International Journal of Contemporary Sciences (IJCS)
Vol. 1, No. 12, 2024: 916-931

The Caffeine Analysis in Tea Bag and Robusta Coffee Using UHPLC Methods

Ahmad Sutomo[1], Muhammad Ridwan[2], Vicko Suswidiantoro[3]*, Iga Mayola Pisacha[4], Annajim Daskar[5], Afi Sania Rosanti[6]
Faculty of Health, Aisyah University of Pringsewu
Corresponding Author: Vicko Suswidiantoro
vicko.suswidiantoro@aisyahunivesity.ac.id

A R T I C L E I N F O

Keywords: Caffein, UHPLC, Optimation Methods, Coffee, Tea Bag

Accepted : 09, August
Revised : 11, September
Accepted: 29, October

A B S T R A C T

Caffeine, a natural psychostimulant, enhances arousal by inhibiting the effects of adenosine on the dopamine pathway. This study aimed to analyze and compare caffeine concentrations in seven robusta coffee samples (coded RC) and five black tea bags (coded BT) using both qualitative and quantitative methods. Qualitative tests employed Dragendorff reagents for coffee and Parry reagents for tea, confirming caffeine presence through colorimetric changes. Quantitative analysis utilized reverse-phase UHPLC, with a mobile phase of aquabidestilata and methanol (60:40), a C18 stationary phase at 40°C, and detection at 272 nm. Validation parameters demonstrated strong performance, with an RSD of 0.573%, a linear correlation coefficient of 0.998 for concentrations between 100–250 ppm, a LoD of 1.02 ppm, a LoQ of 3.10 ppm, and 99.88% accuracy. Results showed robusta coffee sample RC3 contained the highest caffeine concentration at 26.00 mg/serving, while black tea sample BTB3 exhibited 2.44 mg/serving. Despite higher caffeine levels in robusta coffee compared to black tea, both remained below the SNI threshold of 50 mg/serving, confirming their safety for consumption.

INTRODUCTION

Caffeine is a widely consumed stimulant found primarily in coffee and tea. In the context of tea bags and Robusta coffee, it becomes important to analyze the caffeine content to ensure quality control and meet consumer expectations. Ultra-High Performance Liquid Chromatography (UHPLC) is frequently employed for this purpose, offering precise and rapid quantification. Studies have demonstrated that UHPLC provides superior sensitivity and faster retention times, especially compared to traditional methods like HPLC (High Performance Liquid Chromatography) (Morales et al., 2020). For instance, caffeine concentrations in Robusta coffee were observed to vary depending on geographical factors and bean preparation, typically ranging between 0.445% to 2.18% by weight using UHPLC methods. Similarly, caffeine levels in tea also vary, influenced by the type of tea and its processing (Pires et al., 2021). Coffee and tea are globally popular beverages, each offering unique flavors and cultural significance. Tea's appeal in Indonesia, driven by its fragrance, taste, and simple preparation, reflects a preference for various types categorized by fermentation levels, including black, oolong, green, and white tea (Sundalian & Nugrahani, 2018; Ramdan et al., 2022). Fermentation significantly influences caffeine content, with black tea typically containing the highest levels (Wardani & Fernanda, 2016). Indonesia is also a leading coffee producer, ranking third worldwide in 2022, with provinces like Lampung contributing significantly to its Robusta coffee output, known for high caffeine content and distinct bitterness (BPS, 2021; USDA, 2023). Research indicates that Robusta coffee exhibits a caffeine concentration between 2,741 mg/L to 3,814 mg/L, offering a sharper flavor profile compared to Arabica coffee (Aditya et al., 2015; Prasetyo et al., 2020). These findings underscore the importance of production methods in shaping the chemical composition and taste of both tea and coffee.

Caffeine, a natural alkaloid present in both tea and coffee, acts as a stimulant of the central nervous system, contributing to increased alertness and reduced fatigue. Its concentration varies significantly across plant types, with environmental and processing factors playing crucial roles. For instance, caffeine levels in tea can range from 2% to 4% depending on the variety—white and green teas often contain more caffeine than black teas due to differences in processing and leaf maturity. Similarly, Robusta coffee beans tend to exhibit higher caffeine content compared to Arabica beans, offering a more robust flavor and stronger stimulant effects (Horžić et al., 2009; Athayde et al., 2000). Analytical studies emphasize the use of chromatographic techniques, such as UHPLC, to accurately measure caffeine concentrations, as the precision of these methods is essential for quality control and consumer health assessments (Fernando & Soysa, 2016). These findings highlight the importance of precise caffeine quantification in various beverages, providing insights into their impact on human health.

The Ultra-High Performance Liquid Chromatography (UHPLC) method is highly effective in analyzing caffeine due to its ability to provide rapid, precise results with minimal sample preparation. UHPLC typically operates under higher pressure than conventional HPLC, allowing for faster analysis and better

resolution with smaller particle sizes in the columns. For caffeine analysis, isocratic elution is commonly used, involving a mobile phase composed of methanol or acetonitrile mixed with water or a buffer. A reversed-phase column, such as the TSK-GEL ODS-100V or ODS-140HTP, is often employed, as it ensures sharp peak resolution for small molecules like caffeine (Sharma et al., 2005). The temperature for optimal caffeine separation is usually maintained between 25°C and 40°C, with a detection wavelength around 274 nm, which is ideal for identifying caffeine peaks (Tzanavaras & Themelis, 2007). In recent studies, caffeine eluted at about 1.9 to 5 minutes, depending on column length and particle size, ensuring high-throughput analysis even in complex mixtures such as coffee or tea extracts (Douglass, 2007).

Caffeine, an alkaloid derived from xanthine, plays a significant role in enhancing cognitive and physical performance, yet excessive intake can result in adverse effects, from minor musculoskeletal issues to severe metabolic and gastrointestinal disorders (Belguidoum et al., 2014; McLellan et al., 2016; Wilson, 2018). Adolescents, particularly males, are among the most frequent consumers, raising concerns about long-term health risks (Pant, 2023; Rahmatika, 2023). While numerous analytical methods are available for quantifying caffeine—including UV/Vis spectroscopy, gas chromatography, FTIR, HPLC, mass spectrometry, and electrochemical approaches—HPLC remains preferred for its high sensitivity and accuracy (Sereshti et al., 2014; Shiferaw et al., 2018; Aprilia et al., 2018). Despite the wealth of research on caffeine quantification and health impacts, there is limited comparative analysis using advanced UHPLC methods across different beverages, such as tea and Robusta coffee. This study aims to fill that gap by exploring caffeine content across these two popular beverages, providing more precise data that could inform both public health strategies and product quality control.

The objective of this study is to determine the optimal conditions for analyzing caffeine content using UHPLC and to compare the caffeine concentrations in tea and Robusta coffee. By employing UHPLC, known for its superior precision and rapid detection, the study aims to provide more reliable data on the caffeine variations between these two beverages. Understanding these differences can contribute to enhanced product quality control and offer insights into their health implications, supporting public health awareness and guiding consumer choices.

The following is the framework of this research:

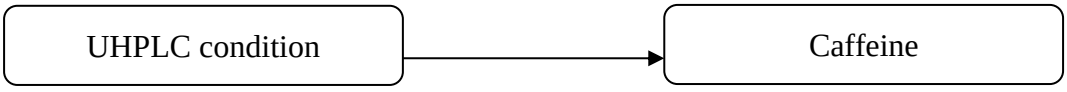

Figure 1. Conceptual Framework

METHODOLOGY

This study employed saturated sampling, targeting all available Robusta coffee and black tea bags from supermarkets in Pringsewu Regency. A total of seven Robusta coffee powder samples, labeled as RC1 to RC7, and five black tea bag samples, labeled as BTB1 to BTB5, were included. The criteria for selection

required that the coffee samples be native Robusta from Lampung province, while the black tea bags contained pure black tea without additives, ensuring consistency in sample quality (BPS, 2021). These diverse samples enabled comprehensive analysis of caffeine content across both beverages.

The research utilized a reverse-phase UHPLC system equipped with a C18 octadecyl silica column (250 x 4.6 mm, 5 µm pore size) to perform both qualitative and quantitative analyses. Coffee and tea samples were extracted using optimized reflux techniques to prevent caffeine degradation. For coffee, the extraction process involved refluxing for 1 hour, followed by centrifugation with zinc ferrocyanide reagents, and chloroform extraction to isolate caffeine crystals (Rahmawati et al., 2021). Tea samples underwent multiple chloroform extractions and were ultrasonicated at 36°C to ensure complete dissolution of caffeine crystals. Each extract was prepared for UHPLC injection by filtration through 0.45 µm syringe filters (Widhyani et al., 2021; Bina et al., 2020).

The UHPLC analysis was conducted using a mobile phase consisting of a 60:40 mixture of aquabidestillate and methanol under isocratic conditions at 1.0 mL/min flow rate. The caffeine concentration was determined based on the Area Under Curve (AUC) values obtained from chromatograms detected at a wavelength of 272 nm (Rahmawati et al., 2021). The data were processed by inserting AUC values into a linear regression equation ($y = b(x) + a$) to calculate caffeine content. The study's replication process ensured accurate measurement, with caffeine concentrations expressed as %w/w for comparison across the coffee and tea samples (Mutmainnah, 2017; Fajara, 2017).

RESULT AND DISCUSSION

This study utilized Lampung Robusta coffee powder obtained from Pringsewu Regency, focusing on removing non-caffeine components such as tannins, proteins, and lipids, which can disrupt UHPLC analysis by forming colloidal particles (Nollet & Toldrá, 2015). To achieve this, the coffee samples were subjected to a reflux extraction process, chosen for its suitability in handling coarse-textured samples that resist direct heating (Utami et al., 2023). Reflux extraction ensures the optimal isolation of caffeine by maintaining a consistent temperature for one hour, balancing sufficient extraction without risking the thermal degradation of caffeine (Rahmawati et al., 2021). The caffeine's semi-alkaloid nature and limited hydrophilic carbonyl groups necessitate careful temperature management to maximize its solubility in water-based solvents.

In addition to reflux extraction, zinc ferrocyanide reagents were employed to clarify the coffee extracts by precipitating large molecular components that could interfere with UHPLC analysis (Padilla, 2017; Zhang et al., 2022). Zinc acetate and potassium ferrocyanide were sequentially added to induce the formation of a precipitate, isolating non-caffeine compounds while leaving caffeine in the filtrate (Rahmawati et al., 2021; Wade & Aini, 2021). This step ensures the removal of both colloidal materials and pigments that might cause turbidity and obstruct accurate analysis (Nollet & Toldrá, 2015). By using this multi-step preparation process, the study ensures that the caffeine isolated is

suitable for precise quantification using UHPLC. The reaction resulting in colloidal formation from the combination of zinc acetate and potassium ferrocyanide in the sample is illustrated in the below figure (Rahmawati et al., 2021):

$$\mathbf{Zn2 + [Fe(CN)_6]^{4-} \rightarrow Zn_2[Fe(CN)_6]\downarrow}$$

This study utilized centrifugation to enhance the separation of zinc ferrocyanide precipitates from the coffee filtrate, with the centrifugation conducted at 4000 rpm for five minutes (Rahmawati et al., 2021). This process was essential to eliminate non-caffeine colloids that could interfere with the UHPLC analysis. Higher centrifugation speeds enhance sedimentation, whereas lower speeds reduce it, affecting the clarity of the filtrate (Holkar et al., 2019; Sulaiman et al., 2023). Following centrifugation, the filtrate was treated with 10 N NaOH, transforming caffeine into a stable salt suitable for further extraction (Rahmadona et al., 2022).

Caffeine, as a weak monoacidic base, readily dissolves in organic solvents like chloroform after alkali treatment. Chloroform was chosen for its efficiency in isolating caffeine due to its low boiling point of 61–62 °C, which ensures rapid evaporation without degrading caffeine molecules (Roossenda & Sunarto, 2016). This ensures that the caffeine extracted remains stable during the evaporation process, as caffeine's decomposition point is well above chloroform's boiling point at 178 °C (Atkins & Paula, 2014; Clayden et al., 2012). Using chloroform instead of other solvents like diethyl ether or n-hexane further optimizes caffeine isolation (Rahmawati et al., 2021).

In preparing black tea samples, a similar reflux extraction was applied, followed by the addition of $Pb(CH_3COO)_2$ to precipitate tannins and other heavy compounds that could interfere with caffeine isolation (Mutmainnah, 2017; Widhyani et al., 2021). The pH of the filtrate was adjusted with NaOH to ensure caffeine's transition to a free base form, facilitating its subsequent extraction into chloroform (Constructor et al., 2020). The extraction process was repeated three times to ensure maximal recovery of caffeine from the tea filtrate.

During extraction, chloroform formed two distinct phases, with caffeine dissolving into the chloroform phase (bottom layer), while other aqueous components remained in the upper layer (Ramdan et al., 2022). The chloroform extracts were combined and evaporated at 90°C to eliminate residual solvents. This temperature ensured complete chloroform removal without compromising caffeine's structure (Irawati et al., 2018; Nugraheni et al., 2017).

The final stage involved caffeine crystallization, where the caffeine crystals obtained from tea and coffee exhibited needle-like morphologies with a brownish-yellow hue (Yunida et al., 2021). This appearance confirmed the purity and identity of the isolated caffeine, aligning with previous research that characterized caffeine crystals with similar physical properties (Builder et al., 2020; Anonymous et al., 2021). The crystallization process thus ensured that the caffeine isolated from both coffee and tea samples was of the highest purity, ready for quantitative analysis.

The qualitative examination of the robusta coffee sample employs the

Dragendorff reaction. The research findings are presented in Table 1. The colour reaction test utilising Dragendorff reagent indicated that samples RC1, RC2, RC3, RC4, RC5, RC6, and RC7 tested positive for caffeine.

Table 1. Results of qualitative tests of robusta coffee

Sample code	Dragendorff reagent (orange precipitate)	
	Positive	Negative
RC1	+	
RC2	+	
RC3	+	
RC4	+	
RC5	+	
RC6	+	
RC7	+	
Total	**7**	**0**

Annotation: RC (Robusta coffee

The sample's interaction with Dragendorff reagent, indicated by the formation of an orange precipitate, confirms the presence of caffeine through the creation of a potassium-alkaloid complex. This reaction occurs due to the interaction between the nitrogen atoms in caffeine's alkaloid structure and potassium ions from potassium tetraiodobismutate (III) [BiI4] complexes, resulting in coordinated covalent bonds (Halimatussakdiah et al., 2018; Azizah et al., 2019). The potassium-alkaloid complex formation highlights the reagent's effectiveness in detecting alkaloids by leveraging its metal-ion interactions, a phenomenon also observed in other alkaloid assays (Kayaputri et al., 2014; Auliah et al., 2024).

Dragendorff reagent, which contains bismuth, serves as a reliable tool for alkaloid identification. It is produced by dissolving bismuth nitrate in hydrochloric acid to prevent hydrolysis, ensuring the stability of bismuth ions (Alviani et al., 2022). In this study, the observed orange precipitate aligns with previous research findings, confirming that the substitution of nitrogen ligands with iodine ions in the reagent facilitates precipitation, making the Dragendorff reagent a valuable tool in caffeine and alkaloid detection (Rahmawati & Gustiani, 2023; Wigati et al., 2018). These results reinforce the consistency of caffeine assays using this method across various studies. Simultaneously, the qualitative analysis of black tea samples yielded results as presented in Table 2.

Table 2. Results of qualitative test of black tea bags

Sample code	Parry reagent (Moss green color)	
	Positive	Negative
BTB1	+	
BTB2	+	
BTB3	+	
BTB4	+	
BTB5	+	
Total	**5**	**0**

Annotation: RC (Robusta coffee

The qualitative analysis of caffeine in five black tea bag samples confirmed the presence of caffeine using Parry reagents, yielding a positive green coloration. This outcome resulted from the interaction of cobalt ions (Co^{2+}) with

the nitrogen moiety of caffeine, producing the characteristic green hue (Fajriana & Fajriati, 2018; Anonymous et al., 2021). Acidic solutions were introduced during testing to convert caffeine into its salt form, enhancing its reactivity with the color reagent (Wilantari et al., 2018). Additionally, precision testing was conducted using standard caffeine solutions at concentrations of 100, 150, 200, and 250 ppm. The method's reliability was validated with a relative standard deviation (RSD) below 2%, indicating high precision and confirming the approach's validity for caffeine quantification (Sarmento et al., 2020).

Table 3. Percent (%) RSD Result

Replication	AUC			
	100	150	200	250
1	196.097	318.260	416.360	522.844
2	195.330	315.870	410.379	522.623
3	194.017	315.396	411.699	519.433
4	194.137	314.623	414.853	521.181
5	195.814	313.457	415.659	523.966
6	195.580	310.575	405.533	516.304
7	193.309	306.611	402.235	525.138
X	**194.897**	**313.5417**	**410.9597**	**521.6412**
SD	**1.065254**	**3.854306**	**5.367458**	**2.989331**
%RSD	**0.54657**	**1.22928**	**1.306079**	**0.573062**

The accuracy and precision of an analytical method are essential for determining its reliability (Tyas, 2021). Precision is evaluated by the recovery percentage, also known as % Recovery, which reflects how well the method can reproduce results (Harmita et al., 2019). According to Tyas (2021), an acceptable recovery range typically falls between 80% and 120%, ensuring that the method provides consistent and accurate outcomes within these limits. The results of the accuracy test for the analytical method are presented in Table 4.

Table 4. Accuracy results

Concentration (PPM)	Peak Area (AUC)	% *Recovery*	Average % *Recovery*
100	196.097	97.429	99.881
150	318.260	102.714	
200	416.360	99.779	
250	522.844	99.605	
y = 2.1567 – 14.029			

Accuracy is a critical component of method validation, reflecting how closely the analytical results align with the actual concentrations of the analyte (Ayuni, 2022). The % Recovery data, presented in Table 4, demonstrated favorable results within the acceptable range of 80-120%, validating the UHPLC method for precise caffeine analysis. Additionally, the limits of detection (LoD) and quantification (LoQ) were determined using signal-to-noise ratios of 3:1 and 10:1, respectively, through linear regression analysis of the caffeine standard curve. The LoD was identified as 1.02, indicating the lowest concentration detectable by the method, while the LoQ, calculated at 3.10, represents the minimum concentration measurable with accuracy and precision under defined conditions. These results confirm the robustness of the UHPLC method for caffeine quantification.

Table 5. LoD and LoQ results

Concentration (ppm)	Lod	LoQ
100		
150	1.02	3.10
200		
250		

The chromatogram in Figure 2 provides the data needed to construct a calibration curve correlating caffeine concentration with the Area Under the Curve (AUC). The linear regression equation derived from the standard caffeine curve is y = 2.1567x - 14.029, where y represents AUC and x indicates the caffeine concentration, with an r-value of 0.998. This high r-value demonstrates strong linearity, meeting the method validation requirements (Sarmento et al., 2020). Using this equation, the caffeine concentration in Robusta coffee samples was calculated by inputting the AUC values from their chromatograms. Among the samples, RC3 exhibited the highest caffeine concentration, confirming the robustness of the UHPLC method in distinguishing caffeine levels across different coffee samples.

Table 6. Analysis of caffeine content in black tea samples.

Sample Code	AUC	Concentration (µg/ml)	Rate % (w/b)	1x serving @8g coffee (mg)	1 day (mg)
RC1	13.529	12.777	0.028	2.299	9.199
RC2	17.811	14.763	0.033	2.657	10.629
RC3	153.746	77.792	0.175	14.002	56.010
RC4	114.649	59.664	0.134	10.739	42.958
RC5	33.548	22.060	0.049	3.970	15.883
RC6	6.407	9.475	0.021	1.705	6.822
RC7	24.904	18.052	0.040	3.249	12.997

The data indicate that the caffeine concentration in Robusta coffee is significantly higher than in black tea bags, influenced by various environmental and agricultural factors such as altitude, temperature, rainfall, and nutrient availability in coffee plantations (Komes & Vojvodić, 2014). Additionally, the roasting process plays a critical role, as higher roasting temperatures reduce caffeine levels in coffee beans (Fajriana et al., 2018; Virhananda et al., 2022). Similarly, the caffeine content in tea is impacted by factors including plant maturity, leaf age, cultivation conditions, and production methods, with carbon dioxide levels also contributing to caffeine variations (Maria & Nofita, 2018). Both Robusta coffee and black tea bag samples analyzed in this study were found to have caffeine levels within acceptable legal limits, meeting the guidelines set by the FDA and SNI 01-7152-2006, which cap caffeine intake at 150 mg per day and 50 mg per serving. If caffeine concentrations were higher, decaffeination would be necessary to reduce potential health risks, but none of the samples exceeded the regulatory threshold, ensuring compliance and safety.

CONCLUSION AND RECOMMENDATION

This study concludes that the caffeine content in Robusta coffee is significantly higher than that in black tea, as confirmed by qualitative testing using Dragendorff reagent, which produced an orange precipitate for coffee and

a moss green solution for tea. The method validation showed high precision, with an RSD of <2%, a recovery percentage of 99.88%, and strong linearity with an r-value of 0.998. Additionally, the limits of detection (LoD) and quantification (LoQ) were determined to be 1.02 and 3.10, respectively, confirming the reliability of the UHPLC method. Among the tested samples, the RC3 Robusta coffee exhibited the highest caffeine concentration at 26.00 mg per serving, while the black tea sample BTB3 showed a maximum concentration of 2.44 mg per serving. These results demonstrate that the caffeine levels in both beverages are within safe consumption limits. Future research is recommended to explore caffeine content from other sources for a broader understanding of its presence across different products.

ADVANCED RESEARCH

Further research could focus on exploring the variations in caffeine content across different processing methods or growing conditions for robusta coffee and black tea, which may influence caffeine concentration. Additionally, investigating the impact of other bioactive compounds in robusta coffee and black tea on health outcomes could provide a more comprehensive understanding of their benefits and risks. Future studies might also examine consumer caffeine metabolism variations, considering factors such as age, health status, or genetic differences, which could affect individual responses to caffeine intake. Furthermore, expanding the study to include other regions or coffee and tea varieties would allow for broader comparisons and insights. Lastly, assessing consumer awareness and understanding of caffeine content and its effects could support public health efforts to promote safe and informed consumption.

REFERENCES

Aditya, I.W., Nocianitri, K.A. and Yusasrini, N.L.A. (2015). Study on Caffeine Content of Coffee Powder, pH Value, and Aroma and Taste Characteristics of Male (Peaberry coffee) and Female (Flat beans coffee) Arabica and Robusta Brewed Coffee. Journal of Food Science and Technology (Itepa), 5(1), 1-12.

Agustine, P., Damayanti, R.P. and Putri, A. (2021). Characteristics of Caffeine Extract in Several Coffee Varieties in Indonesia: Review. Jurnal Arti, 6, 78-89.

Alviani, S., Adelia, Fajri, R., Amri, Y. and Amna, U. (2022). Phytochemical Screening of Benalu Coffee Leaf Extract (Scurrula parasitica L.) Gayo Plateau. QUIMICA: Journal of Science and Applied Chemistry, 4(1), 9-14. https://doi.org/10.33059/jq.v4i1.4360

Annissa, S., Musfiroh, I., & Indriati, L. (2020). Perbandingan Metode Analisis Instrumen HPLC dan UHPLC : Article Review. Farmaka, 17(3), 189-197.

Annuryanti, F., Zahroh, M. and Purwanto, D.A. (2019). Effect of Temperature and Amount of Brewing on Dissolved Caffeine Levels in Dry Green Tea Products by KCKT Method. Indonesian Journal of Pharmacy and Pharmaceutical Sciences, 5(1), 30. https://doi.org/10.20473/jfiki.v5i12018.30-35

Aprilia, F.R., Ayuliansari, Y., Putri, T., Azis, M.Y., Camelina, W.D. and Putra, M.R. (2018). Analysis of the Caffeine Concentration Contained in Traditional Coffee (Kopi Gayo and Kopi Lombok) Using UV/Vis Spectrophotometry and HPLC. BIOTIKA Scientific Journal of Biology, 16(2), 40.

As'ad, M.H. and Mulyo Aji, J.M. (2020). Factors that affect the preferences of consumers of modern coffee shops in Bondowoso. JSEP (Journal of Social and Agricultural Economics), 13(2), 182 -199. https://doi.org/10.19184/jsep.v13i2.16441

Atkins, P. and Paula, J. de. (2014). Physical chemistry. In Journal of the Franklin Institute (10th ed., Vol. 272, Issue 1). Oxford University Press. https://doi.org/10.1016/0016-0032(61)90671-8

Auliah, N., Bachri, N., and Sr, M. A. (2024). Development Of Miana (Coleus scutellarioides) Leaf N- Hexane Fraction Emulgel Formulation As An Antibacterial Against Propionibacterium acnes Causes Acne (Acne vulgaris). Indonesian Journal of Pharmaceutical Research, 6(1).

Ayuni, B.F. (2022). Validation Of Caffeine Analysis Method In Latte Coffee With Uv-Vis Spectrophotometry. Analytical: Analytical and Environmental Chemistry, 7(02), 155-164.

Aziz, Y.S., Astuti, S.D. and Wardani, T.S. (2021). Phytopharmaceuticals. Pustaka

Baru Press.

Azizah, Z., Misfadhila, S. and Oktoviani, T.S. (2019). Phytochemical Screening and Antioxidant Activity Test of Methanol Extract of Traditional Processed Coffee Powder of Sungai Full-Kerinci and Aro Wood Tea Using the DPPH Method (1, 1-Diphenyl-2-Pyrrylhydrazil). Higea Journal of Pharmacy, 11(2), 105-112.

Baehaki, F., Nurhamidah, Y. and Purwaeni. (2023). Analysis of Caffeine Levels in Packaged Coffee in Indonesia. Asian Journal Of Health And Applied Sciences, 2(1), 14-19.

Belguidoum, K., Amira-Guebailia, H., Boulmokh, Y. and Houache, O. (2014). HPLC coupled with UV-vis detection for quantitative determination of phenolic compounds and caffeine in different brands of coffee in the Algerian market. Journal of the Taiwan Institute of Chemical Engineers, 45(4), 1–7. https://doi.org/10.1016/j.jtice.2014.03.014

BPS. (2021). Indonesian Coffee Statistics 2021.

Budi, I.M. and Mawardi, A. (2021). Molecular Identification of Genetic Kinship of Wamena Coffee Based on Random Amplified Polymorphic DNA (RAPD) Mark. Papuan Journal of Biology, 13(1), 8-18. https://doi.org/10.31957/jbp.1322

Cappelletti, S., Daria, P., Sani, G. and Aromatario, M. (2015). Caffeine: cognitive and physical performance enhancer or psychoactive drug? Curr Neuropharmacol, 13(1), 71-78.

Clayden, J., Greeves, N. and Stuart, W. (2012). Organic Chemistry. In Oxford University Press (2nd ed.). https://doi.org/10.1080/02603598808072315

Demissie, E.G., Workeneh, G. and Alebe, A. (2016). UV/Vis Spectrometer Determination of Caffeine in Green Coffee Beans From Hararghe Ethiopia. Chemistry and Chemical Engineering, Biotechnology, Food Industry, 17(2), 109 -123.

Fajara, B.E.P. and Susanti, H. (2017). HPLC determination of caffeine in coffee beverage. IOP Conference Series: Materials Science and Engineering, 259(1). https://doi.org/10.1088/1757-899X/259/1/012011

Fajriana, N. H., and Fajriati, I. (2018). Caffeine Content Analysis Of Arabica Coffee (Coffea Arabica L.) At The Variation Of Roasting Temperature In Ultra Violet Spectrophotometry. Analytical and Environmental Chemistry, 3(02), 148–162.

Fajriana, N.H., Fajriati, I., Kimia, J., Sains, F., Teknologi, D., Islam, U., Sunan, N. and Yogyakarta, K. (2018). Analisis Kadar Kafein Kopi Arabika (Coffea

arabica L.) Pada Variasi Temperatur Sangai Secara Spektrofotometri Ultra Violet. Analit: Analytical and Environmental Chemistry, 3(02), 148-162.

Fitriana, L., L, T.A., S, A.D.S. and Raharjo, D. (2024). Formulation and Antibacterial Activity Test of Ethanol Cream Preparation of Mangosteen Fruit Peel Extract (Garcinia mangostana Linn.) Against Propionibacterium Acnes ATCC 1335 Bacteria. Journal of Pharmaceutical and Health Sciences Research, 2(1).

Gopinandhan, T., Banakar, M., Ashwini, M., and Basavaraj. (2014). International Journal of Current Research in Chemistry and Pharmaceutical Sciences. International Journal of Current Research in Chemistry and Pharmaceutical Sciences, 2(1). https://doi.org/10.13140/RG.2.2.11561.44644

Halimatussakdiah, Amna, U. and Wahyuningsih, P. (2018). Preliminary Phytochemical Analysis And Larvicidal Activity Of Edible Fern (Diplazium esculentum (Retz.) Sw.) EXTRACT AGAINST Culex. Natural Journal, 18(3), 141=147. https://doi.org/10.24815/jn.v18i3.11335

Harmita, K., Harahap, Y., and Supandi. (2019). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

Holkar, C.R., Jadhav, A.J. and Pinjari, D.V. (2019). A critical review on the possible remediation of sediment in cocoa/coffee flavored milk. Trends in Food Science and Technology, 86, 199=208. https://doi.org/10.1016/j.tifs.2019.02.035

Hujjatusnaini, N., Indah, B., Afitri, E., Widyastuti, R. and Ardiansyah. (2021). EXTRACTION.

Irawati, D., Styawan, A.A. and Nurhaini, R. (2018). Determination of caffeine levels in Oolong tea (Camellia sinensis) by water-free titration method. Proceedings of the University Research Colloquium, 464-469.

Itana, O. and Negussa, D. (2019). Electrochemical Determination of Caffeine in Laagee Coffee (Ethiopia) With Electropolymerization of Alizarin on Glassy Carbon Electrode. International Journal of Scientific & Engineering Research, 10(12), 962-978.

Kayaputri, I.L., Sumanti, D.M., Djali, M., Indiarto, R. and Dewi, D.L. (2014). Phytochemical Study Of Cocoa Bean Shell Extract (Theobroma cacao L.). Chimica et Natura Acta, 2(1), 83-90. https://doi.org/10.24198/cna.v2.n1.9140

Komes, D.and Vojvodić, A. (2014). Effects of Varieties and Growing Conditions on Antioxidant Capacity of Coffee. Processing and Impact on Antioxidants in Beverages, 77-85. https://doi.org/10.1016/B978-0-12-404738-9.00009-X.

Maria Ulfa, A., & Nofita. (2018). Perbandingan Kadar Kafein Dalam Seduhan Kopi Bubuk Dan Teh Bubuk Dengan Metode Spektrofotometri Uv. Jurnal Analis Farmasi, 3(3), 215-222.

Maria Ulfa, A.and Nofita. (2018). Perbandingan Kadar Kafein Dalam Seduhan Kopi Bubuk Dan Teh Bubuk Dengan Metode Spektrofotometri Uv. Jurnal Analis Farmasi, 3(3), 215-222.

McLellan, T.M. Caldwell, J.A. and Lieberman, H.R. (2016). A review of caffeine's effects on cognitive, physical, and occupational performance. Neuroscience and Biobehavioral Reviews, 71, 294-312. https://doi.org/10.1016/j.neubiorev.2016.09.001

Ministry of Health. (1979). Indonesian Pharmacopoeia Edition III. Ministry of Health of the Republic of Indonesia, Jakarta.

Ministry of Health. (2020). Indonesian Pharmacopoeia Edition VI. Ministry of Health of the Republic of Indonesia.

Mutmainnah, N. (2017). Determination of Temperature and Optimal Time for Brewing Green Tea Stems (Camelia sinensis L.) Against the Content of Antioxidants, Caffeine, Tannins and Catechins.

Nollet, L. M.L. and Toldra, F. (2015). Handbook of Food Analysis Edition III (Third). CRC Press Taylor Francis Group.

Nugraheni, F.T., Dewi, M. and Septiyana, R. (2017). Comparison of Caffeine Crystal Yield in Coffee Beans (Coffea arabica L.) and Chocolate (Theobroma cacao L.) Using the Reflux Method. Scholar Journal of Pharmacy, 1(1), 41-48. https://doi.org/10.31596/cjp.v1i1.6

Padilla Mercado, J.B. (2017). Development Of A Teaching Coulometry Instrument For The Direct Determination Of Sulfur Compounds And Zinc Indirectly.

Pant, A. and Maheshwari, R. (2023). Caffeine Effect On Physical And Mental HealthMental Health Of College Students. World Journal Of Pharmaceutical Research, 12(1), 1734-1754. https://doi.org/10.20959/wjpr20231-26863

Pascawinata, A., Andriansyah and Bismanevi, R. (2021). Effect of speed and length of blood centrifugation time on platelet count in the process of making rich fibrin platelets. B-Dent: Journal of Dentistry, Baiturrahmah University, 8(3), 285-292. https://doi.org/10.33854/jbd.v8i3.604

Perbina, D.I., Ilsanna, C., Simorangkir, D.M., Nerdy and Simarta, H. (2020). Determination of caffeine levels in black tea powder circulating in the old Deli market using UV spectrophotometry. Journal of Pharmaceutical and Herbal Research, 3(1), 21-28. https://doi.org/10.36656/jpfh.v3i1.313

Pisacha, I. M., Putri, D. K., Ardiansyah, & Miftausakina, T. (2023). Penetapan Panjang Gelombang Maksimum, Kurva Baku Dan Kadar Kafein Pada Teh Menggunakan U-HPLC. Jurnal Farmasi Universitas Aisyah Pringsewu, 2(2), 80–85.

Pokhrel, P., Shrestha, S., Rijal, S.K. and Rai, K.P. (2016). A simple HPLC Method for the Determination of Caffeine Content in Tea and Coffee. Journal of Food Science and Technology Nepal, 9, 74-78. https://doi.org/10.3126/jfstn.v9i0.16200

Prasetyo, D., Sitorus, S. and Gunawan, R. (2020). The Use of UV and HPLC Spectrophotometers in the Analysis of Caffeine Content of Arabica and Robusta Coffee. Atomic Journal, 05(2), 76-80.

Rahmadona, S.P., Marzuki, H. and Darhani, C.R. (2022). Identification of Caffeine in Solid Preparation Male Stamina Enhancement Herbal Medicine by Klt-Densitometry. Proceedings of the National Seminar on Applied Science and Technology, 5, 449-458.

Rahmatika, Azrifirwan, and Taib, G. (2023). Analysis of Caffeine Content of Robusta Coffee Powder from the Coffee Powder Industry in Pasaman Regency. AJARCDE (Asian Journal of Applied Research for Community Development and Empowerment), 7(2), 28–32. https://doi.org/10.29165/ajarcde.v7i2.252

Rahmawati, A.I., Wirasti and Rejeki, H. (2021). Analysis of caffeine content in pure coffee powder products produced in Pekalongan Regency using the Performance High-Performance Liquid Chromatography (HPLC) method. Kajen Journal, 5(01), 61-78. https://doi.org/10.54687/jurnalkajenv5i01.6

Rahmawati, I., and Gustiani, L. T. (2023). Analysis of Caffeine in Arabica Coffee (Coffea arabica L.) Gununghalu Light Roasting, Medium Roasting, and Dark Roasting Techniques, 1(2), 66-73.

Ramdan, S.R.K., Yusuf, A.L. and Setiawan, A. (2022). Isolation and identification of caffeine from the leaves of green, black, and oolongoolong leaves using UV Vis spectrophotometry. PHARMACY GENIUS, 01(01), 62-73.

Ramola, B., Kumar, V., Nanda, M., Mishra, Y., Tyagi, T., Gupta, A. and Sharma, N. (2019). Evaluation, comparison of different solvent extraction, cell disruption methods, and hydrothermal liquefaction of Oedogonium macroalgae for biofuel production. Biotechnology Reports, 22. https://doi.org/10.1016/j.btre.2019.e00340

Roossenda, K. and Sunarto. (2016). The Effectiveness Of Solvent In Extraction And Determination Of Caffeine In Typical Regional Soft Drink Using Uv-Vis Spectrophotometer. Department of Chemistry Education, FMIPA,

Yogyakarta State University.

Sarmento, Z., Rangdi, O., De Sena, B. and Dewi, K. (2020). Determination of Paracetamol and Caffeine Levels by Performance High-Performance Liquid Chromatography (HPLC) Method. Cakra Kimia Indonesian E-Journal of AppliedApplied Chemistry, 8(2), 99-104.

Sereshti, H. and Samadi, S. (2014). A rapid and simple determination of caffeine in teas, coffees, and eight beverages. Food Chemistry, 158, 8-13. https://doi.org/10.1016/j.foodchem.2014.02.095

Shiferaw, M., Adane, L. and Alemayehu, I. (2018). Determination of Caffeine Content of Bale Coffee Using HPLC Analysis. Food Science and Quality Management, 73, 23-32.

Sulaiman, I., Marsyanda and Erika, C. (2023). Reduction of caffeine levels in the process of clarified coffee using centrifugation. IOP Conference Series: Earth and Environmental Science, 1182(1), 1-9. https://doi.org/10.1088/1755-1315/1182/1/012045

Sundalian, M. and Nugrahani, I. (2018). Determination of Caffeine Levels in Indonesian Black Tea Products Using FTIR Spectrophotometer. Indonesian Journal of Pharmaceutical Science and Technology, 7(2), 15-21.

Suwiyarsa, I.N., Nurhayati, S. and Hamzah, B. (2018). Analysis Of Caffeine Levels In Local Ground Coffee Circulating In Palu City. Journal of Kim Academic, 7(4), 189-192.

Tyas, D.P. (2021). Validation of the analysis method and determination of caffeine content using the reverse-phase kckt method in black tea samples from PTPN XII Lawang, East Java.

USDA. (2023). Production - Coffee 2022 / 2023. 24–27.

Utami, D.M., Tutik and Ulfa, A.M. (2023). Determination of Flavonoid, Alkaloid, and Phenolic Levels of Methanol Extract of Shallot Peel (Allium cepa L.) Using the Socletasis and Reflux extraction methods. Malahayati Journal of Pharmacy, 6(1), 90-102. https://doi.org/10.33024/jfm.v6i1.8356

Virhananda, M.R.P., Suroso, E., Nurainy, F., Satyajaya Jurusan Teknologi Hasil Pertanian, W., Pertanian, F.and Lampung, U. (2022). Analisis Kadar Asam Klorogenat dan Kafein Berdasarkan Perbedaan Lokasi Penanaman dan Suhu Roasting Pada Kopi Robusta (C. canephora Pierre). Agroindustri Berkelanjutan, 1(2), 245-252.

Wahyuni, M. and Aini, S. (2021). Synthesis of Prussian Blue (Fe4[Fe(CN)6]3) dye made of natural iron sand. Chemistry Journal of Padang State University, 10(1). https://doi.org/10.22437/jop.v3i2.5730

Wahyuni, S. and Marpaung, M.P. (2020). Determination of Total Alkaloid Levels of Yellow Root Extract (Fibraurea Chloroleuca Miers) Based on the Difference in Ethanol Concentration by UV-VIS Spectrophotometry Method. Dalton: Journal of Chemistry and Chemical Science Education, 3(2), 52-61. https://doi.org/10.31602/dl.v3i2.3911

Wardani, R.K. and Fernanda, M.A.H.F. (2016). Analysis of Caffeine Levels from Black Tea, Green Tea, and White Tea Powder (Camellia sinensis L.). Journal of Pharmacy and Science, 1(1), 15-17. https://doi.org/10.53342/pharmasci.v1i1.48

Weldegebreal, B., Redi-Abshiro, M. and Chandravanshi, B.S. (2017). Development of new analytical methods for the determination of caffeine content in aqueous solution of green coffee beans. Chemistry Central Journal, 11(1), 1-9. https://doi.org/10.1186/s13065-017-0356-3

Widhyani, R., Rahmasari, K.S., Kristiyanti, R. and Slamet. (2021). Determination of Caffeine Levels in Packaged Dry Tea for Tea Industry Production in Pekalongan. Journal of Pharmaceutical Sciences, 12(1), 29-35.

Wigati, E.I., Pratiwi, E., Nissa, T.F. and Utami, N.F. (2018). Phytochemical Characteristics and Antioxidant Activity of Robusta Coffee Beans (Coffea canephora Pierre) from Bogor, Bandung, and Garut by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Phytopharmacology Journal of Pharmaceutical Sciences, 8(1), 59-66.

Wilantari, P.D., Putri, Putra, Ngraha, Syawalistianah, Prawitasari and Samirani. (2018). Isolation of Caffeine by Sublimation Method from Ethyl Acetate Fraction of Camelia sinensis Leaf Powder. Udayana Journal of Pharmacy, 7(2), 53-62. https://doi.org/10.24843/jfu.2018.v07.i02.p03

Willson, C. (2018). The clinical toxicology of caffeine: A review and case study. Toxicology Reports, 5, 1140-1152. https://doi.org/10.1016/j.toxrep.2018.11.002

Yenrina, R. (2015). Methods of Analysis of Food Ingredients and Bioactive Components. In Andalas University Press.

Zhang, H., Sun, C. and Ge, M. (2022). Review of the Research Status of Cost-Effective Zinc–Iron Redox Flow Batteries. Batteries, 8(11), 1-33. https://doi.org/10.3390/batteries8110202.