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Effect of Roasting Temperature on Arabica Coffee Caffeine Content by UV-Vis Spectrophotometry

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Abstract. Coffee is a major agricultural commodity in Indonesia, with caffeine as its principal bioactive compound influencing product quality and consumer health. Roasting affects caffeine levels, but data on its impact in locally produced Arabica coffee remain limited. This study evaluated the effect of roasting temperature on caffeine content in Arabica coffee beans from Pulosari District, Central Java, using UV-Visible spectrophotometry as a cost-effective alternative to HPLC. Coffee samples roasted at 200 °C, 210 °C, and 215 °C for nine minutes were analysed. Caffeine was extracted via reflux and sublimation, identified by thin-layer chromatography (TLC), and quantified using UV-Vis spectrophotometry. TLC confirmed caffeine presence in all samples with Rf values (0.65–0.66) matching the standard. The UV-Vis method showed excellent linearity ($r = 0.9981$), precision (RSD = 0.39%), accuracy (95.90–98.93%), and acceptable LOD (0.730 ppm) and LOQ (2.435 ppm). Caffeine content decreased significantly with increasing roasting temperature ($p < 0.05$), from 0.181% at 200 °C to 0.139% at 215 °C. These results demonstrate that roasting temperature significantly influences caffeine retention, and UV-Vis spectrophotometry is a reliable tool for caffeine quantification in small-scale coffee processing.

Keywords : Arabica coffee, caffeine content, roasting temperature, UV-Vis spectrophotometry

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Introduction

Coffee is one of Indonesia's most strategic plantation commodities, playing a vital role in both the national economy and cultural identity. In addition to serving as a primary source of income for farmers, coffee offers substantial added value through its wide variety of processed products. Arabica coffee (Coffea arabica L.) stands out among various coffee types because of its refined flavor, pronounced acidity, and rich aroma, which make it highly sought after in both local and global markets. [1], [2]. The rising popularity of Arabica coffee has increased public and scientific interest in its bioactive compounds, particularly caffeine.

Caffeine is the primary alkaloid found in coffee beans and functions as a stimulant of the central nervous system. Its concentration is influenced by several factors, including varietal type, geographical origin, environmental conditions, and post-harvest processing, particularly roasting [3]–[5]. Roasting initiates a series of chemical reactions that significantly alter the chemical composition of coffee, including its caffeine content. Previous studies have shown that higher roasting temperatures tend to reduce caffeine levels, primarily due to thermal degradation or volatilization [6]. However, this trend may vary depending on the coffee's origin and the analytical method used.

As a pharmacologically active compound, caffeine exhibits biphasic effects: in low to moderate doses, it enhances alertness and concentration; in contrast, chronic or excessive intake may lead to adverse outcomes such as insomnia, tachycardia, and anxiety [7], [8]. Therefore, determining the caffeine content in coffee products is essential not only for consumer safety but also for ensuring product quality and regulatory compliance.

Pulosari District, located in Pemalang Regency, Central Java, is an emerging center for Arabica coffee cultivation. Situated at 800–1000 meters above sea level on the slopes of Mount Slamet, the region offers ideal agroecological conditions for growing high-quality Arabica coffee [9]. Nevertheless, coffee production in this area remains largely small-scale, relying on home-based roasting processes with varying temperatures, and currently lacks scientific documentation on caffeine content. This represents a significant research gap with practical implications for quality assurance and consumer health considerations.

Although High-Performance Liquid Chromatography (HPLC) is the gold standard for caffeine analysis, it requires sophisticated instrumentation, specialized expertise, and high operational costs. As an alternative, UV-Visible spectrophotometry offers several advantages such as simplicity, speed, cost-efficiency, and sufficient sensitivity, which makes it particularly suitable for use in educational laboratories and small-scale industry applications [10], [11].

In this context, the present study aims to determine the caffeine content in Arabica coffee produced in Pulosari District at various roasting temperatures using UV-Vis spectrophotometry. The results are expected to contribute to the establishment of quality standards for local coffee products and provide a scientific basis for optimizing caffeine preservation in small-scale coffee processing.

Experimental

Instruments. Reflux apparatus, UV-Visible Spectrophotometer (Shimadzu), TLC silica gel 60 GF254 plates (Merck), analytical balance (Pioneer), Büchner funnel, evaporating dish, bunsen burner, standard laboratory glassware, tweezers, filter paper, clamp, microsyringe pipette (Dragonlab), and spatula.

Materials. Caffeine anhydrous (p.a), chloroform ($CHCl_3$), distilled water, lead(II)acetate, ethanol 96%, and three samples of roasted Arabica coffee powder from Pulosari District.

Sample Collection and Preparation. The coffee samples were obtained from small-scale Arabica coffee producers in Pulosari District, Pemalang Regency, Central Java. A purposive sampling technique was used with the following criteria: 1) The coffee must be of the Arabica variety; and 2) The roasting temperature must differ between producers.

Caffeine Isolation Using Reflux Extraction. Caffeine was isolated from the coffee samples using a reflux extraction method. A total of 40 grams of finely ground coffee was mixed with 350 mL of distilled water and heated at 100 °C for 30 minutes in a round-bottom flask equipped with a reflux condenser. After heating, the mixture was filtered through a Büchner funnel lined with filter paper and allowed

to cool to room temperature. Subsequently, 40 mL of lead(II)acetate solution was added dropwise to the filtrate with constant stirring until no further precipitation occurred. The resulting precipitate was removed by a second filtration. The clear filtrate was then transferred to a separatory funnel and subjected to liquid-liquid extraction using chloroform in three stages (15 mL, 15 mL, and 10 mL). The chloroform layer, which contained the extracted caffeine, was collected from each stage and combined in an evaporating dish for further processing [12].

Caffeine Crystallization via Sublimation. The chloroform extract was sublimated using a Bunsen burner. A glass funnel lined with perforated filter paper was placed over the evaporating dish for 15 minutes. The caffeine crystals adhering to the filter paper were collected and weighed.

Caffeine Identification Using Thin-Layer Chromatography (TLC). Caffeine identification was carried out using thin-layer chromatography (TLC). The mobile phase consisted of a chloroform and ethanol mixture in a 99:1 (v/v) ratio, with a total volume of 20 mL. The stationary phase was a silica gel 60 GF254 plate, which was pre-activated by oven-drying at 45 °C for 3 minutes. Samples and a standard caffeine solution were carefully spotted onto the TLC plate and allowed to dry. The plate was then placed in a saturated TLC chamber containing the mobile phase and developed until the solvent front reached the upper limit. After development, the plate was dried and visualized under UV light at 254 nm. The retention factor (Rf) values of the sample spots were calculated and compared to the standard to confirm the presence of caffeine [13].

Caffeine Quantification Using UV-Visible Spectrophotometry. Caffeine quantification was performed using UV-Visible spectrophotometry. A stock solution of caffeine at 100 ppm was prepared by dissolving 0.01 g of pro-analysis caffeine in a 100 mL volumetric flask with distilled water. To determine the maximum absorbance wavelength (λmax), a 9 ppm standard solution was scanned within the range of 200–400 nm using a UV-Vis spectrophotometer, with distilled water as the blank. A calibration curve was constructed by preparing a series of standard caffeine solutions with concentrations of 5, 6, 7, 8, and 9 ppm, derived from the 100 ppm stock. The absorbance of each solution was measured at λmax, and a regression equation was obtained by plotting absorbance (y) against concentration (x), using this formula:

$$y=bx+a \qquad (1)$$

where b represents the slope and a the intercept.

For sample analysis, 0.01 g of isolated caffeine crystals from each coffee sample was dissolved in 100 mL of distilled water to obtain a 100 ppm stock solution. This solution was further diluted to 50 ppm, and then to a working solution, which was analyzed at the previously determined λmax. The caffeine concentration in each sample was calculated using the regression equation from the calibration curve. The final caffeine content was expressed as a percentage and calculated using the formula:

$$\text{Caffeine content (\%)} = \frac{(c \times v \times f)}{m} X\ 100\% \qquad (2)$$

where c is the concentration in mg/L, f is the dilution factor, v is the volume of the sample solution in liters, and m is the sample mass in milligrams [14].

Linearity. Linearity was assessed using standard caffeine solutions at concentrations of 5, 6, 7, 8, and 9 ppm, each measured in triplicate. A linear regression equation and the correlation coefficient (r) were generated from the calibration curve. A correlation coefficient of r ≥ 0.99 was considered indicative of good linearity.

Precision. Precision was evaluated by analyzing a 7 ppm standard solution seven times on the same day. The relative standard deviation (RSD) was calculated, with an RSD value ≤ 2% indicating acceptable precision.

$$SD=\sqrt{\frac{(xi-x)^2}{n-1}} \qquad (3)$$

$$RSD=\frac{SD}{Average\ measure\ level} X\ 100\% \qquad (4)$$

Accuracy. Accuracy was determined through recovery testing at concentrations of 6, 7, and 8 ppm, each performed in triplicate (n = 3). Recovery values ranging from 90% to 110% were considered acceptable, indicating good accuracy.

Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD and LOQ were calculated

based on the calibration curve using the standard deviation of the residuals (Sy) and the slope (S) of the regression line. The formulas used were:

$$LOD = \frac{3.3 \times Sy}{S} \quad (5)$$

$$LOQ = \frac{10 \times Sy}{S} \quad (6)$$

where Sy is the standard deviation of the residuals, and S is the slope of the calibration curve.

Result and Discussion

This study evaluated the caffeine content in Arabica coffee beans produced in Pulosari District, Pemalang Regency, Central Java, roasted at varying temperatures (200, 210, and 215 °C) with a fixed duration of nine minutes. Caffeine identification was performed using thin-layer chromatography (TLC), while quantitative analysis was conducted using UV-Visible spectrophotometry.

Caffeine Isolation. Caffeine was successfully isolated from Arabica coffee samples using the reflux extraction and sublimation method described previously. The resulting caffeine crystals varied in color and mass across roasting temperatures. Coffee roasted at 200 °C produced white crystals with the highest mass (0.0771 g), whereas samples roasted at 215 °C yielded semi-brown crystals with the lowest mass (0.0474 g). The yields are summarized in Table 1.

Qualitative Analysis of Caffeine. Thin-layer chromatography (TLC) confirmed the presence of caffeine in all coffee samples. The spots exhibited bright blue fluorescence under UV light at 254 nm, with Rf values closely matching that of the caffeine standard. Minimal variation in Rf values was observed among the samples. The findings are summarized in Table 2 and depicted in Figure 1.

Determination of Maximum Absorbance Wavelength. The maximum absorbance wavelength (λmax) for caffeine was determined to be 273 nm using a 9 ppm standard solution. This wavelength was subsequently used for all spectrophotometric measurements.

Method Validation. The UV-Vis spectrophotometric method demonstrated acceptable performance across key validation parameters. The calibration curve for caffeine (5–9 ppm) produced a regression equation (y = 0.1273x − 0.4323) with a correlation coefficient (r) of 0.9981, indicating excellent linearity. Precision testing using a 7 ppm stand-

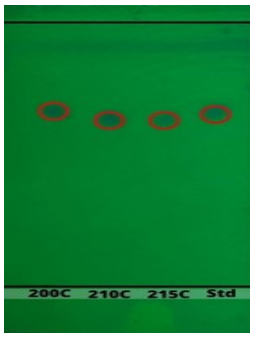

Figure 1. TLC chromatogram of caffeine in coffee samples under UV light (254 nm)

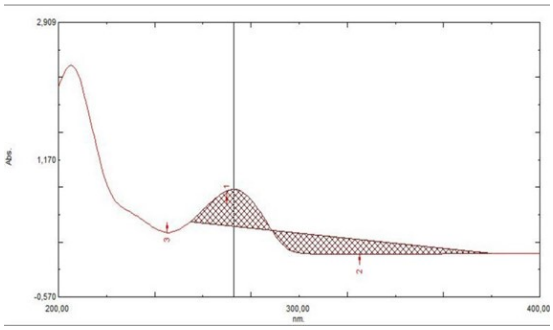

Figure 2. Maximum absorbance wavelength of caffeine (λmax = 273 nm)

Table 1. Characteristics and yield of caffeine crystals from Arabica coffee samples

Roasting Temperature (°C)	Crystal Color	Crystal Mass (g)	Yield (%)
200	White	0.0771	0.0019
210	White	0.0678	0.0016
215	Semi-brown	0.0474	0.0011

Table 2. Rf values and fluorescence characteristics of caffeine in coffee samples

Sample	Rf Value	Spot Color
Caffeine standard	0.66	Bright blue
Coffee roasted at 200 °C	0.66	Bright blue
Coffee roasted at 210 °C	0.65	Bright blue
Coffee roasted at 215 °C	0.65	Bright blue

ard solution (n = 7) resulted in a relative standard deviation (RSD) of 0.39%. Accuracy was assessed through recovery tests at three concentration levels, yielding values between 95.90% and 98.93%. The method's limit of detection (LOD) and limit of quantification (LOQ) were 0.730 ppm and 2.435 ppm, respectively.

Caffeine Content in Arabica Coffee Samples. The caffeine content of Arabica coffee samples decreased progressively with increasing roasting temperature. Coffee roasted at 200 °C exhibited the highest caffeine concentration (0.181%), while the lowest concentration was observed in samples roasted at 215 °C (0.139%). The results are presented in Table 4.

One-way ANOVA revealed a significant difference in caffeine content among the three roasting temperatures ($p = 0.000$). Post-hoc Tukey HSD tests confirmed significant pairwise differences between all groups ($p < 0.05$).

This study provides valuable insights into the influence of roasting temperature on caffeine content in Arabica coffee beans from Pulosari District, Central Java. The isolation method efficiently extracted caffeine across all roasting levels, decreasing yields as the temperature increased. This trend is consistent with previous findings that reported a similar decline in caffeine content at higher roasting temperatures, likely due to partial volatilisation and thermal degradation of caffeine during roasting [3], [15].

The observed change in crystal colour, from white at 200 °C to semi-brown at 215 °C, may be attributed to Maillard reactions and caramelisation processes at elevated temperatures. These reactions produce melanoidins and other colored compounds that can alter the appearance of the caffeine crystals and potentially co-precipitate with them during isolation [16], [17].

Thin-layer chromatography (TLC) was performed using a mobile phase of chloroform and ethanol in a 99:1 (v/v) ratio. This solvent system was selected because non-polar chloroform effectively carried caffeine along the plate. At the same time, a small proportion of ethanol increased polarity slightly, facilitating optimal migration and resolution on the silica gel stationary phase. Using this optimised mobile phase, TLC successfully confirmed the presence of caffeine in all coffee samples. The Rf values of the coffee samples (0.65–0.66) matched the Rf value of the caffeine standard, indicating that all samples contained caffeine [18].

Table 4. Caffeine content in Arabica coffee samples at different roasting temperatures

Roasting Temperature(°C)	Caffeine Concentration (mg/L)	Caffeine Content (%)
200	9.088 ± 0.115	0.181
210	7.506 ± 0.020	0.149
215	6.974 ± 0.041	0.139

The UV-Vis spectrophotometric method demonstrated robust analytical performance, characterised by high linearity ($r = 0.9981$), precision (RSD = 0.39%), and accuracy (95.90–98.93%). The high linearity confirms that caffeine absorbance was directly proportional to its concentration over the tested range, meeting the acceptance criterion ($r \geq 0.990$) [19]. The low RSD value (< 2%) reflects excellent repeatability, while recovery values (95–99%) fall within the acceptable range for quantitative methods (90-100%), confirming the method's accuracy [3]. These validation parameters align with international standards and support UV-Vis spectrophotometry as a rapid, cost-effective alternative to more complex techniques such as HPLC for determining caffeine in coffee samples.

Quantitative analysis revealed a significant decrease in caffeine concentration with increasing roasting temperature. One-way ANOVA confirmed that the differences in caffeine content among the three

Table 3. Precision and accuracy data for caffeine quantification using UV-Vis spectrophotometry

Parameter	Concentration (ppm)	Replication (n)	Mean ± SD (%)	RSD (%)	Recovery (%)
Precision	7.0	7	7.0015 ± 0.0276	0.39	-
Accuracy	6.0	3	5.8939 ± 0.0412	-	98.93
	7.0	3	6.8994 ± 0.0251	-	98.56
	8.0	3	7.8813 ± 0.0335	-	95.90

Note: Precision was evaluated at 7 ppm, and accuracy was assessed at three concentration levels

roasting temperatures were statistically significant ($p = 0.000$). Post-hoc Tukey HSD tests showed significant pairwise differences between all groups ($p < 0.05$), indicating that each roasting level had a distinct effect on caffeine retention. Coffee roasted at 200 °C exhibited the highest caffeine content (0.181%), while the lowest concentration was observed at 215 °C (0.139%). These findings corroborate previous reports of temperature-dependent reductions in caffeine levels during roasting [5]. This decline may be attributed to caffeine's relatively low sublimation point (~178 °C), which facilitates volatilisation at high temperatures, and its susceptibility to oxidative degradation under prolonged heating in an oxygen-rich environment, leading to partial breakdown into other alkaloid derivatives [20], [21].

This study is particularly relevant for small-scale coffee producers in Pulosari District, where roasting practices are often unstandardized. Optimising roasting temperature is essential to preserve caffeine content, as it affects not only the sensory attributes of coffee but also its functional role as a mild stimulant that contributes to consumer preference.

Future research should investigate additional variables, including roasting duration, bean size, and initial moisture content, to better understand their combined effects on caffeine stability. Advanced analytical techniques such as HPLC and GC-MS are recommended for comparative validation and to explore changes in other bioactive compounds during roasting.

Conclusion

This study demonstrated that roasting temperature significantly affects caffeine content in Arabica coffee from Pulosari District, decreasing levels as temperature increases. The validated UV-Vis spectrophotometric method showed high linearity, precision, and accuracy, supporting its use as a reliable analytical tool. These findings are essential for improving quality control in small-scale coffee production and contribute to optimising roasting practices to preserve caffeine as a functional and commercial component. UV-Vis spectrophotometry offers a cost-effective method suitable for wider educational and industrial applications.

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Author Contributions

Mia Cornelia Chandra conducted the experiments, analysed the data, and drafted the manuscript. Adita Silvia Fitriana and Rani Prabandari provided academic supervision and methodological guidance and critically reviewed the manuscript for scientific content and accuracy. All authors approved the final version of the manuscript.

Additional Information

No additional information is available for this article.

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