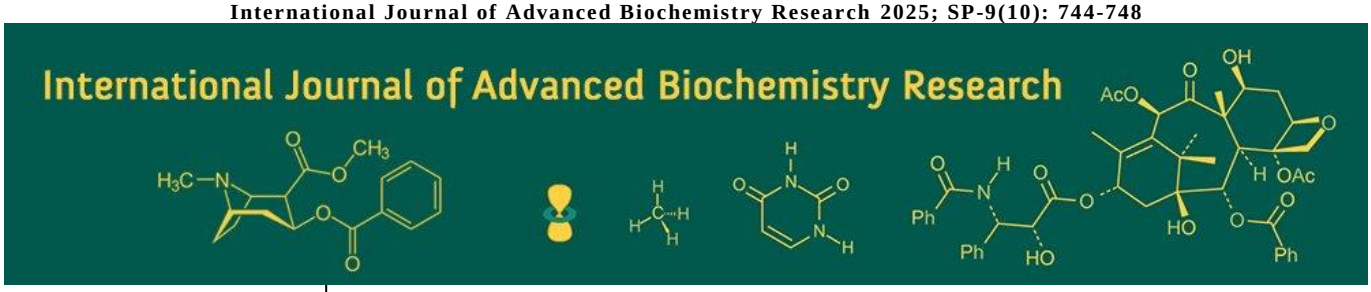

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Effect of coffee species and roasting levels on biochemical composition and antioxidant properties

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Abstract
Coffee species and roasting levels are major determinants of the biochemical composition and bioactive potential of coffee. This study investigated the effects of three coffee species-Arabica (*Coffea arabica*), Robusta (*Coffea canephora*) and Liberica (*Coffea liberica*) across light, medium and dark roasting levels on caffeine, chlorogenic acid, antioxidant activity and flavonoid content. Significant differences were observed among species and roasting treatments. Robusta exhibited the highest caffeine and antioxidant activity, Arabica showed intermediate levels and Liberica recorded lower caffeine content with appreciable antioxidant potential, particularly under light and medium roasting. Roasting progressively reduced chlorogenic acid and flavonoid content, with dark roasting causing the greatest losses. Significant species × roasting interactions indicated that intrinsic biochemical profiles and roast intensity jointly influence bioactive compound retention. These findings highlight the potential of Liberica, alongside Arabica and Robusta, for producing coffee with reduced caffeine content and enhanced antioxidant properties, providing valuable insights for specialty coffee development and health-focused consumption.

Keywords: Liberica, coffee species, roasting, caffeine, chlorogenic acid, antioxidant activity, flavonoids

Introduction
Coffee is among the most widely consumed beverages worldwide, valued for its stimulating effect, complex aroma and diverse flavor profile. Beyond consumption, coffee holds significant cultural and economic importance, representing one of the most traded agricultural commodities globally (Oliveira and Oliveira, 2023) [23]. Its biochemical composition is influenced by species, cultivation environment, post-harvest processing and particularly roasting, which plays a pivotal role in defining coffee quality.

Globally, coffee production exceeds 10 million tonnes annually, concentrated in tropical regions such as Brazil, Vietnam, Colombia and Ethiopia (Anon., 2023) [2]. India ranks seventh in production, with Karnataka contributing nearly three-fourths of the output, mainly Arabica and Robusta varieties (Anon., 2024) [3].

The genus *Coffea* includes over 120 species, yet commercial cultivation is dominated by *C. arabica*, *C. canephora* (Robusta)and to a lesser extent, *C. liberica* (Davis *et al*., 2012) [7]. Arabica is prized for its mild flavor, bright acidity and aromatic complexity (Silvarolla *et al*., 2004) [27], while Robusta offers higher caffeine content and a stronger, bitterer flavor (Farah, 2012) [10]. Liberica, though less widely cultivated, produces large beans with distinctive fruity and floral notes and demonstrates adaptability to hot, humid conditions (Davis *et al*., 2022) [8].

Roasting transforms green coffee beans into chemically and organoleptically distinct products through complex reactions, including Maillard reactions, Strecker degradation and pyrolysis. These reactions influence caffeine, chlorogenic acids, flavonoids and other bioactive compounds, affecting acidity, bitterness and antioxidant potential (Ruosi *et al*., 2012; Fadai *et al*., 2017) [26, 9]. Roast level profoundly modulates these compounds: light roasts retain higher levels of thermolabile bioactives, while dark roasts promote degradation and formation of new compounds (Stiefel *et al*., 2022; Perdana *et al*., 2018) [28, 24].

Despite extensive research on Arabica and Robusta, studies examining the combined effects of coffee species and roasting on biochemical composition, including Liberica, remain limited. Understanding these interactions is crucial for optimizing bioactive compound retention and developing coffee products with tailored health benefits.

Therefore, the present study aimed to investigate the effects of three coffee species (*C. arabica*, *C. canephora*, *C. liberica*) and roasting levels (light, medium, dark) on key biochemical parameters, including caffeine, chlorogenic acid, total antioxidant activity and flavonoid content. This work emphasizes the comparative potential of Liberica alongside Arabica and Robusta, particularly in the context of developing coffee products with reduced caffeine content and enhanced health-promoting bioactive compounds. The findings aim to provide insights for optimizing species selection and roasting strategies to improve coffee quality, flavour and functional benefits, supporting both specialty coffee development and health-oriented consumption.

Materials and Methods: A factorial completely randomized design (FCRD) was employed to study the effects of coffee species and roasting levels on biochemical composition. The first factor, coffee species, comprised three levels: Arabica (*Coffea arabica*), Robusta (*C. canephora*) and Liberica (*C. liberica*). The second factor, roasting level, included three treatments: light roast, medium roast and dark roast. This resulted in nine treatment combinations, each replicated three times. First-grade Arabica and Robusta green cherry beans were procured from a certified curing works in Chikkamagalur, while dry cherry Liberica beans were sourced from a commercial curing unit in Chikkamagalur.

Roasting Procedure

Green beans of *Coffea arabica, C. canephora* and *C. liberica* were roasted separately to three levels using a PROBAT 5 kg drum-type commercial roaster equipped with digital temperature controls. Roasting profiles for each species are summarized in Table 1. After roasting, beans were air-cooled, degassed and stored in airtight containers.

Table 1: Roasting profiles of coffee species

Species	Roast level	Initial Temperature (°C)	First crack (min: s)	End time (min: s)	Final Temperature (°C)
Arabica	Light	170	8:00	9:30	190
Arabica	Medium	180	8:00	10:08	197
Arabica	Dark	185	9:00	11:23	205
Robusta	Light	190	9:10	10:17	200
Robusta	Medium	190	9:30	11:00	200
Robusta	Dark	200	10:00	11:34	205
Liberica	Light	150	8:05	9:40	180
Liberica	Medium	150	8:36	10:25	175
Liberica	Dark	175	8:00	11:40	202

Handling during roasting and post-processing

During roasting, beans were continuously agitated for uniform heat exposure and roasting progress was monitored using temperature sensors and sensory cues (colour change, aroma, first crack). After roasting, beans were rapidly cooled by forced air, stored in airtight containers and allowed to degas at room temperature for 24 h.

Grinding and storage

Roasted beans were ground to medium-coarse size using a commercial burr grinder (Mahlkönig VTA 6S) as per SCA standards. Ground samples were stored in foil pouches with one-way degassing valves at room temperature (20-22 °C).

pH of coffee brew

The pH of the coffee extract was determined using a digital pH meter. Ten grams of roasted and ground coffee sample were extracted with 100 ml of distilled water at 90±2°C and filtered through Whatman No. 1 filter paper. The filtrate was cooled to room temperature and the pH was measured by immersing the electrode of the pH meter (calibrated with buffer solutions of pH 4.0 and 7.0) into the extract. The readings were recorded in triplicate and the average value was computed (Kim *et al*., 2024) [17].

Caffeine Content (%)

Caffeine content was estimated using High-Performance Liquid Chromatography (HPLC) following the AOAC Official Method 995.1 (AOAC, 2000) [4], standardized by the Coffee Quality Division, Coffee Board of India. One gram of roasted coffee powder was extracted with Milli-Q water and filtered through a 0.45 µm membrane filter. HPLC analysis was carried out using a C18 reversed-phase column with a methanol: water (24:76 v/v) mobile phase, UV detection at 272 nm and a flow rate of 1.0 mL/min.

Caffeine concentration in the sample was calculated from the standard calibration curve using the formula:

$$\text{Caffeine content (\%)} = \frac{C \times V}{W} \times 100$$

Where,

- C = concentration obtained from calibration curve (mg/ml)
- V = final volume of extract (ml)
- W = weight of sample (mg)

Results were expressed on a dry weight basis as a percentage (%).

Chlorogenic Acid Content (%)

Chlorogenic acid content was determined by UV-Visible spectrophotometry following AOAC Official Method 957.04 (AOAC, 2000) [4]. Defatted coffee powder was extracted with hot water and the absorbance of the extract was recorded at 324 nm against prepared chlorogenic acid standards (4-16 ppm). Chlorogenic acid content was calculated using the formula:

$$\text{Corrected value} = C_0 - \frac{C_1 - 0.00045}{4} \times 100$$

Where,
C_0 = absorbance of untreated sample
C_1 = absorbance of lead acetate-treated sample
Values were derived from the standard curve and expressed as percentage (% dry weight basis).

Total antioxidant activity (TAA), (%)
The antioxidant activity of the roasted coffee extracts was estimated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by Priftis *et al*. (2015) [25]. Coffee extracts (0.1 mL) were mixed with 3.9 mL of 0.4 mM DPPH solution, incubated in the dark at room temperature for 30 min and absorbance was measured at 515 nm using a UV-Visible spectrophotometer against a methanol blank. The radical scavenging activity of the sample was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{A0-Ae}{A0} \times 100$$

Where,
A_0 = Absorbance of the DPPH solution without sample (control)
A_e = Absorbance of the DPPH solution with coffee extract
Each sample was analysed in triplicate and the results were expressed as mean ± standard deviation.

Flavonoid content (mg QE/mL or mg/g)
Flavonoid content was quantified following Gomez *et al.* (2016) [13] using quercetin as the standard. The extract was reacted sequentially with $NaNO_2$, $AlCl_3$ and NaOH and the final volume was adjusted with distilled water. Absorbance was measured at 510 nm using a UV-Vis spectrophotometer and results were expressed as mg quercetin equivalents per gram (mg QE/g) of coffee powder. Flavonoid content was calculated from a standard curve prepared with quercetin as the reference and expressed as milligrams of flavonoids per gram of coffee sample (mg/g) using the formula:

$$\text{Flavonoid content (mg QE/g or mg/g)} = \frac{\text{(Concentration from graph in mg QE/ml} \times \text{Volume of extract in ml)}}{\text{Weight of sample in g}}$$

Results and Discussion
pH of coffee brew
Significant differences in pH were observed among coffee species. Robusta exhibited the highest pH (5.35), followed by Arabica (5.13) and Liberica (5.09). The elevated pH in Robusta is attributed to its lower concentrations of small organic acids such as citric and malic acids, which are primary contributors to coffee acidity, despite higher levels of chlorogenic acid, a weaker phenolic acid. These findings align with previous reports by Kim *et al*. (2024) [17] and Langi *et al*. (2023) [19], who noted lower organic acid content and consequently higher pH values in Robusta compared to Arabica. Roasting significantly increased pH, with dark roast recording the highest value (5.32), followed by medium (5.17) and light roast (5.09). This trend reflects the thermal breakdown of organic acids at higher roasting intensities. This observation is in agreement with Halim *et al*. (2022) who reported a clear elevation in pH as roasting levels increased due to acid degradation. The pH varied significantly due to the combined effects of species and roasting levels with Robusta-dark showing the highest pH (5.49), while Liberica-light recorded the lowest (4.97). This interaction reflects the combined effects of species-specific acid profiles and roasting-induced acid degradation. Similar findings were reported by Kim *et al*. (2024) [17] and Langi *et al*. (2023) [19].

Caffeine content (%)
Caffeine content differed significantly among species. Robusta (2.70%) contained nearly double the caffeine of Arabica (1.46%) and more than Liberica (1.10%), reflecting genetic regulation of caffeine biosynthesis across species. These findings are consistent with Ky *et al*. (2001) [18] and Awwad *et al*. (2021) [5], who documented interspecies variation in caffeine levels among commercial cultivars. Roasting exerted minimal influence on caffeine (1.75-1.77%), with slight increases in darker roasts due to concentration from moisture loss rather than degradation. These findings were supported by Awwad *et al*. (2021) [5], Alamri *et al*. (2022) [1] and Fuller and Rao (2017) [12]. A significant interaction between species and roasting level was observed in caffeine content. Robusta medium roast exhibited the maximum caffeine (2.75%), while Liberica light roast showed the lowest (1.05%). Thus, species largely governs caffeine content, while roasting mainly modifies it through concentration effects. These findings are consistent with those reported by Farikha *et al*. (2025) [11], Jung *et al*. (2021) [16] and Ky, *et al*. (2001) [18].

Chlorogenic acid content (%)
Species varied significantly in CGA, with Robusta (5.04%) and Liberica (5.03%) both exceeded Arabica (4.54%). The higher levels in Robusta and Liberica are attributed to stronger phenolic biosynthesis pathways as reported by Fuller and Rao (2017) [12] and Ky *et al*. (2001) [18]. Roasting reduced CGA content progressively: light (5.57%) > medium (5.05%) > dark (3.99%) due to thermal degradation via hydrolysis and decarboxylation of these heat-labile compounds. Similar findings were reported by Awwad *et al*. (2021) [5] and Moon *et al*. (2009) [22]. A significant interaction effect was observed between species and roasting level. Robusta under light roast recorded the highest CGA content (5.970%), followed by Liberica-light (5.850%), while the lowest was found in Arabica-dark (3.870%). This trend reflects species differences in initial CGA levels and their thermal degradation during roasting. Robusta and Liberica, with higher inherent CGA, retained more CGA under light roast, whereas Arabica, having lower starting levels, showed greater losses under dark roast. These findings align with the reports of Sualeh *et al*. (2020) [29] and Moon *et al*. (2009) [22].

Antioxidant activity (%)
Antioxidant activity varied significantly among species, with Robusta (90.10%) > Arabica (81.82%) > Liberica (76.07%), reflecting its higher CGA and total phenolic content (Aytar & Aydin, 2013) [6]. Roasting influenced antioxidant capacity, with light roast (84.26%) > medium (83.40%) > dark (80.33%). The decline is likely due to the degradation of heat-sensitive compounds such as

chlorogenic acids and flavonoids. Although roasting generates Maillard reaction products with antioxidant potential, these compounds may not fully compensate for the loss of native phenolics at darker roasting levels as reported by Gorecki and Hallmann (2020) [14] and Priftis *et al*. (2015) [25]. The highest antioxidant activity (93.27%) was recorded in Robusta under medium roast while, Liberica-dark recorded the lowest (72.22%). This is likely due to an optimal balance between the preservation of phenolic compounds and the formation of antioxidant Maillard reaction products. These results align with the observations of Mahalingam and Zaidi (2024) [21] and Tarigan *et al*. (2022) [30], who reported that moderate roasting enhances antioxidant activity in Robusta-rich blends by promoting both retention and transformation of bioactive compounds.

Flavonoid content (mg/g): Flavonoid content varied significantly among species, with Robusta (3.00 mg/g) recording the highest, followed by Arabica (2.43 mg/g) and Liberica (2.08 mg/g). This difference reflects species-specific variation in phenolic metabolism and flavonoid biosynthesis, with Robusta known for stronger phenolic accumulation (Mahalingam & Zaidi, 2024) [21]. Roasting significantly reduced flavonoids due to their heat-labile nature, declining from 2.93 mg/g in light roast to 2.15 mg/g in dark roast. The losses result from oxidation, polymerization and structural breakdown during thermal exposure, consistent with Laukaleja *et al*. (2022) [20] and Jung *et al*. (2021) [16]. The species × roasting interaction was evident: Robusta-light recorded the highest flavonoid content (3.45 mg/g), whereas Liberica-dark was lowest (1.85 mg/g). This demonstrates that both inherent species characteristics and roast intensity jointly influence flavonoid retention, as also highlighted by Gorecki & Hallmann (2020) [14].

Table 2: Biochemical properties of coffee species under different roasting levels

Treatments	pH	Caffeine (%)	Chlorogenic acid content (%)	Antioxidant Activity (%)	Flavonoid Content (mg/g)
C1-Arabica (*Coffea arabica*)	5.13^{b}	1.464^{b}	4.543^{b}	81.82^{b}	2.43^{b}
C_2-Robusta (*Coffea canephora*)	5.35^{a}	2.703^{a}	5.043^{a}	90.10^{a}	3.00^{a}
C3-Liberica (*Coffea liberica*)	5.09^{c}	1.100^{c}	5.036^{a}	76.07^{c}	2.08^{c}
S.Em. ±	0.01	0.002	0.004	0.34	0.005
CD (1%)	0.03	0.010	0.016	1.39	0.02
R_1-Light	5.09^{c}	1.750^{b}	5.576^{a}	84.26^{a}	2.93^{a}
R_2-Medium	5.17^{b}	1.751^{b}	5.056^{b}	83.40^{a}	2.44^{b}
R_3-Dark	5.32^{a}	1.770^{a}	3.990^{c}	80.33^{b}	2.15^{c}
S.Em. ±	0.01	0.002	0.004	0.36	0.005
CD (1%)	0.03	0.010	0.016	1.39	0.02
T_1 (C_1R_1)	5.06^{d}	1.520^{c}	4.910^{e}	82.87^{d}	2.95^{b}
T_2 (C_1R_2)	5.12^{d}	1.380^{e}	4.850^{f}	80.48^{e}	2.35^{e}
T_3 (C_1R_3)	5.21^{c}	1.490^{d}	3.870i	82.11^{d}	2.00^{f}
T_4 (C_2R_1)	5.25^{b}	2.680^{b}	5.970^{a}	90.36^{b}	3.45^{a}
T_5 (C_2R_2)	5.32^{b}	2.750^{a}	5.240^{c}	93.27^{a}	2.95^{b}
T_6 (C_2R_3)	5.49^{a}	2.680^{b}	3.920^{h}	86.67^{c}	2.60^{c}
T_7 (C_3R_1)	4.97^{e}	1.050^{h}	5.850^{b}	79.55^{e}	2.40^{d}
T_8 (C_3R_2)	5.06^{d}	1.120^{g}	5.080^{d}	76.44^{f}	2.00^{f}
T_9 (C_3R_3)	5.25^{b}	1.140^{f}	4.180^{g}	72.22^{g}	1.85^{g}
S.Em. ±	0.01	0.004	0.006	0.59	0.010
CD (1%)	0.04	0.018	0.028	2.42	0.04

Conclusion

The study demonstrated that coffee species and roasting levels significantly influence the biochemical composition of coffee brew. Robusta exhibited the highest caffeine and antioxidant activity, Arabica showed moderate levels, while Liberica presented lower caffeine content with appreciable antioxidant potential, particularly under light and medium roasting. Roasting progressively decreased chlorogenic acid and flavonoid content, with dark roasting causing the greatest losses. Significant species × roasting interactions indicate that both inherent biochemical profiles and roast intensity jointly determine caffeine concentration and bioactive compound retention. These findings highlight the potential of Liberica, along with Arabica and Robusta, for developing coffee products optimized for lower caffeine content and enhanced antioxidant benefits, offering practical insights for specialty coffee formulation and health-oriented consumption.

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