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 range of 5-40 ppm. The linear correlation coefficient (r²) 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 sublimination 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
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:

\[
\text{LOD} = \frac{3.3 \text{ } \sigma}{s} \\
\text{LOQ} = \frac{10 \text{ } \sigma}{s}
\]

Where \(s\) is the slope of the calibration curve and \(\sigma\) 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.
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.

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 fulfills 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...
The 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 as...
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


