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Determination of caffeine in Robusta coffee beans with different roasting method using UV-Vis spectrophotometry

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Abstract

Caffeine is one of the substances in coffee. Several factors affect the caffeine content in coffee, such as harvesting, post-harvesting, roasting, drying, and storage. This study aimed to compare the roasting methods to caffeine content from two samples of robusta coffee beans (from two different areas in east Java, Indonesia) and determine the caffeine using UV-Vis spectrophotometry. Light (180°C), medium (210°C), and dark (240°C) roast profiles were used. This method was validated pertaining to linearity, precision and accuracy studies, limit of detection (LOD) and limit of quantification (LOQ). The result of the validation method showed the specificity of caffeine in coffee with dichloromethane as solvent at a Wavelength of 275 nm. The linearity showed linear results at a concentration of 5-40 ppm. The linear correlation coefficient (r^2) was 0.9997. The LOD and LOQ were 0.57 ppm and 1.90 ppm, respectively. The accuracy method showed % recovery in the range of 97.9-99.6%. The precision results showed % relative standard deviation of between 0.9-1.0%. The result showed that the highest caffeine content was found in the light roast profile up to 8%. Based on the statistical test, there is no difference in caffeine content between the two samples ($p>0.05$). In conclusion, the different roasting methods affect the caffeine content ($p<0.05$). The caffeine content will decrease as the roasting temperature increases. The method was established to be simple, linear, precise, accurate as well as sensitive and can be applied to determining the caffeine content in coffee.

1. Introduction

Coffee is one of the most popular and highly consumed drinks, especially in Indonesia. Recent studies have shown that consumption of 2 up to 3 cups of coffee per day has beneficial effects on human health, including cardiovascular health, several types of cancer, psychoactive response, neurodegenerative diseases, metabolic disorders, and liver functions (Dórea and da Costa, 2005; Bawazeer and Alsobahi, 2013; Grosso *et al*., 2017; Jeon *et al*., 2019; Dong *et al*., 2020; Chieng and Kistler, 2021). Coffee consists of several bioactive compounds, such as trigonelline, chlorogenic acid, tannic acid, nicotinic acid, quinolinic acid, pyrogallic acid, and caffeine (Kim *et al*., 2012; Khalid and Ahmad, 2016; Muhammed *et al*., 2021).

Several factors affect the quality of coffee beans, such as harvesting, post-harvesting, processing, drying, and storage. However, roasted coffee beans are one of the most important factors influencing the final quality of the drink. Roasting is, therefore, among the steps that require significant attention because only in roasting that the characteristic coffee flavour, aroma, and colour formed (Pires *et al*., 2021; Ratanasanya *et al*., 2021). In general, roasting coffee beans is divided into three profiles: light, medium, and dark, with a temperature range of 150°C - 250°C. Some research studied the effect of roasting on caffeine content in Robusta coffee beans. According to Hečimović *et al*. (2011) and Casal *et al*. (2000), the roast temperature affects caffeine content. High temperature caused caffeine degradation. During the roasting process, caffeine compounds will decrease their content due to sublimation and dragging by vapour (Hečimović *et al*., 2011; Ee Shan *et al*., 2016; Severini *et al*., 2018).

Several studies analyzed caffeine in coffee using the HPLC method (Casal *et al.*,2000; Hečimović *et al*., 2011; Jeszka-Skowron *et al*., 2016) and the UV-Vis Spectrophotometry (Dobrinas *et al*., 2013; Gizachew Demissie *et al*., 2016; Tadesse Wondimkun, 2016). The UV-Vis Spectrophotometry method was selected because it is easy to use, efficient in cost, sensitive, accurate, and precise (Belay *et al*., 2008; Hečimović *et*

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al., 2011; Navvara *et al*., 2017). However, there is no research on caffeine analysis on robusta coffee beans from Indonesia with different roasting methods using UV-Vis Spectrophotometry. Therefore, this study was conducted. In this research, the effect of the roasting method on caffeine content will be studied. This is to ensure people can consider the roasting process of the caffeine content in the coffee they will consume.

2. Materials and methods

2.1 Materials

The sample of Robusta coffee (*Coffea canephora*) beans from Kawi Mountainside and Dampit, Malang, East Java, Indonesia. The chemicals: dichloromethane pro-analysis (Sigma-Aldrich, Darmstradt, Germany), aquadest from Makmur Sejati store in Malang, Indonesia and caffeine standard (Tokyo Chemical Industry, Japan).

2.2 Apparatus

Analytical balance (Shimadzu AUW220, Japan), coffee roaster (Global Agro model C03J3), coffee grinder (Bartex Type JY09A-4), sieve wire mesh no. 60, hotplate (IKA C MAG HS 7) and UV-1800 UV-Vis Spectrophotometer from Shimadzu, Japan.

2.3 Roasting coffee beans

This research refers to Tewabe Gebeyehu (2015) method with modification of weighing samples. The coffee beans weighed about 320 g for each profile (light, medium, dark). Then the samples were roasted at temperatures of 180°C, 210°C, and 240°C to produce light, medium, and dark roast profiles Then ground it into powder and screened by sieve.

2.4 Sample extraction

Sample extraction in this study refers to the method (Tewabe Gebeyehu, 2015). Coffee powder weighed 50 mg and dissolved in 100 mL of distilled water. Then, the coffee solution was heated at 90°C and stirred for 30 mins using a magnetic stirrer. Next, the coffee solution was filtered into a 100 mL volumetric flask, and aquadest was added to the boundary mark. Then, 25 mL was taken and extracted with 25 mL dichloromethane using a separating funnel. The aqueous phase was extracted three times again with 25 mL of dichloromethane for each extract. The results of the organic phase were put into a 100 mL volumetric flask, and dichloromethane was added to the boundary mark. This solution was used as a test solution. The procedure of sample extraction was replicated 4 times for each profile.

2.5 Method validation

2.5.1 Specificity

This test was carried out by measuring the spectrum of caffeine standards in the range of 200-400 nm, then compared with the spectrum of coffee samples in each roast profile.

2.5.2 Linearity

The linearity was carried out by the accurately weighed amount of caffeine standard (25 mg) then dissolved in dichloromethane to obtain 5 concentrations namely 5, 10, 20, 30 and 40 ppm.

2.5.3 Accuracy and precision

The caffeine standard was weighed at 50 mg and dissolved in 50 mL of distilled water. After that, 4, 5, and 6 mL were taken for concentrations of 80%, 100%, and 120%, then put into a 100 mL volumetric flask and aquadest was added to the boundary mark. The solution was heated, filtered, and extracted with dichloromethane four 4 times. The organic phase was collected in a 100 mL volumetric flask quantitatively. The procedure was repeated three times for each concentration. The absorbance of the test solution was measured by measuring the test solution at the wavelength of maximum absorbance of 275 nm. Then calculated % recovery and % RSD values.

2.5.4 Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were obtained based on the standard deviation of the response and slope of the calibration. To evaluate LOD and LOQ, five different concentrations were assessed 5, 10, 20, 30 and 40 ppm. In this study, the LOD and LOQ of the methods were calculated as:

$$LOD = 3.3\frac{\sigma}{s} \qquad LOQ = 10\frac{\sigma}{s}$$

Where s is the slope of the calibration curve and σ is the standard deviation of the y-intercept of the regression line (Ihsan et al., 2020).

2.5.5 Determination of caffeine content in Robusta coffee beans

The absorbance of the test solution was measured by measuring the test solution at the wavelength of maximum absorbance of 275 nm. The caffeine concentration of the test solution was calculated by plotting the caffeine standard curve. The caffeine content in the sample is expressed in %w/w.

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$$\text{Caffeine Content}\left(\%\frac{w}{w}\right) = \frac{\text{caffeine mass obtained}}{\textit{sample mass}} \times 100\%$$

3. Results and discussion

3.1 Specificity

This test was conducted by observing the maximum wavelength in the 200-400 nm range. The previous researchers who analyzed caffeine found that caffeine was detected at 277 nm and 273.5 nm (Dobrinas *et al*., 2013; Gizachew Demissie *et al.,* 2016). In this study, the wavelength of maximum absorbance of caffeine standard was obtained at 275 nm. Similarly, coffee samples obtained spectra at wavelengths of 271.50 nm (light), 271.10 nm (medium), and 274.20 nm (dark). The results show the similarity of the spectrum of the caffeine standard to the caffeine analyte in the sample. This method was specific for determining caffeine in the sample at an analytical wavelength of 275 nm. The spectrum of caffeine standards with coffee samples is shown in Figure 1.

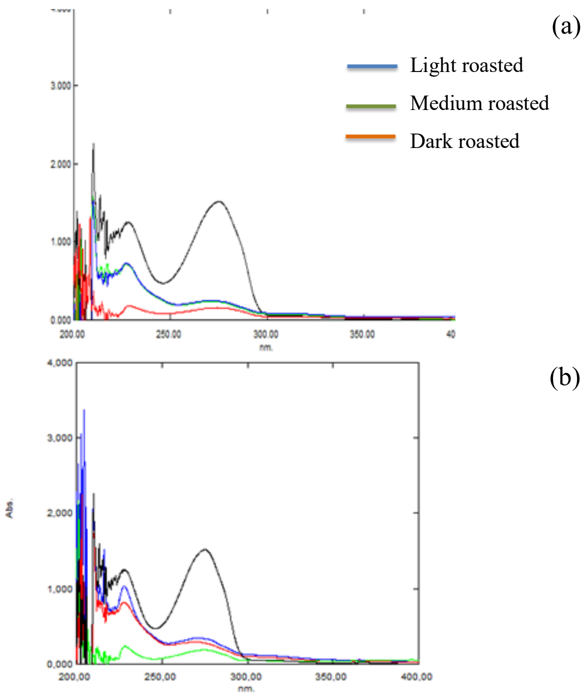

Figure 1. The spectrum of caffeine standard with Robusta coffee from (a) Kawi and (b) Dampit.

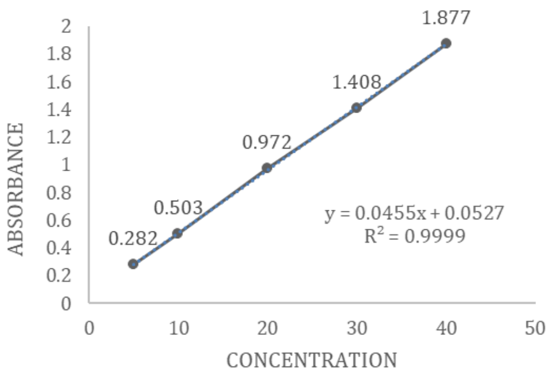

Figure 2. Calibration curve of caffein standard.

3.2 Linearity

Linearity was obtained from the standard caffeine solution with five concentrations such as 5, 10, 20, 30, and 40 ppm and absorbances were measured at a wavelength of 275.2 nm. The linear standard equation was obtained $y = 0.0455x + 0.0527$ with r^2 of 0.9999. The graph of linearity is presented in Figure 2. Based on the determination coefficient (r^2) in Figure 2, found that the tests performed were considered good and acceptable according to the terms $r^2 \geq 0.99$ (AOAC, 2002).

3.3 Limit of detection and limit of quantification

LOD and LOQ were used to determine the limits of the data The data is above the boundary and can be observed with a linear equation. The LOD and LOQ values were 0.570 ppm and 1.901 ppm, respectively. If compared with other researchers like Dobrinas *et al.* (2013), the acquisition values of LOD and LOQ were not significantly different (LOD was 0.85 ppm and LOQ was 1.52 ppm).

3.4 Accuracy and precision

Accuracy was done to determine the closeness between the analysis results with the actual analyte content. Accuracy was expressed with % recovery. Precision was obtained from the calculation of % RSD which indicates the accuracy of the measurement. In this study, the accuracy of each concentration fulfils the requirements in the range of 95-102% and it was found that % RSD of each concentration met the requirements of below 1.5% (AOAC, 2002). The results of % recovery and % RSD are presented in Table 1.

3.5 Caffeine content

The average caffeine content (%w/w) in the coffee sample from Kawi for each profile was 8.80% for light, 7.42% for medium, and 5.71% for the dark profile. In comparison, the coffee sample from Dampit has an average of 8.19% for light, 7.36% for medium, and 5.65% for dark profile. The data were tested with a One-Way Analysis of Variance (ANOVA) using Minitab 18 (United States). Based ANOVA test, there are no differences ($p>0.05$) between caffeine content on samples from Kawi and Dampit, and the caffeine content from each roast profile (light, medium, dark) is significantly different ($p<0.05$). The caffeine contents by the light roasting method are highest. It is shown that the roasting process affects the caffeine content in coffee beans. The longer time and the higher temperature used during roasting will decrease the caffeine content (Mumin *et al.,* 2006). Another cause of high caffeine content was the possibility of interference from the chlorogenic acid compound that took when the

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Table 1. Value of % Recovery and RSD.

Concentration	Absorbance	% Recovery	Mean of % Recovery	SD	% RSD
80%	0.500	97.9	98.6	0.09	0.92
	0.508	99.7			
	0.502	98.4			
100%	0.605	96.7	97.9	0.13	1.05
	0.614	98.3			
	0.616	98.7			
120%	0.737	99.9	99.6	0.14	0.92
	0.728	98.6			
	0.740	100.3			

extraction process took place. The caffeine extraction process in coffee makes it possible to interfere with the chlorogenic acid compound in coffee (Belay *et al*., 2008; Esquivel and Jiménez, 2012; Navarra *et al*., 2017). Heat treatment during roasting should be able to break the bond of the chlorogenic acid from the caffeine to produce caffeine in a free base condition hence the caffeine content in coffee beans decreases. Therefore, controlling the length of time and the roast temperature became an important factor in affecting the caffeine content that coffee beans will produce.

Caffeine content (%w/w) in the sample of robusta coffee beans can be seen in Table 2. Based on Table 2, the caffeine content in robusta coffee was 5-8%. The acquisition of caffeine contents was above the quality requirements of the Indonesian National Standard (SNI) which is 0.45 – 2% w/w (SNI, 2004). Caracostea *et al.* (2020) also analysed the caffeine content in Robusta coffee using UV-Vis spectrophotometer. The caffeine content in Robusta coffee was found 1.82% w/w. According to Skowron *et al.* (2016), the caffeine content of robusta coffee beans in Indonesia was 8.17%. That caffeine content was higher than in other countries such

Table 2. Results of caffeine content in robusta coffee beans from Kawi and Dampit

Sample	Roast Profile	Absorbance	Concentration (µg/mL)	Content (% w/w)	Mean of Content (% w/w)
Robusta coffee from Kawi	Light	0.556	11.10	8.84	8.80±0.63
		0.536	10.60	8.50	
		0.515	10.20	8.13	
		0.588	11.80	9.40	
		0.571	11.40	9.12	
	Medium	0.485	9.50	7.60	7.42±0.28
		0.469	9.15	7.32	
		0.484	9.48	7.55	
		0.482	9.43	7.55	
		0.456	8.86	7.07	
	Dark	0.382	7.23	5.76	5.71±0.21
		0.370	6.69	5.57	
		0.384	7.27	5.80	
		0.367	6.89	5.50	
		0.389	7.38	5.89	
Robusta coffee from Dampit	Light	0.528	10.40	8.26	8.19 ±0.39
		0.502	9.87	7.81	
		0.558	11.10	8.82	
		0.509	10.00	7.96	
		0.519	10.20	8.12	
	Medium	0.484	9.48	7.55	7.36±0.29
		0.446	8.64	6.90	
		0.477	9.32	7.44	
		0.488	9.57	7.62	
		0.472	9.21	7.31	
	Dark	0.382	7.23	5.78	5.65±0.11
		0.373	7.03	5.62	
		0.379	7.16	5.70	
		0.374	7.05	5.63	
		0.366	6.87	5.50	

as Vietnam, India, and Uganda, which were 3 - 7.4%. This matter can be caused by robusta coffee beans' content that varies depending on the type or variety and the surrounding environment.

4. Conclusion

The roasting methods have an effect on caffeine content. The light roasting method gives the highest caffeine content. This method was validated pertaining to linearity, precision and accuracy studies, LOD and LOQ. The method was established to be simple, linear, precise, accurate as well as sensitive and can be applied to determining the caffeine content in coffee.

Conflict of interest

The authors declare no conflict of interest.

References

AOAC. (2002). AOAC International Methods Committee Guidelines for Validation of Qualitative and Quantitative Official Methods of Analysis. Washington, DC, USA: Association of Official Analytical Chemists.

Bawazeer, N.A. and Alsobahi, N.A. (2013). Prevalence and Side Effects of Energy Drink Consumption among Medical Students at Umm Al-Qura University, Saudi Arabia. *International Journal of Medical Students International Journal of Medical Students*, 1(3), 104-108. https://doi.org/10.5195/ijms.2013.215

Belay, A., Ture, K., Redi, M. and Asfaw, A. (2008). Measurement of caffeine in coffee beans with UV/vis spectrometer. *Food Chemistry*, 108(1), 310–315. https://doi.org/10.1016/j.foodchem.2007.10.024

Caracostea, L.-M., Sirbu, R. and Busurico, F. (2020). Determination of Caffeine Content in Arabica and Robusta Green Coffee of Indian Origin. *European Journal of Medicine and Natural Sciences*, 6397, 16–24.

Casal, S., Oliveira, M.B. and Ferreira, M.A. (2000). Analytical, Nutritional and Clinical Methods Section HPLC/diode-array applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. *Food Chemistry*, 68(4), 481-485. https://doi.org/10.1016/S0308-8146(99)00228-9

Chieng, D. and Kistler, P.M. (2021). Coffee and tea on cardiovascular disease (CVD) prevention. *Trends in Cardiovascular Medicine*, 32(7), 399-405. https://doi.org/10.1016/j.tcm.2021.08.004

Dobrinas, S., Soceanu, A., Popescu, V., Stanciu, G. and Smalberger, S. (2013). Optimization of A UV-Vis Spectrometric Method For Caffeine Analysis in Tea, Coffee and Other Beverages. *Scientific Study and Research. Chemistry and Chemical Engineering, Biotechnology, Food Industry*, 14(2), 71–78.

Dong, X., Li, S., Sun, J., Li, Y. and Zhang, D. (2020). nutrients Association of Coffee, Decaffeinated Coffee and Caffeine Intake from Coffee with Cognitive Performance in Older Adults: National Health and. *Nutrients*, 12(3), 840. https://doi.org/:10.3390/nu12030840

Dórea, J.G. and da Costa, T.H.M. (2005). Is coffee a functional food? *British Journal of Nutrition*, 93(6), 773–782. https://doi.org/10.1079/bjn20051370

Ee Shan, O., Zzaman, W. and Yang, T.A. (2016). Tropical Agricultural Science Impact Of Different Temperature-Time Profiles During Superheated Steam Roasting On Some Physical Changes Of Robusta Coffee. *Pertanika Journal of Tropical Agricultural Science*, 39(3), 311-320.

Esquivel, P. and Jiménez, V.M. (2012). Functional properties of coffee and coffee by-products. *Food Research International*, 46(2), 488–495. https://doi.org/10.1016/j.foodres.2011.05.028

Gizachew Demissie, E., Workeneh, G. and Arayaselassie Abebe, S. (2016). UV/Vis Spectrometer Determination Of Caffeine In Green Coffee Beans From Hararghe, Ethiopia, Using Beer-Lambert's Law And Integrated Absorption Coefficient Techniques. *Scientific Study and Research,* 17(2), 109-123.

Grosso, G., Godos, J., Galvano, F. and Giovannucci, E.L. (2017). Coffee, Caffeine, and Health Outcomes: An Umbrella Review. *Annual Review of Nutrition*, 37, 131–156. https://doi.org/10.1146/annurev-nutr-071816-064941

Hečimović, I., Belščak-Cvitanović, A., Horžić, D. and Komes, D. (2011). Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. *Food Chemistry*, 129(3), 991–1000. https://doi.org/10.1016/j.foodchem.2011.05.059

Ihsan, B.R.P., Hafid, A.F., Primaharinastiti, R., Widyawaruyanti, A. and Yuwono, M. (2020). Development and validation of hplc method for determination of andrographolide in raw material and tablet of ethyl acetate fractions of *Andrographis paniculata*. *Research Journal of Pharmacy and Technology*, 13(9), 4291-4296. https://doi.org/10.5958/0974-360X.2020.00758.1

Jeon, J.S., Kim, H.T., Jeong, I. H., Hong, S.R., Oh, M.S., Yoon, M.H., Shim, J.H., Jeong, J.H. and Abd El-Aty, A.M. (2019). Contents of chlorogenic acids and caffeine in various coffee-related products. *Journal of Advanced Research*, 17, 85–94. https://doi.org/10.1016/j.jare.2019.01.002

Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K. and De Peña, M.P. (2016). Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *European Food Research and Technology*, 242(8), 1403–1409. https://doi.org/10.1007/s00217-016-2643-y

Khalid, A. and Ahmad, S. (2016). Determination of Caffeine in Soft and Energy Drinks Available in Market by using UV/Visible Spectrophotometer. *Family Medicine and Medical Science Research*, 5 (4), 3–8. https://doi.org/10.4172/2327-4972.1000206

Kim, J., Lee, S., Shim, J., Kim, H.W., Kim, J., Jang, Y.J., Yang, H., Park, J., Choi, S.H., Yoon, J.H., Lee, K.W. and Lee, H.J. (2012). Caffeinated coffee, decaffeinated coffee, and the phenolic phytochemical chlorogenic acid up-regulate NQO1 expression and prevent H_2O_2-induced apoptosis in primary cortical neurons. *Neurochemistry International*, 60(5), 466–474. https://doi.org/10.1016/j.neuint.2012.02.004

Muhammed, B.L., Seid, M.H. and Habte, A.T. (2021). Determination of caffeine and hydrogen peroxide antioxidant activity of raw and roasted coffee beans around habru woreda, ethiopia using UV-Vis spectroscopy. *Clinical Pharmacology: Advances and Applications*, 13, 101–113. https://doi.org/10.2147/CPAA.S311032

Mumin, A., Abdul Mumin, M., Farida Akhter, K., Zainal Abedin, M. and Zakir Hossain, M. (2006). Determination and Characterization of Caffeine in Tea, Coffee and Soft Drinks by Solid Phase Extraction and High Performance Liquid Chromatography (SPE-HPLC). *Malaysian Journal of Chemistry*, 8(1), 45-51.

Navarra, G., Moschetti, M., Guarrasi, V., Mangione, M.R., Militello, V. and Leone, M. (2017). Simultaneous determination of caffeine and chlorogenic acids in green coffee by UV/Vis spectroscopy. *Journal of Chemistry*, 2017, 6435086. https://doi.org/10.1155/2017/6435086

Pires, F.de C., Pereira, R.G.F.A., Baqueta, M.R., Valderrama, P. and Alves da Rocha, R. (2021). Near -infrared spectroscopy and multivariate calibration as an alternative to the Agtron to predict roasting degrees in coffee beans and ground coffees. *Food Chemistry*, 365, 130471. https://doi.org/10.1016/j.foodchem.2021.130471

Ratanasanya, S., Chindapan, N., Polvichai, J., Sirinaovakul, B. and Devahastin, S. (2021). Model-based optimization of coffee roasting process: Model development, prediction, optimization and application to upgrading of Robusta coffee beans. *Journal of Food Engineering*, 318, 110888. https://doi.org/10.1016/j.jfoodeng.2021.110888

Severini, C., Derossi, A., Ricci, I., Caporizzi, R. and Fiore, A. (2018). Roasting Conditions, Grinding Level and Brewing Method Highly Affect the Healthy Benefits of A Coffee Cup. *International Journal of Clinical Nutrition and Dietetics*, 4, IJCND-127. https://doi.org/10.15344/2456-8171/2018/127

SNI. (2004). SNI 01-3542-2004: Kopi Bubuk. Retrieved from website: http://sispk.bsn.go.id/SNI/DaftarList?q=SNI+01-3542-2004%3A+Kopi+Bubuk

Tadesse Wondimkun, Z. (2016). The Determination of Caffeine Level of Wolaita Zone, Ethiopia Coffee Using UV-visible Spectrophotometer. *American Journal of Applied Chemistry*, 4(2), 59-63. https://doi.org/10.11648/j.ajac.20160402.14

Tewabe Gebeyehu, B. (2015). Determination of Caffeine Content and Antioxidant Activity of Coffee. *American Journal of Applied Chemistry*, 3(2), 69-76. https://doi.org/10.11648/j.ajac.20150302.16

