Determination of Caffeine Content at the Difference in Arabica and Robusta Coffee Roast Levels Using HPLC

I Farikha A [1], L Eka [2], Ngatinem [3], A Rahayuningtyas[2], B.Ulum[1]*

[1] Directorate of Laboratory Management Research Facilities and Science Technology Areas
[2]Center Research of Appropriate Technology, National Research and Innovation Agency
[3] Center Research of Medicinal Raw Materials and Traditional Medicine, National Research and Innovation Agency

Abstract. Coffee is one of the refreshing plants that can provide a stimulating effect for consumption. Two types of coffee that are popular among the public are Arabica and Robusta. Coffee **contains** caffeine. Consumption of large amounts of caffeine can have an impact on human health. The development of research so that development is carried out by determining caffeine from different roasting levels which are Light, medium and Dark. This study aims to determine the caffeine content in Arabica and Robusta coffee at different roasting levels using the HPLC (High-Performance Liquid Chromatography) method. The samples used as much as 5 grams. The method used is the determination of caffeine using HPLC (High-Performance Liquid Chromatography). Then taken to be injected into a 1.5 ml vial and ready to be injected into the HPLC device using HPLC Agilent 1260 Infinity II, C18 and UV Vis detector with a wavelength of 275 nm. The mobile phase used was distilled water and methanol with a flow rate of 1 ml/min. The results of this study determined caffeine levels in Arabica with roasting levels of light 0.0779, medium 0.0893, and dark 0.0909 mg/g. While Robusta Coffee contains caffeine at a roasting light level of 0.1399, medium 0.1432 and dark of 0.2115 mg/g.

1 Introduction

Coffee is one of the refreshing plants that if processed can be one of the refreshing drinks. Coffee in the world is known to have 3 types, namely Arabica, robusta and Liberia/Excelsa. The type of coffee has regional characteristics depending on the coffee that is grown. Coffea arabica accounts for 70% of world coffee production, arabica accounts for 70% of world coffee production, and C. canephora and Coffea liberica account for the rest [1]. Coffee is one of the most popular drinks in the world. Initially, coffee was produced and consumed only in Middle Eastern countries, but now it has spread all over the world and has become a popular drink in America and Europe [2].

* Corresponding author: farikha2605@gmail.com

Over the past ten years, numerous research has concentrated on physiologically active compounds, particularly alkaloids in beverages, due to their potential health benefits for humans [3]. Methylxanthine is an alkaloid frequently present in food. According to[4], animals and plants inherently synthesize these chemicals, specifically caffeine, theobromine, and theophylline, which are the most extensively researched. These chemicals are predominantly located in food items such as coffee beans, cocoa beans, and tea leaves [5]. Caffeine is an alkaloid commonly present in coffee beans and leaves. Moreover, it is also present in tea leaves and cacao seeds.

Caffeine (1,3,7-trimethyl xanthine) is an alkaloid naturally found in the beans, leaves, and fruits of cocoa, coffee, tea, and over sixty other plant species. The widespread availability of many plants has contributed to the enduring global popularity of caffeine and caffeinated goods. Caffeine, a naturally occurring substance, is the most extensively consumed legal psychostimulant today and is found in various foods and beverages. Caffeine possesses a distinctive flavor and is used in soft drinks as a flavoring agent to enhance their overall quality. Caffeine, while utilized as a flavor enhancer, raises significant health safety concerns for consumers at present. The caffeine in coffee is recognized for its diverse physiological effects, including enhanced alertness, less weariness, improved long-term memory and focus, and augmented physical performance. Excessive intake may correlate with hypertension, anxiety, hyperactivity, and headaches [6].

Coffee storage can be done with green beans or in the form of beans after roasting or powder. This will affect the quality of the coffee. The roasting process greatly determines the quality of the coffee. Roasted coffee can be divided into 3 levels, namely light, medium and dark. This difference will determine the content of alkaloids and caffeine in roasted coffee. Determination of caffeine levels can be done using the High-Performance Liquid Chromatography (HPLC) method. This method allows the separation and analysis of caffeine compounds from other compounds in coffee. In addition to the difference in roasting, the type of coffee also has a different caffeine content. This study aims to determine the caffeine content in 2 types of coffee with 3 roast levels using the HPLC method.

2 Method

The tools used include Erlenmeyer, beaker glass, funnel, analytical scale, sonicator, roaster, HPLC Agilent 1260 Infinity II with Diode Array Detector (DAD) and HPLC C18 Column. The ingredients used include 2 varieties of coffee, Arabica and Robusta which have been roasted at 3 levels light, medium, and dark roasting. Other materials used include filter paper, and water for chromatography, as the Motion phase uses a mixture of 40% water and 60% methanol and a standard series of caffeine 0, 20, 50, 75 and 100 ppm.

2. 1 Sample Preparation

Roast 2 types of Arabica and robusta coffee at 3 different levels (light, medium, dark) then grind the coffee into powder. Perform caffeine extraction from coffee grounds, weigh 0.3 grams of coffee grounds for example, add aquadest water solvent in Erlenmeyer, and keep in a water bath for 30 minutes. Cool and strain the extract solution. Take 1 ml for each sample and add water for chromatography on a 10 ml flask to the cut-off mark. Then sonicator for 15 minutes.

2. 2 Determination of Caffeine Levels using HPLC

Take about 1.5 ml of sample then strain with 0.45 µl into the vial. Inject the extract solution into the HPLC column. Perform a standard curve calibration until a regression line is obtained. The process of separating and measuring caffeine content uses a flow rate of 1 ml/minute, a wavelength of 275 nm, and an injection volume of 10 µl. the time used until it stops is 10 minutes. The temperature of the column is 40^0C, and the phase of motion used is water and methanol with a ratio of 40% : 60%.

The sample solution will be measured for absorption at the maximum length of the sample then an area of the sample and standard will be recorded. The concentration of caffeine is determined based on the regression equation of the standard calibration curve. The calculation of the caffeine content in coffee based on the standard curve of caffeine can be done using the following formula:

$$Caffeine\ Content\ \left(\frac{mg}{g}\right) = \left(\frac{soluble\ concentration\left(\frac{mg}{L}\right) x\ \ dilute\ volume\ (L)\ x\ FP}{weigh\ (g)}\right)$$

Remarks:

Concentration mg/L : from the result of linear regression (y=bx+a)

FP : Dilution Factor

3 Results and Discussion

Coffee cultivation greatly affects the quality of coffee in terms of taste, aroma and chemical content. In addition, the coffee processing process also greatly determines the coffee yield that can be consumed. Coffee that has been harvested in the form of cherries is then selected by soaking in water, then if the coffee is good, it will sink to the bottom of the water and the floating one has little nutritional content. After the coffee selection process, it is then dried until the moisture content reaches 15 to 20 percent. Then the coffee is separated from the husk and the beans. Until now, there has not been too much use of dried coffee skin. As for coffee beans after being separated from the skin, they can be stored after reaching a moisture content of 12% or less than 15%. This is to prevent multiple it damages to the coffee beans.

This study uses selected Subang arabica and robusta coffee. Coffee in the form of green beans is then roasted until the green color becomes light, medium and dark. This process is to determine the caffeine content of each roast level of each type of coffee using the HPLC method. The HPLC method is the most efficient and accurate in determining caffeine levels.

After the standard and sample are ready in the vial, the process will be carried out on the HPLC tool. The results showed that the caffeine content in coffee varied depending on the type of coffee and the roast level. Robusta coffee generally has the highest caffeine content, followed by Arabica and Liberica coffees. The longer the roast level, the higher the caffeine content in the coffee. This is because the roasting process can increase the concentration of caffeine by evaporating water and other compounds.

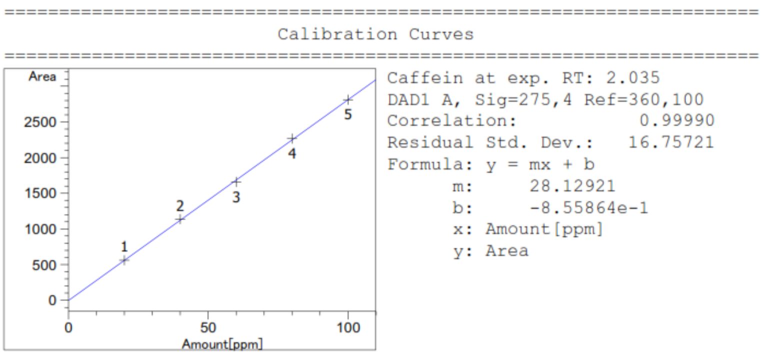

Fig 1. Calibration Curve Graph Caffeine Standard Regression Analysis

The ideal flow rate of eluent is established at 1.0 ml/min to identify the caffeine standard and determine the rate for all analyses. The coated UV spectrum exhibits a favourable response at 275 nm for caffeine. Under optimised conditions, caffeine was isolated with a resolution above 4, and the retention duration was determined to be 2.035 minutes (Figure 1). The calibration curve for caffeine was constructed using the area of chromatographic peaks, demonstrating exceptional linearity, as illustrated in the figure, with a high regression factor of 0.9999. The linear regression equation is y = 28.12921x - 8.55864.

Table 1. Results of Analysis of Caffeine Levels of Arabica Coffee and Robusta with Differences in Roasting Levels

Varietis	Level Roasting	Average from 3 repeat	RPD
A	L	0.0779	0.0008
	M	0.0893	0.0011
	D	0.0909	0.0037
R	L	0.1399	0.0014
	M	0.1432	0.0021
	D	0.2115	0.0030

The results of this study show that each roasting level has a difference in caffeine content. This can be due to the roasting process. The longer the coffee is roasted, the higher the caffeine content. Although the difference is not significant enough, there are differences in content. Coffee that is roasted longer undergoes a discoloration process which means that the caffeine is stronger with a high concentration. In addition to the roast level, each type of coffee has its characteristics. The difference can be caused by factors such as the environmental conditions of coffee growing, the processing process and the storage process. Arabica coffee has its characteristics among the stronger and softer aromas in terms of taste. In contrast to robusta which has a softer aroma and is not too strong but has a stronger coffee taste. When robusta coffee beans are roasted, a stronger flavor will appear. The coffee produced in each region also has a distinctive taste from its region. Robusta coffee with a dark roast level has a higher caffeine content than arabica and coffee levels below.

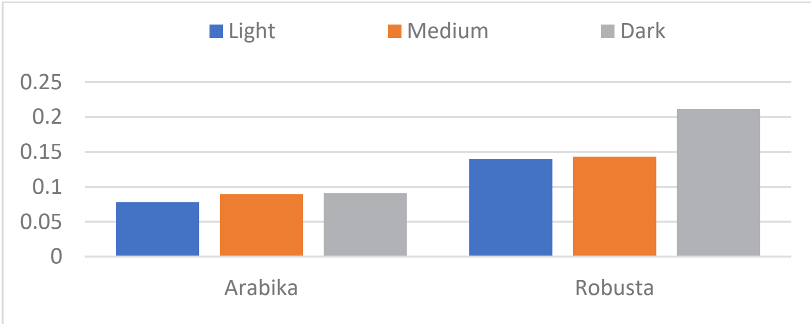

Fig 2. Result of Average Caffeine Content

The results showed that the caffeine content in coffee varied depending on the type of coffee and the roast level. Robusta coffee has the highest caffeine content, followed by Arabica and Liberica coffees. The darker the roast level of the coffee, the higher the caffeine content.

The difference in caffeine levels in coffee is influenced by the process during coffee making, such as the amount of caffeine lost due to damage or dissolution in boiling water and the high temperature in the coffee bean roasting process. Caffeine itself is a pseudo-alkaloid and belongs to the type of purine alkaloid (xanthine) which has two carbon rings with four nitrogen atoms and has chemical properties that are easily decomposed by heat and light in the presence of oxygen [7]. This research is in line with the caffeine level in black ground coffee is less than 5 mg/g [8].
This research is also supported by research conducted by Tjahjani and research [9], which proves that caffeine levels will decrease in the process of roasting coffee using high temperatures. Reviewed from the Pharmacopoeia Indonesia Edition IV[10], if consuming coffee can have the effect of shrinking blood vessels which can acutely increase blood pressure with the occurrence of rhythm disorders (temporarily) and if drinking >5 cups a day can increase the risk of heart infarction by up to 70%, especially in women with angina pectoris. Drinking coffee too often for a long period results in an increase in bad fats or LDL (low-density lipoprotein) which can cause high blood pressure [11].

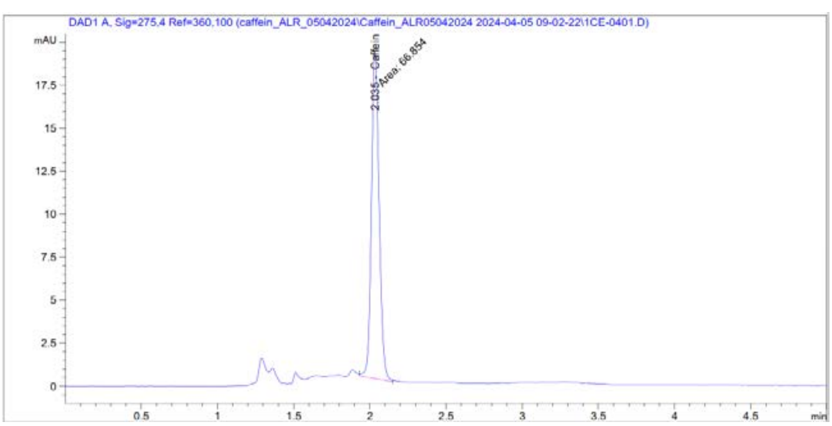

Fig 3. Chromatography Result of sample AM1

4 Conclusion

The HPLC method has been proven to be effective in determining the caffeine content in coffee with 2 types of coffee and 3 roast levels. The results of this study can provide important information for the public in choosing coffee with caffeine levels that suit their needs and preferences. The results of this study show that the caffeine content in coffee varieties depends on the type of coffee and the roast level.

References

1. G. Khamitova, S. Angeloni, G. Borsetta, J. Xiao, F. Maggi, G. Sagratini, S. Vittori, and G. Caprioli, Food Chemistry **314**, 126220 (2020)
2. K. Ota, Journal of International Economic Studies **32**, 43 (2018)
3. D. Horžić, D. Komes, A. Belščak, K. K. Ganić, D. Iveković, and D. Karlović, Food Chemistry **115**, 441 (2009)
4. J. P. Monteiro, M. G. Alves, P. F. Oliveira, and B. M. Silva, Molecules (Basel, Switzerland) **21**, (2016)
5. B. Srdjenovic, V. Djordjevic-Milic, N. Grujic, R. Injac, and Z. Lepojevic, Journal of Chromatographic Science **46**, 144 (2008)
6. T. M. McLellan, J. A. Caldwell, and H. R. Lieberman, Neuroscience & Biobehavioral Reviews **71**, 294 (2016)
7. J. Depaula and A. Farah, Beverages **5**, (2019)
8. I. Zarwinda and D. Sartika, Lantanida Journal **6**, 180 (2019)
9. N. H. Fajriana and I. Fajriati, Analit: Analytical and Environmental Chemistry **3**, 148 (2018)
10. Kementriaan Kesehatan RI, Direktorat Jenderal Kefarmasian dan Alat Kesehatan (1995)
11. J. P. Reis, C. M. Loria, L. M. Steffen, X. Zhou, L. van Horn, D. S. Siscovick, D. R. J. Jacobs, and J. J. Carr, Arteriosclerosis, Thrombosis, and Vascular Biology **30**, 2059 (2010)