and nutritional qualities and is a significant source of antioxidants in consumers' diets, in addition to fruits and vegetables.[1] Recent studies have revealed bioactive effects associated with regular coffee consumption, altering its perception from a luxury stimulant to a health-promoting natural product, especially when consumed in moderation.[2] This habitual coffee intake has led to a reduced risk of Alzheimer's disease, cardiovascular disease, Parkinson's disease, type 2 diabetes mellitus, and liver cirrhosis. It also enhances cognitive and physical performance by mitigating oxidative stress, protecting cells, and reducing inflammation in vitro.[3,4] These benefits are attributed to its biologically active substances, including chlorogenic acids, caffeic and ferulic acids, catechin, epicatechin, anthocyanins, and alkaloids such as caffeine, trigonelline, and melanoidin.[5]

*Coffea liberica*, although less commercially favoured than *Coffea arabica* and *Coffea canephora*, is a recognised coffee species with two different varieties, such as *Coffea liberica* var. dewevrei and *Coffea liberica* var. liberica.[6] In Malaysia, *Coffea liberica* is widely cultivated due to the favourable climatic conditions. Compared with *Coffea arabica* and *Coffea canephora*, previous research shows that green beans of this variety have greater antioxidant activity.[7] Moreover, *Coffea liberica* has demonstrated possible anti-angiogenic activity. However, this specialty of Malaysia has garnered relatively little attention, resulting in a lack of established phytochemical studies.[8]

Roasting is an essential process that contributes to the formation of distinctive aroma, flavour, and the darker colour of brewed coffee.[9] Throughout the roasting process, coffee beans undergo various complex chemical reactions that alter the final product's chemical traits and sensory properties. Moreover, roasted beans exhibit varied chemical properties depending on processing time and temperature.[10,11] Studies demonstrate that the phenolic antioxidants contributing to the antioxidant properties of coffee diminish with increased roasting intensity, primarily caused by the degradation of chlorogenic acids, which are abundant in green beans.[12]

In recent decades, metabolomics has emerged as a valuable technology for the large-scale determination of the chemical composition of food matrices, facilitating a variety of downstream applications.[13] Specifically, it combines bioinformatics methods with high-throughput analytical techniques to produce comprehensive information about a diverse array of metabolites and their accumulation trends in relation to the biological phenomena being studied.[14] According to research, the downstream processes of coffee, such as cherry processing, roasting, grinding, and brewing techniques, strongly impact the chemical composition and overall quality of the beverage.[15] Metabolomics has been utilised in coffee processing to examine alterations in chemical composition resulting from pyrolysis and Maillard reactions, among other factors. This underscores the importance of identifying metabolite markers that differentiate the changes occurring during roasting.[16] This method has been applied in various coffee processing operations. For example, to differentiate among coffee cherry varieties and colours,[17] coffee beans from diverse regions,[18] and various coffee brewing methods.[19]

Liquid chromatography-mass spectrometry (LC/MS) is one of the metabolomics techniques known for its remarkable selectivity and sensitivity, thus providing it as an effective platform for metabolite profiling.[20] LC/MS has been utilised to profile medium-polar and high molecular weight compounds, for example, chlorogenic acid. Besides that, it is also used to analyse the non-volatile metabolites associated with coffee brewing, such as polyphenols, diterpenes, and alkaloids.[21] Studies utilising LC/MS-based untargeted metabolomics to examine the metabolite profiles of *Coffea liberica* across different roasting levels are insufficient, despite the growing interest in coffee metabolomics. While most of the research focuses on *Coffea arabica* and *Coffea canephora*, there is a lack of published data that investigates the changes in the metabolite profiles and antioxidant potential of *Coffea liberica* across various roasting levels. A comprehensive understanding of this phenomenon is essential for analysing metabolite changes induced by the roasting process, offering insights that may improve final product quality, as the levels and conditions of coffee roasting significantly affect

the composition of beneficial bioactive compounds in *Coffea liberica*, a potential health-promoting food. Hence, this research aimed to explore the antioxidant activity and the presence of bioactive compounds in *Coffea liberica* at different roasting levels (green beans, light, medium, dark, and very dark-roasted) using various antioxidant assays and an LC/MS-QTOF profiling method. The research also focused on identifying which roasting level enhances the antioxidant capacity of bioactive constituents, which could contribute to the growing interest in its potential as a nutritional and therapeutic food application.

## Materials and methods

### Sample preparation

Green and roasted beans (light, medium, dark, and very dark) of *Coffea liberica* were purchased from MyLiberica Enterprise in Simpang Renggam, Malaysia. The roasted beans were classified based on roasting temperature provided by the supplier: light roasted (210˚C), medium roasted (220˚C), dark roasted (230˚C), and very dark roasted (240˚C). The beans were ground to a medium coarse using an electrical coffee grinder (Model HB-990, China). The coffee beans were brewed with hot water (93 ± 1˚C) using the V60 pour-over method with a coffee: water ratio of 1:10. Initially, 10% of hot water was poured over the ground coffee and allowed to pre-infuse for 30 seconds. Subsequently, the hot water was applied in concentric circles and shaken manually. The extraction duration was approximately 3 minutes.[22]

## Antioxidant analyses

### Total phenolic content

The total phenolic content of the coffee brew was determined using the Folin-Ciocalteu method with slight modifications.[23] Briefly, diluted coffee brew was mixed with the Folin-Ciocalteu reagent, followed by 5 minutes of incubation. Subsequently, a 7.5% sodium carbonate solution was added to the mixture and incubated at 45˚C in the dark. After 30 minutes, the absorbance was measured at 765 nm using a microplate reader (BMG POLARstar Omega, Ortenberg, Germany). All measurements were conducted in triplicate. Gallic acid (0–200 $\mu$g/mL) was used as the standard. The total phenolic content values were presented in mg/g weight of gallic acid equivalents (GAE).

### Total flavonoid content

The total flavonoid content was measured using the method described by Chang et al.[24] Briefly, 900 $\mu$L of methanol, 60 $\mu$L of 10% aluminium chloride, and 60 $\mu$L of 1N sodium acetate were added to the diluted coffee brew sample. The absorbance was measured using a microplate reader (BMG POLARstar Omega, Ortenberg, Germany) at 420 nm after 30 minutes. Quercetin (0–1000 $\mu$g/mL) was used for a standard curve. The total flavonoid content values were expressed as mg/g of quercetin equivalents (QE).

### 1,1,-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity assay was conducted following the method by Blois.[25] The coffee brew samples were prepared in different concentrations. To summarise, the diluted coffee brew sample was mixed with a 0.45 mM DPPH solution. The mixture was agitated vigorously and left undisturbed. After 30 minutes, the absorbance (Abs) was measured at a wavelength of 517 nm using a microplate reader BMG POLARstar Omega, Ortenberg, Germany. The results are presented in accordance with the $IC_{50}$ value, with lower $IC_{50}$ values indicating higher antioxidant activity.

### Ferric reducing antioxidant power (FRAP)

The FRAP of coffee brew was measured using the procedure described by Benzie and Strain,[26] with slight modifications. Briefly, 10 mM TPTZ in 40 mM HCl, 20 mM iron (III) chloride hexahydrate, and 300 mM acetate buffer (pH 3.6) in a ratio of 1:1:10 were mixed to prepare the FRAP solution. Then, the diluted coffee brew sample (100 $\mu$L) was added to 3mL of FRAP solution. The mixture was incubated for 30 minutes at 37˚C before being measured at 593 nm. Trolox (0 to 300 $\mu$g/mL) was used as a standard. The reducing capacity was presented in mg/g of Trolox equivalents (TE).

### 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

The ABTS assay was measured in accordance with the method described by Re et al.,[27] with slight modifications. The ABTS radical cation was produced by reacting 7 mM of ABTS powder with 2.45 mM of potassium persulfate, and was kept overnight in the dark. The ABTS radical cation was diluted with 50% methanol to achieve an absorbance of 0.7 ± 0.2. Briefly, 300 $\mu$L of diluted coffee brew was

combined with 3 mL of ABTS solution. The absorbance (Abs) was measured at 745 nm after 6 minutes. The percentage of radical scavenging activity (RSA) was calculated using the formula below Eq. (1);

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (1)$$

## Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of the coffee brew was measured following the method described by Manjusha and Merugu,[28] with slight modifications. A 2 mM hydrogen peroxide solution was prepared by mixing 50% (w/v) of hydrogen peroxide with phosphate buffer (0.1 M, pH 7.4). Briefly, the diluted coffee brew was mixed with 800 $\mu$L of phosphate buffer and 1.2 mL of 2 mM hydrogen peroxide solution. After 10 minutes, the absorbance (Abs) was measured at 230 nm. The percentage of hydrogen peroxide radical scavenging activity (RSA) was calculated using the formula Eq. (1).

### *Metabolite profiling by LC/MS-QTOF*

The bioactive compounds in the coffee brew were analysed using LC/MS coupled with quadrupole time-of-flight (QTOF) (model 6520, Agilent Technologies, USA). The diluted coffee brew samples (5 mg/mL) were separated using a ZORBAX C18 column (100 mm × 2.1 mm × 1.8 $\mu$m, Agilent Technologies, USA), maintained at 40°C. The mobile phase used was water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B), with mobile phase B increasing from 5 to 95%. The flow rate was set to 0.25 mL/min employing a linear gradient comprising water. The duration of each run was 30 minutes. The electrospray ionisation (ESI) source was configured with a capillary voltage (V Cap) of 4000 V, a fragmentor at 125 V, and a skimmer at 65 V. The nebuliser was set to 45 psig, utilising high-purity nitrogen as the drying gas at a flow rate of 12 L/min. The data were collected in positive ESI mode, with a mass range scanned from 100 to 1000 m/z. Two reference masses, 121.0509 m/z ($C_5H_4N_4$) and 922.0098 m/z ($C_{18}H_{18}O_6N_3P_3F_{24}$), were continuously administered to monitor and verify the accuracy of mass detection for the compounds. The QC sample was prepared from the pooled aliquots of all coffee brew samples and was injected at the beginning, middle, and end of the run to assess the performance and ensure reproducibility of the system.[29]

### *Statistical analyses*

All data were analysed with IBM SPSS v27 for Windows (IBM SPSS Inc., Chicago, IL) and presented as mean ± standard deviation (SD). A one-way ANOVA was used to analyse sample differences, followed by Tukey's HSD Test at a 95% confidence level ($p < 0.05$). Antioxidant activities were analysed using Excel 2021 software (Microsoft Inc., USA) to calculate the coefficient of determination ($r^2$). Agilent Mass Hunter Qualitative Analysis B.05.00 software (Agilent Technologies, Santa Clara, CA, USA) was used to process and analyse mass spectrometry data. The data were analysed using Metaboanalyst 6.0 for multivariate analyses, such as Principal Component Analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and a heat map. Additionally, Variable Importance in Projection (VIP) values and fold change (FC) were used to analyse significant alterations in metabolites between groups.

## Results and discussion

### *Antioxidant activities of Coffea liberica brews*

In this study, the reducing capacity of *Coffea liberica* brews was evaluated for green and roasted beans, at various roasting levels (light, medium, dark, and very dark roasted), employing the Folin-Ciocalteu method for total phenolic content, total flavonoid content, and the FRAP methods. Furthermore, free-radical scavenging properties were evaluated using the ABTS, DPPH, and $H_2O_2$ scavenging assays. The results are shown in Table 1. This study comprehensively evaluated the antioxidant activity of *Coffea liberica* through various antioxidant assays. This arises from the fact that each assay functions distinctly, utilising diverse reaction mechanisms such as hydrogen atom transfer (HAT), single electron transfer (SET), and metal chelation. They exhibit varied responses to hydrophilic, lipophilic, pigments, and Maillard reaction products. As a result, this approach enhances the evaluation of antioxidants and increases both the accuracy and reliability of the findings.[30,31].

The total phenolic content of the coffee brews is shown in Table 1. This assay was employed to quantify the secondary metabolites that are detected in plants and plant-derived foods and beverages.[23] Results showed that the total phenolic content differed significantly across roasting levels. The highest total phenolic content was observed in the light-roasted brew (38.27±0.51 mg/g GAE), followed by medium, very dark, and dark-roasted coffees, while green bean brew showed the lowest (29.76 ± 0.54 mg/g GAE).

**Table 1.** The antioxidant activities of coffee brews prepared from green and roasted *Coffea liberica* at varying roasting levels.

| | Antioxidants activities | | | | | |
|---|---|---|---|---|---|---|
| Coffee brew | Total phenolic content **(mg/g GAE)** | Total flavonoid content **(mg/g QE)** | DPPH **($IC_{50}$, $\mu$g/mL)** | FRAP **(mg/g TE)** | ABTS **(% RSA)** | $H_2O_2$ **(% RSA)** |
| Green bean | 29.76±0.54$^{d}$ | 3.87 ±0.03$^{b}$ | 174.72 ±0.91$^{b}$ | 169.23± 0.20$^{b}$ | 22.99 ±0.89$^{d}$ | 15.85± 0.54$^{a}$ |
| Light-roasted | 38.27±0.51$^{a}$ | 3.96 ±0.03$^{a}$ | 146.14 ±0.94$^{a}$ | 199.48 ±0.28$^{a}$ | 32.55± 0.64$^{a}$ | 13.16 ±0.15$^{b}$ |
| Medium-roasted | 32.28±0.85$^{c}$ | 2.60± 0.02$^{c}$ | 247.94 ±3.34$^{d}$ | 164.27 ±0.40$^{c}$ | 24.24 ±0.60$^{c}$ | 11.68± 0.20$^{c}$ |
| Dark-roasted | 32.95±0.67$^{c}$ | 2.45 ±0.04$^{d}$ | 243.77 ±3.57$^{d}$ | 145.01 ±0.43$^{d}$ | 27.60 ±0.32$^{b}$ | 8.43 ±0.25$^{d}$ |
| Very dark-roasted | 34.02±0.60$^{b}$ | 2.62 ±0.05$^{c}$ | 224.89 ±4.59$^{c}$ | 136.80 ±0.52$^{e}$ | 26.79 ±0.75$^{b}$ | 3.62± 0.08$^{e}$ |

The roasting process induces thermal degradation of phenolic compounds, which are susceptible to high temperatures, resulting in a decrease in total phenolic content as roasting intensity increases.[12,32] This trend was also similar to several research findings.[1,32,33] Roasting can decrease specific phenolic compounds, such as chlorogenic acids, while simultaneously initiating complex chemical reactions that may improve antioxidant potential by releasing bound phenolics and forming Maillard reaction products. This may explain the higher total phenolic content observed in light-roasted beans relative to green beans.[34] Notably, the results obtained were lower than those reported by Seow et al.[8] The reason for this could be the variation in the extraction method employed. The preparation conditions, including the temperature of the water, duration of the extraction, levels of roasting, and other parameters, impacted the antioxidant activity values.[35] The results suggest that polyphenolic compounds are influenced by coffee roasting, where light roasting enhances the level of bioactive compounds, before degradation at high roasting levels.[33]

The total flavonoid content values were in the range of 2.45–3.96 mg/g QE. The light-roasted brew exhibited the highest flavonoid content (3.96 ± 2.78 mg/g QE), followed by green bean, medium, and very dark, while the dark-roasted brew had the lowest TFC content (2.45 ± 3.71 mg/g QE). A study by Lee et al.[36] reported that the coffee roasted at the lowest temperature exhibited the highest total flavonoid content. Additionally, Cho et al.[37] also found that the content of flavonoids was the highest in light roasting, followed by green beans, medium, and dark-roasted. However, in this study, very dark roasting showed higher total phenolic and total flavonoid content than medium and dark roasting. According to Ripper et al.,[38] as roasting temperature and duration increase, certain antioxidants may diminish due to thermal degradation, while other antioxidant compounds may be produced or intensified through the Maillard reaction. Furthermore, the presence of Maillard reaction products, especially melanoidins, has caused an increase in the content of total phenols to some extent.[39] Poljsak et al.[40] suggested that thermal processing can modify the structure of existing antioxidants or facilitate the formation of new ones.

Antioxidant compounds result in either a stable or enhanced overall antioxidant capacity of coffee beans in spite of the potential depletion of certain active compounds. Similarly, Mestanza et al.[41] also observed that the initial increase in phenolic compounds and antioxidant capacity of coffee beans during roasting is due to temperature, which promotes the release and formation of antioxidant compounds, with variations depending on the variety.

According to the DPPH assay, the roasting levels of the coffee brews significantly influenced the antioxidant activity. The $IC_{50}$ values for coffee brews ranged from 146.14 $\mu$g/mL to 247.94 $\mu$g/mL, with light-roasted coffee (146.14±0.94 $\mu$g/mL) brew exhibiting the highest percentage of inhibition, which was indicated by the lowest $IC_{50}$ value, followed by green bean, very dark, and dark, while the medium-roasted coffee brew exhibited the highest $IC_{50}$ value (243.77±3.57 $\mu$g/mL). This finding aligns with the report by Cho et al.[37] and Herawati et al.,[42] which indicated that light-roasted coffee exhibited the highest DPPH value. Furthermore, a previous study demonstrated that variations in antioxidant activity, including both increases and decreases, correlate with higher degrees of roasting.[43] This phenomenon can be attributed to the initial changes that occur during the roasting process, wherein lower molecular weight phenolic compounds are released, thereby enhancing antioxidant activity. Moreover, the degradation and structural alterations of these bioactive compounds may take place during more advanced stages of roasting, leading to a modification in antioxidant activity.[44]

Meanwhile, for the FRAP assay, the light-roasted brew showed the significantly highest reducing power (199.48±0.28 mg/g TE). The reducing power exhibited a significant decrease with increasing roasting levels, with very dark-roasted coffee brew showing the lowest reducing power (136.80±0.52 mg/g

TE). Our results are in agreement with previous findings, where light-roasted coffee exceeded the green beans and decreased with the increment in roasting levels.[1,33] This is due to the free radical scavenging capacity of coffee's chlorogenic acid and melanoidins, which is diminished with higher roasting degrees.[45]

Similar to FRAP, the statistical analysis demonstrated a significant effect between light-roasted and the other coffee brews for ABTS. The light-roasted exhibited the highest percentage of radical scavenging activity (32.55 ± 0.64%), indicating higher extraction of active compounds against $ABTS^+$ radical cation in contrast to the green bean, which had the lowest radical scavenging activity (22.99 ± 0.89%). No significant differences were observed between dark and very dark-roasted coffee brews. The results were similar to a previous study, where roasted beans exhibit higher antioxidant activity than green beans.[46] Moreover, van der Werf et al.[47] indicated that the ABTS radical scavengers generated in roasted coffee are phenolic derivatives rather than Maillard derivatives, and that enhanced antioxidant activity results from lower roasting temperatures.

Hydrogen peroxide ($H_2O_2$) is a reactive oxygen species (ROS) that can function as an oxidant or a reducing agent. Although it has minimal reactivity, it can be harmful at elevated concentrations.[48] The present study demonstrated that the *Coffea liberica* brews were able to scavenge the ROS. The percentage of inhibition was in the range of 3.62–15.85%, where green beans demonstrated significantly the highest radical scavenging activity (RSA) (15.85 ± 0.54%), and it decreased when the roasting levels increased. Previous studies by Alonso-Salces et al.[49] and Bicho et al.[50] demonstrated that chlorogenic acids, cinnamoyl−amino acid conjugates, and caffeine are higher in green beans, which may improve the scavenging ability of reactive oxygen species (ROS). The findings highlight the significant impact of roasting conditions on the antioxidant capacity of coffee, suggesting that green and lightly roasted coffees may offer more effective protection against oxidative stress caused by $H_2O_2$. Besides that, Rodrigues et al.[51] reported that coffee had a comparable $H_2O_2$ scavenging capacity to that of 5-caffeoylquinic acid and higher than 5-hydroxymethylfurfural.

The findings indicated that light-roasted coffee enhances the formation or alteration of specific bioactive compounds during the initial roasting stages, exhibiting significantly greater antioxidant activity compared to other roasted coffees. Furthermore, it is also suggested that the degradation of the most bioactive compounds in coffee may occur during the latter phases of the roasting process.

### *Metabolite profiling of Coffea liberica across different roasting levels*

Metabolomics data is highly dimensional and massive, requiring multiple analyses to accurately identify different metabolites, which involves combining univariate and multivariate methods of analysis.[52] In total, 95 metabolites were significantly ($p < 0.05$) detected in the *Coffea liberica* brew and were categorized according to the compound classes, such as phenolic acids (24 metabolites), flavonoids (13 metabolites), alkaloids (4 metabolites), amino acids (12 metabolites), heterocyclic compounds (23 metabolites), organic acids (6 metabolites), and others (13 metabolites), as shown in Table 2.

In this study, various phenolic acids were identified. Chlorogenic acids (CGA) and their derivatives, including coumarin, pyrocatechol, 1-O-caffeoylquinic acid, 3-feruloyquinic acid, 3-feruloyl-1,5-quinolactone, 3-O-Caffeoyl-4-O-methylquinic acid, 3-O-p-Coumaroylquinic acid, 4-Caffeoyl-1,5-quinolactone, and cryptochlorogenic acid. CGA is the main hydrocinnamic acid present in coffee, formed through the esterification of quinic acid and certain trans-cinnamic acids (caffeic acid, coumaric acid, ferulic acid).[53] Besides being the most abundant, it plays a crucial role in shaping the flavour profile of bitter and astringent, which affects the overall quality of the coffee.[54] Furthermore, Zhai et al.[55] reported that the primary CGA found in coffee includes caffeoylquinic acids, dicaffeoylquinic acids, p-coumaroylquinic acids, and feruloylquinic acids.

Proteins are composed of various amino acids, which are primarily responsible for the unique aroma generated during the roasting process. Amino acids and reducing sugars serve as precursors in the development of aroma and flavours, participating in Maillard reactions that result in the formation of various volatile compounds, thereby enhancing the flavour and colour of coffee brew.[56] As the temperature of roasting increases, amino acid levels decrease, as demonstrated by 4-aminophenol, L-Phenylalanine, and N-Methylphenylethanolamine. Alkaloids, which are nitrogen-containing compounds, contribute to the bitterness of coffee and exhibit numerous biological properties.[18] In this study, caffeine, arecoline, and paraxanthine were detected in *Coffea liberica* brews. Notably, this study also identified an abundance of heterocyclic compounds, including pyrazine, pyridines, azoles, and furans, as well as their isomeric forms, that are essential for the development of coffee aroma and flavour in the coffee. For example, pyrazines are the primary class that contributes to

**Table 2.** Fold change differences of the metabolite detected in the *Coffea liberica* brews with different roasting levels, ANOVA (p<0.05).

| Name of compound | Fold change | | | | p-value |
|---|---|---|---|---|---|
| | Light vs Green | Light vs Medium | Light vs Dark | Light vs Very dark | |
| **Phenolic acid and derivatives** | | | | | |
| Coumarin-4-carboxylic acid | -1.66 | 2.52 | –1.58 | 2.52 | $3.70E^{-04}$ |
| 3-Feruloylquinic acid | NA | 1.03 | 1.58 | 2.08 | $7.00E^{-17}$ |
| 3-O-Caffeoyl-4-O-methylquinic acid | NA | 1.12 | 1.70 | 2.11 | $2.32E^{-17}$ |
| 6-Hydroxy-R-acenocoumarol | 2.35 | NA | NA | NA | $1.68E^{-16}$ |
| 1-O-Caffeoylquinic acid | NA | 1.80 | 2.71 | 2.92 | $7.79E^{-18}$ |
| Sinapyl alcohol | 3.59 | 1.02 | 1.59 | 1.02 | $6.43E^{-05}$ |
| Cryptochlorogenic acid | NA | 1.61 | 2.70 | 2.89 | $1.81E^{-13}$ |
| 4-Caffeoyl-1,5-quinolactone | 2.42 | NA | 1.04 | 1.20 | $5.91E^{-09}$ |
| 3-O-Caffeoyl-1-O-methylquinic acid | NA | 1.40 | 3.43 | 3.27 | $2.94E^{-08}$ |
| Gerberinol | –1.02 | 1.42 | 2.00 | 1.30 | $3.55E^{-07}$ |
| 3-O-p-Coumaroylquinic acid | NA | 1.03 | 2.46 | 3.63 | $7.42E^{-09}$ |
| 4-Feruloyl-1,5-quinolactone | 2.38 | NA | NA | NA | $1.53E^{-08}$ |
| 4-Ethyl-2,6-dimethoxyphenol | 2.52 | NA | NA | NA | $8.28E^{-07}$ |
| 3-Hydroxycoumarin | NA | 1.66 | 2.41 | 2.64 | $7.26E^{-12}$ |
| Caffeic acid | NA | 1.79 | 3.09 | 2.83 | $3.49E^{-11}$ |
| 3-Feruloyl-1,5-quinolactone | 2.35 | NA | NA | NA | $8.97E^{-12}$ |
| 5-Caffeoylquinic acid | NA | 1.42 | 2.18 | 2.41 | $5.04E^{-15}$ |
| Coumarin | NA | 1.31 | 3.35 | 3.36 | $5.29E^{-13}$ |
| 3-O-Caffeoylshikimic acid | 2.34 | NA | NA | 1.29 | $5.03E^{-08}$ |
| Scopoletin | 2.82 | NA | NA | NA | $4.48E^{-04}$ |
| 3-Caffeoyl-1,5-quinolactone | 2.32 | NA | NA | 1.46 | $1.63E^{-05}$ |
| 2,6-Dimethoxy-4-methylphenol | NA | –2.89 | –2.50 | –2.67 | $7.88E^{-06}$ |
| 4-Methylumbelliferone | 1.79 | NA | 1.57 | 2.35 | $6.41E^{-12}$ |
| Pyrocatechol | 2.45 | 3.29 | 4.78 | 4.57 | $4.80E^{-11}$ |
| **Flavonoids and derivatives** | | | | | |
| Amentoflavone | NA | –2.46 | –3.55 | –2.43 | $9.90E^{-07}$ |
| (-)-epicatechin-3'-O-glucuronide | 3.13 | –2.45 | –1.51 | –1.84 | $1.50E^{-04}$ |
| 4',5,6,7,8-Pentahydroxy-3'-methoxyflavone | 1.95 | –4.52 | –4.40 | –4.68 | $1.51E^{-09}$ |
| 8-Methoxygravelliferone | 2.43 | NA | NA | NA | $1.10E^{-06}$ |
| 6-Hydroxyluteolin 6-xyloside | NA | –2.51 | –2.70 | –2.50 | $3.24E^{-06}$ |
| Epicatechin | –2.48 | NA | NA | NA | $2.16E^{-07}$ |
| 5-Hydroxy-3,3',4',6,7,8-hexamethoxyflavone | NA | 2.90 | 2.90 | 2.90 | $1.10E^{-08}$ |
| Isorheagenine | NA | 1.01 | 1.69 | 2.10 | $3.22E^{-14}$ |
| Quercetin | NA | –2.39 | –2.54 | –2.41 | $1.49E^{-08}$ |
| Theaflavic acid | NA | 1.76 | 3.25 | 3.75 | $7.55E^{-12}$ |
| Isorhamnetin | NA | –2.47 | –2.54 | –2.41 | $2.78E^{-07}$ |
| Isomangiferin | 2.43 | 3.43 | 4.19 | 3.93 | $7.04E^{-10}$ |
| 4',7-Di-O-methylcatechin | 2.39 | 2.39 | 2.39 | 2.39 | $1.21E^{-13}$ |
| **Alkaloids** | | | | | |
| Arecoline | 2.49 | 1.29 | 1.02 | 1.45 | $6.86E^{-05}$ |
| Paraxanthine | 2.32 | 1.06 | 1.25 | 2.32 | $2.82E^{-13}$ |
| 7',8'-Dihydro-8'-hydroxycitraniaxanthin | NA | –1.32 | –1.61 | NA | $7.04E^{-06}$ |
| Enprofylline | 2.84 | NA | –1.57 | NA | $8.02E^{-09}$ |
| **Amino acids and derivatives** | | | | | |
| 4-Aminophenol | 5.23 | –1.56 | –1.67 | –1.48 | $8.23E^{-11}$ |
| 4-Hydroxynonenal glutathione | 2.88 | NA | NA | 1.18 | $1.20E^{-04}$ |
| L-Tryptophan | 2.73 | NA | NA | NA | $9.49E^{-03}$ |
| L-Aspartic acid | –2.73 | NA | NA | NA | $3.54E^{-08}$ |
| L-Valine | –1.07 | NA | NA | NA | $1.84E^{-09}$ |
| L-Phenylalanine | NA | –2.37 | –2.38 | –2.60 | $9.14E^{-09}$ |
| 5-Aminopentanal | 3.96 | –1.00 | –1.13 | –1.09 | $3.87E^{-05}$ |
| 4-Hydroxybenzylamine | NA | –2.33 | –2.35 | –2.37 | $2.53E^{-15}$ |
| Cinnamoylglycine | 3.86 | NA | 1.03 | 1.09 | $2.71E^{-03}$ |
| 4-ethylamino-6-isopropylamino-1,3,5-triazin-2-ol | 2.44 | 1.71 | 3.80 | 4.05 | $2.88E^{-11}$ |
| N-Methylphenylethanolamine | 2.43 | –2.09 | –1.69 | –1.83 | $9.49E^{-06}$ |
| Ergothioneine | 2.41 | NA | 2.14 | 2.61 | $4.36E^{-06}$ |

(Continued.)

**Table 2.** Continued.

| Name of compound | Fold change | | | | p-value |
|---|---|---|---|---|---|
| | Light vs Green | Light vs Medium | Light vs Dark | Light vs Very dark | |
| **Heterocyclic compounds** | | | | | |
| 2,6-Dimethylpyridine | 7.54 | –1.20 | NA | NA | 1.11E$^{-03}$ |
| 2-Acetylpyrrolidine | 3.92 | –1.27 | –1.06 | NA | 3.20E$^{-06}$ |
| 2-Methoxy-3-methylpyrazine | 3.64 | –1.32 | –1.17 | NA | 1.22E$^{-03}$ |
| 2-Propionylpyrrole | 3.34 | –1.46 | –1.56 | –1.43 | 1.80E$^{-04}$ |
| 8-Hydroxy-7-methoxy-2H-1-benzopyran-2-one | 3.21 | NA | NA | NA | 1.69E$^{-03}$ |
| Methoxypyrazine | 3.06 | –3.05 | –2.69 | –3.08 | 5.06E$^{-08}$ |
| 5-Ethyl-2-methylpyridine | 2.65 | –2.06 | –2.21 | –2.18 | 7.05E$^{-12}$ |
| 2-Piperidone | 2.63 | NA | NA | NA | 3.00E$^{-04}$ |
| 2-Isopropyl-5-methoxypyrazine | 2.60 | NA | NA | 1.13 | 2.45E$^{-05}$ |
| 2-Ethoxy-3-methylpyrazine | 2.52 | NA | NA | NA | 2.50E$^{-04}$ |
| Trimethylpyrazine | 2.48 | NA | NA | NA | 4.74E$^{-05}$ |
| 2-Heptyl-4,5-dimethylthiazole | 2.42 | –2.14 | –1.99 | –2.19 | 1.57E$^{-03}$ |
| 3-Ethylpyridine | 2.40 | –1.91 | –2.21 | –2.35 | 4.72E$^{-05}$ |
| 4-Butyl-5-propylthiazole | 2.32 | 1.51 | NA | NA | 2.60E$^{-04}$ |
| 2-Acetyl-4-methylpyridine | 2.05 | NA | 1.07 | 1.23 | 3.55E$^{-08}$ |
| 2-Ethyl-3-methylpyrazine | 1.88 | –2.57 | –2.85 | –2.86 | 9.07E$^{-05}$ |
| 2-Acetyl-1-ethylpyrrole | NA | –2.46 | –2.35 | –2.34 | 8.78E$^{-12}$ |
| 2-Isopropyl-3,5-dimethoxy-6-methylpyrazine | NA | –2.41 | –2.53 | –2.52 | 9.65E$^{-06}$ |
| 1-Methylpyrrolo[1,2-a]pyrazine | NA | –1.57 | –1.35 | –1.20 | 5.53E$^{-08}$ |
| 2-Acetyl-3-ethylpyrazine | 1.47 | NA | NA | NA | 1.03E$^{-02}$ |
| 1,2-dimethyl-5,6-dihydroxy-tetrahydroisoquinoline | 3.43 | NA | NA | NA | 8.47E$^{-04}$ |
| 2-[(Isopropylthio)methyl]furan | 2.38 | NA | NA | NA | 3.69E$^{-11}$ |
| Gentiatibetine | 2.43 | NA | NA | NA | 5.09E$^{-09}$ |
| **Organic acids** | | | | | |
| Methylsuccinic acid | –2.83 | –1.87 | –1.47 | –1.58 | 2.52E$^{-05}$ |
| Monomethyl glutaric acid | 2.76 | | NA | –1.01 | 1.87E$^{-05}$ |
| 2-Furoic acid | 2.62 | –1.73 | –1.27 | –1.06 | 1.22E$^{-06}$ |
| Shikimic acid | –2.59 | –2.64 | –3.44 | –2.60 | 1.86E$^{-05}$ |
| Citric acid | NA | NA | 1.06 | 1.61 | 1.43E$^{-04}$ |
| Quinic acid | 2.05 | NA | NA | NA | 9.02E$^{-10}$ |
| **Other compounds** | | | | | |
| 3-Aminosalicylic acid | 2.58 | 3.54 | 2.58 | 2.58 | 3.96E$^{-08}$ |
| Galactitol | –2.73 | 1.08 | 1.47 | NA | 2.35E$^{-11}$ |
| 2-Hydroxyestradiol-3-methyl ether | 2.66 | NA | NA | NA | 3.94E$^{-04}$ |
| 2,6-Diethylaniline | 2.85 | 2.65 | 4.78 | 2.85 | 6.06E$^{-06}$ |
| Indoleacetic acid | 2.49 | –2.73 | –2.51 | –2.74 | 1.89E$^{-07}$ |
| Indole-3-methanamine | 2.43 | –1.02 | NA | NA | 2.92E$^{-05}$ |
| Cyclohexamine | 2.35 | –3.49 | –3.73 | –3.69 | 3.54E$^{-11}$ |
| 4-Hydroxy-2H-pyran-3-carboxaldehyde | 2.22 | NA | NA | NA | 2.86E$^{-08}$ |
| Carboxy-leukotriene B4 | 2.16 | 1.10 | 2.57 | 2.82 | 1.11E$^{-06}$ |
| 2-Hydroxy-3-methyl-9H-carbazole | –1.82 | NA | –1.09 | NA | 1.30E$^{-04}$ |
| 2-Hydroxyadipic acid | –1.46 | NA | NA | NA | 6.81E$^{-07}$ |
| 2,3-Dihydroxyvaleric acid | 1.22 | –5.05 | –5.54 | –5.36 | 3.62E$^{-06}$ |
| 3-Methoxybenzenepropanoic acid | NA | –2.43 | –2.33 | –2.47 | 1.76E$^{-09}$ |

*NA, not available

the distinctive aroma of coffee by imparting earthy, musty, woody, and papery notes.[19]

To further compare the metabolite profiles of *Coffea liberica* subjected to different roasting levels, several unsupervised and supervised multivariate statistical analyses were conducted. The variability of metabolites in *Coffea liberica* across the roasting levels was demonstrated by the PCA plot, as shown in Fig. 1. The two primary principal components (PC1 and PC2) represented a total of 81.2% of the variability within each group, which indicates a distinct discrimination based on the roasting level of the coffee bean. In accordance with the two-dimensional distribution of the unsupervised multivariate PCA plot, a clear separation was observed on green bean and light-roasted coffee brews, significantly highlighting the

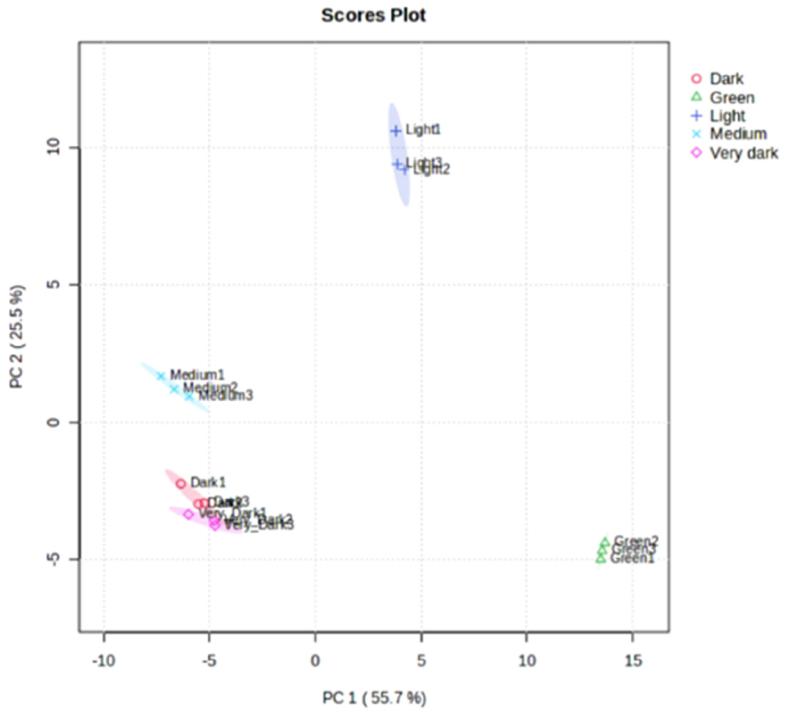



**Fig. 1.** The PCA score plot of coffee brews of green bean and different roasting levels (light, medium, dark, and very dark roasted).

differences in their metabolite profile, while medium, dark, and very dark-roasted coffee brews were dispersed among the groups.

A heatmap hierarchical clustering analysis, as shown in Fig. 2, was used to visually show the top 25 most significant metabolites ($p < 0.05$) accumulated in green beans and different roasting levels. The bright red colour and bright blue colour represent the high and low abundance of the metabolites, respectively, while the y-axis dendogram represents the hierarchical clustering of metabolites based on their abundance. As illustrated by the heatmap, the CGA derivatives such as 3-Feruloyquinic acid, 1-O-Caffeoylquinic acid, 3-O-Caffeoyl-4-O-methylquinic acid, coumarin, and caffeic acid showed a red colour in green beans and faded, shifting towards blue, indicating low abundance as the roasting levels progressed from medium to very dark. CGAs are the most abundant in green beans, their content gradually declines after exposure to intense roasting.[2,43] This may be the reason why the hydrogen peroxide scavenging assay showed the highest values in green beans, which aligns with the findings reported by Sualeh et al.[57]

Furthermore, Isomangiferin, pyrocatechol, 4',7-Di-O-methylcatechin, and paraxanthine were the highest in the light-roasted brew and decreased when roasting levels increased. It is noteworthy that these compounds exhibited a high fold change value when the light-roasted brew was compared with others, which potentially enhances the bioactivity of the light-roasted coffee. CGA demonstrates various health benefits, including significant antioxidant activity, hepatoprotection, hypoglycemic effects, anticarcinogenic, antibacterial, and anti-inflammatory properties. They also contribute to a reduced risk of major diseases such as cardiovascular disease, type 2 diabetes, and Alzheimer's disease.[58,59] Isomangiferin, being a part of the xanthone family, demonstrates considerable potential as a preventive agent for dietary diseases, as evidenced by a variety of antioxidant assays.[60] Furthermore, this flavonoid also demonstrates potential in the prevention of various aspects of cognitive decline.[61] A study reported by Funakoshi-Tago et al.[62] stated that coffee contains a high amount of pyrocatechol that exhibits a strong anti-inflammatory effect. The decomposition of CGA in green beans throughout the roasting process produces quinic and caffeic acids. According to a previous study, pyrocatechol is primarily produced through the breakdown of caffeoylquinic acids that are derived from quinic and caffeic acid.[63]

Table 2 summarizes the discriminant metabolites based on the $\log_2$ fold-change (FC) values of light-roasted against green bean, medium-roasted, dark-roasted, and very dark-roasted. The FC value $> 1$ indicates higher abundance, while FC $< 1$ indicates lower abundance. Given the

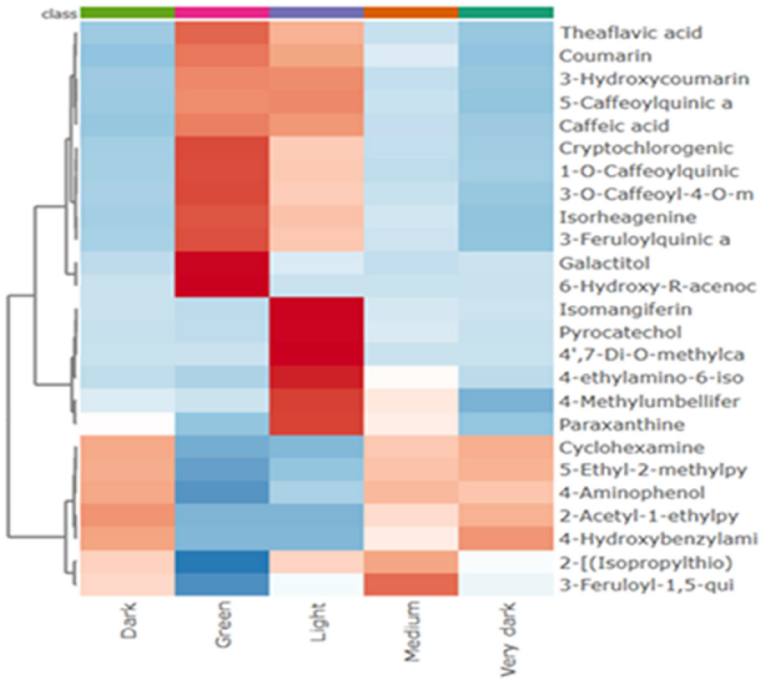
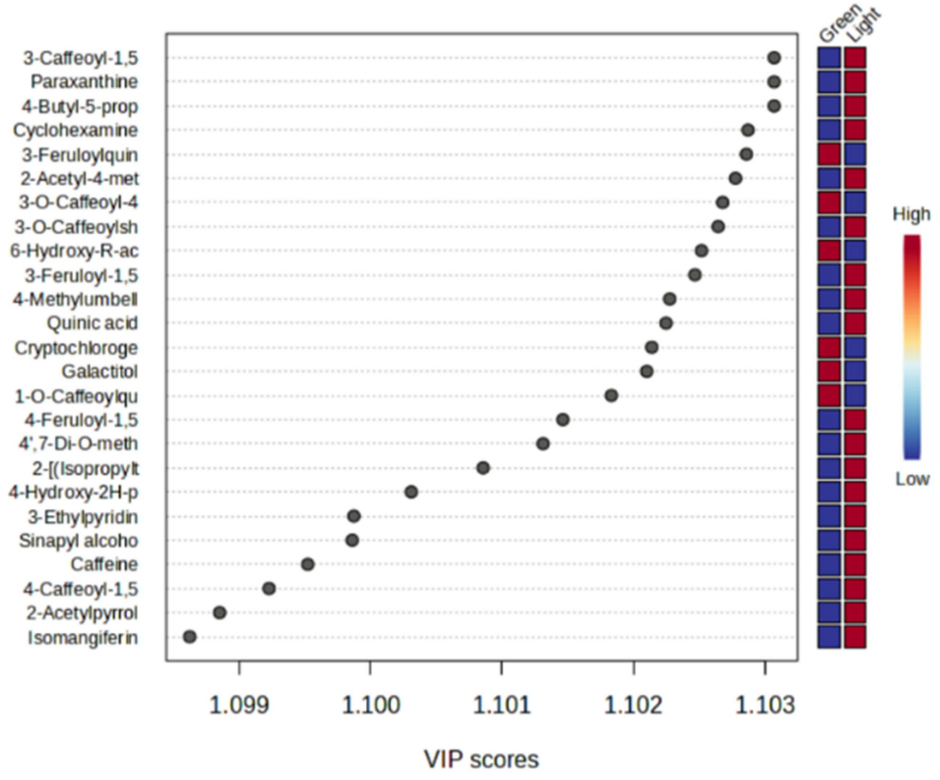


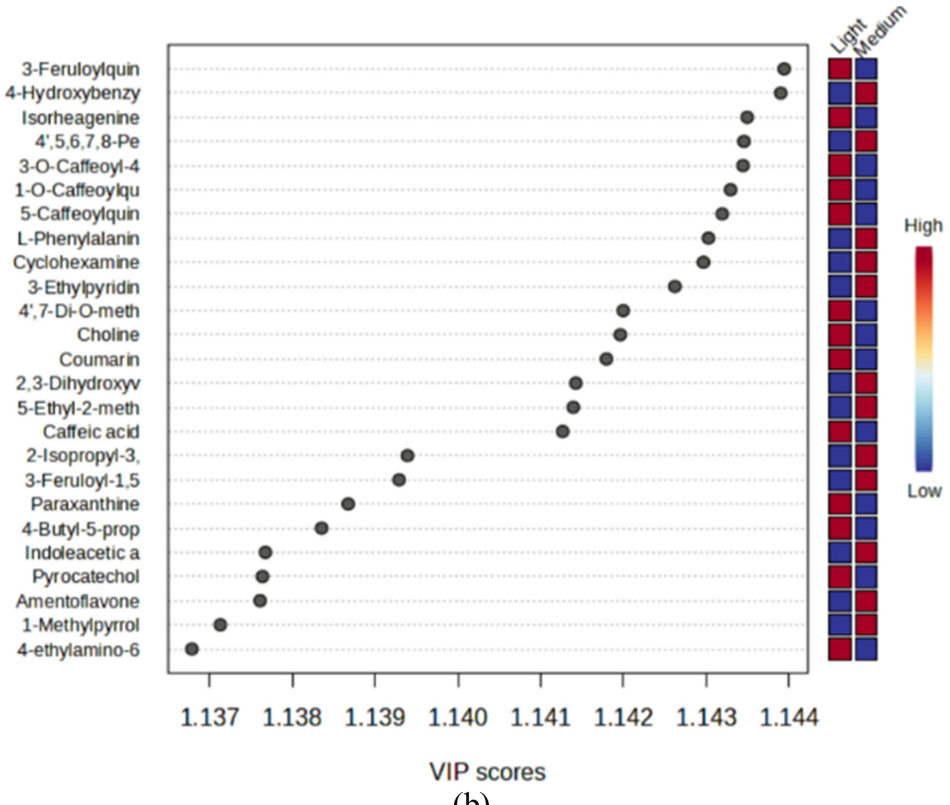

**Fig. 2.** The heatmap hierarchical analysis of green beans and roasted coffee brews with the top 25 discriminating metabolites.

significantly high antioxidant activity observed in this study, a comparison was made between the light-roasted coffee brew and others to explore the alterations in metabolites throughout the early and advanced phases of roasting. Several compounds were identified to be higher in abundance in light-roasted coffee as compared to other roasting levels. These included phenolic acid and its derivatives (pyrocatechol, coumarin, 3-O-Caffeoyl-1-O-methylquinic acid, 3-O-Caffeoyl-4-O-methylquinic acid, 1-O-caffeoylquinic acid, cryptochlorogenic acid, 5-caffeoylquinic acid, 4-Methylumbelliferone), flavonoids (isorheagenine, theaflavic acid, isomangiferin, 4',7-Di-O-methylcatechin), and alkaloids (paraxanthine). These compounds demonstrated a higher abundance based on FC value as the roasting levels progressed from light to very dark. In contrast, several heterocyclic compounds associated with the Maillard reaction, such as pyrazine derivatives, for instance, 2-Methoxy-3-methylpyrazine, methoxypyrazine, as well as 2-[(Isopropylthio)methyl]furan, and azole derivatives, exhibited a lower abundance based on FC values when going from light to very dark roasted. As described by Rocchetti et al.,[64] the formation of compounds in roasted coffee is primarily associated with Maillard or non-enzymatic browning, Strecker degradation, the degradation of individual amino acids, trigonelline, sugars, polyphenols, and lipids, as well as the interactions among all intermediate products. The FC values indicate that thermal degradation leads to a reduction in specific bioactive compounds and the formation of Maillard reaction products.

Supervised OPLSDA was conducted to obtain variable importance in projection (VIP) values as shown in Fig. 3 by comparing different roasting levels (Light vs. Green, Light vs. Medium, Light vs. Dark, Light vs. Very dark) in order to analyse significant changes in metabolites that occurred during the roasting process. The VIP value serves as a parameter to illustrate the cumulative effect of distinct metabolites on a model.[65] According to,[66] metabolites with VIP values exceeding one are considered significant, and this value was employed as an indicator. In discriminating between light-roasted with green bean and other roasted brews, it is worth noting that 3-caffeoyl-1,5-quinolactone, 3-feruloylquinic acid, paraxanthine, cryptochlorogenic acid, 3-O-caffeoyl-4-O-methylquinic acid, 1-O-caffeoylquinic acid, and 5-caffeoylquinic acid were the key discriminant metabolites (VIP≥1.0) of light-roasted brew with other roasting levels. A study by[67] reported that the production of these compounds was significantly influenced by roasting levels and suggested that a light-medium roast generates the highest concentration of lactones in coffee, whereas darker roasts yield a lower amount. In coffee, chlorogenic acid lactones and chlorogenic acid derivatives constitute only 1% of the total phenolic content and exhibit a strong antioxidant potential.[68,69] Besides their contribution to bitter, sweet, or acidic taste, they also display a variety of bioactivities, including anti-inflammatory, antimicrobial, antihyperglycemic, and anticancer effects.[70]

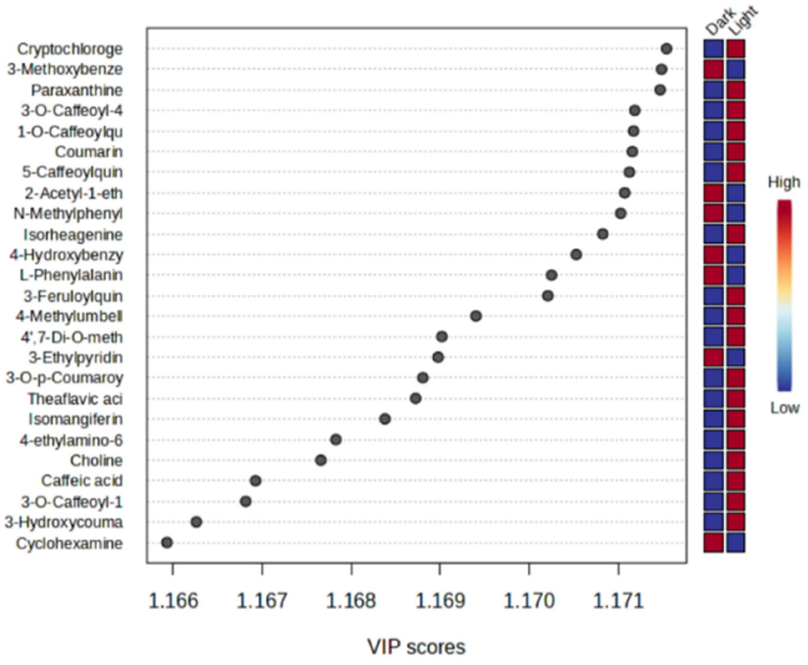



(a)

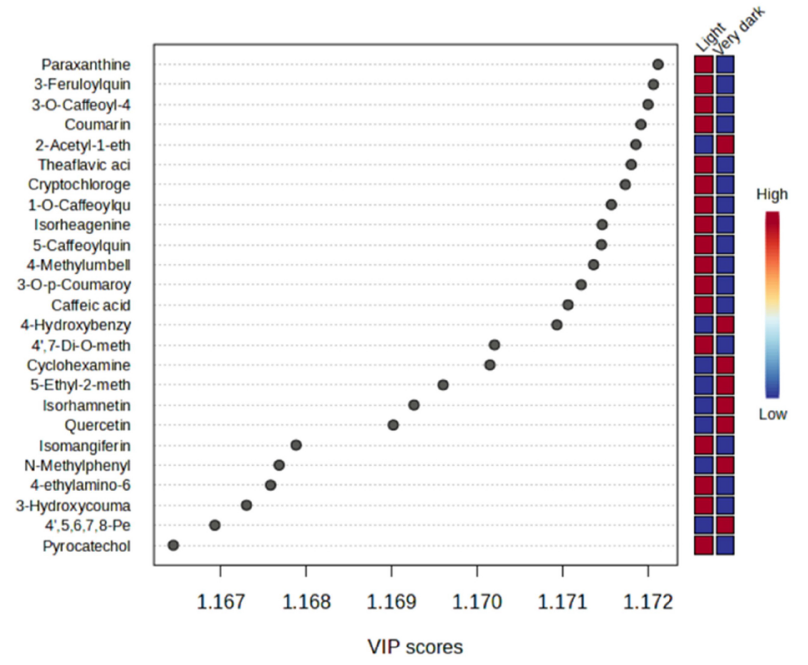



(b)

**Fig. 3.** Continue.




(c)




(d)

**Fig. 3.** Top 25 metabolites with the highest VIP scores (a) Light vs. Green, (b) Light vs. Medium, (c) Light vs. Dark, (d) Light vs. Very dark) with VIP $\geq$ 1.

Moreover, paraxanthine, a primary metabolite of caffeine, exhibits neuroprotective properties similar to caffeine.[71] These properties are essential for the survival of neurons in the central nervous system during the state of oxidative stress.[72] The availability of these bioactive metabolites in light-roasted coffee may enhance the antioxidant properties, suggesting that early roasting stages can facilitate the formation of beneficial compounds. In addition, 5-caffeoylquinic acid and caffeic acid were also detected in the roasted coffee brews. Previous studies have found that 5-caffeoylquinic acid is an effective

free radical scavenger and acts as a xanthine oxidase inhibitor, exhibiting rate constants that surpass those of typical antioxidants like Trolox and butylated hydroxytoluene (BHT) as well as a powerful, potent antioxidant.[73,74] Similarly, caffeic acid has also been reported to have important antioxidant activity.[75]

In summary, the findings showed that the antioxidant activity and metabolite composition of *Coffea liberica* were greatly affected by the roasting process. The potential of *Coffea liberica* as a functional food is further supported by the presence of key metabolites, including 3-O-Caffeoyl-4-O-methylquinic acid, cryptochlorogenic acid, 3-O-Caffeoyl-1-O-methylquinic acid, 1-O-caffeoylquinic acid, 5-caffeoylquinic acid, coumarin, caffeic acid, pyrocatechol, isorheagenine, isomangiferin, 4',7-Di-O-methylcatechin, and paraxanthine. These compounds may significantly contribute to the antioxidant properties. Compared to other coffee brews, light-roasted coffee has been shown to enhance the formation of this compound more effectively, likely as a result of reduced exposure to thermal degradation during the roasting process.

## Conclusion

In conclusion, the antioxidant activity of *Coffea liberica* was assessed at different roasting levels using multiple assays and the metabolites were profiled via LC/MS-QTOF, primarily focusing on the investigation of antioxidant activity and alterations in chemical composition resulting from the roasting process. The results showed that light-roasted coffee had the highest values for total phenolic acid, total flavonoid acid, DPPH, FRAP, and ABTS in comparison to other roasting levels. Additionally, metabolite profiling identified various metabolites, including phenolic acids, flavonoids, alkaloids, and their derivatives. The retention of compounds such as CGA and flavonoids emphasises the significant chemical distinctions between light-roasted and other samples. This implies that the bioactive compounds are most effectively maintained or enhanced through the initial roasting stage. The metabolomic findings from this study may serve as a significant reference for future investigations into health-promoting compounds in *Coffea liberica*. Further research should be focused on targeted metabolomics as well as the quantification of these bioactive compounds.

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## Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical clearance: The project was approved by the local ethical committee at Universiti Teknologi Mara (UiTM).

## Authors' contribution statement

N.A. acquired the data, performed the analysis, and drafted the manuscript. L.K.T. contributed to data interpretation, and performed revision and proofreading. M.Z.S., E.M.Y., and Y.H.S. contributed to revision and proofreading. M.S.R. contributed to data interpretation. L.J.S. performed data analysis and interpretation. E.K.S. conceived and designed the study, performed the analysis and interpretation, secured the grant funding, supervised the project, and contributed to revision and proofreading. All authors reviewed and approved the final manuscript.

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# التغيّرات المعتمدة على درجة التحميص في النشاط المضاد للأكسدة والملفات الأيضية لحبوب *Coffea liberica*: الكشف عن المركّبات الحيوية النشطة وخصائصها الوظيفية

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## الخلاصة

على الرغم من النشاط المضاد للأكسدة المرتفع الذي تم الإبلاغ عنه في حبوب *Coffea liberica* الخضراء، فإنّ الأبحاث المتعلقة بالتغيّرات في تحليل الطيف الأيضي عبر مستويات التحميص المختلفة ما تزال محدودة في الأدبيات. لذلك، هدفت هذه الدراسة إلى تقييم النشاط المضاد للأكسدة والملفات الأيضية لكلٍّ من الحبوب الخضراء ومستويات التحميص المختلفة لمستخلص *Coffea liberica*، وذلك باستخدام اختبارات متعددة للقدرة المضادة للأكسدة، وهي: إجمالي المحتوى الفينولي، إجمالي محتوى الفلافونويدات، اختبارDPPH ، واختبار القدرة الاختزالية للحديد(FRAP) ، واختبارABTS ، واختبار القدرة على اصطياد بيروكسيد الهيدروجين، بالإضافة إلى تحليل الكروماتوغرافيا السائلة المقترن بقياس الطيف الكتلي وزمن طيران الرباعي الأقطاب (LC/MS-QTOF) وقد أظهرت النتائج أن القهوة المحمّصة تحميصًا خفيفًا احتفظت بأعلى نشاط مضاد للأكسدة عبر معظم الاختبارات. كما تم تحديد 95 مستقلبًا ذا دلالة إحصائية، شملت فئات مختلفة من المركّبات مثل الأحماض الفينولية، والفلافونويدات، والأحماض الأمينية، والقلويدات، والمركّبات الحلقية غير المتجانسة، والأحماض العضوية، وغيرها. وكانت مشتقات حمض الكلوروجينيك والفلافونويدات والقلويدات هي المستقلبات الرئيسة ($p<0.05$) التي تميّز مستخلص *Coffea liberica* عبر مستويات التحميص المختلفة. وتُظهر هذه النتائج أن القهوة المحمّصة تحميصًا خفيفًا تمتلك القدرة على تعزيز تكوّن وتحوّر المركّبات الحيوية النشطة التي قد توفّر فوائد صحية، مما يبرزها كمصدر غذائي طبيعي ذي فوائد صحية محتملة.

**الكلمات المفتاحية:** النشاط المضاد للأكسدة، *Coffea liberica*، LC/MS-QTOF، التحليل الأيضي، مستويات التحميص.