Identification of Chlorogenic Acid, Caffeine, Melanoidin, Sucrose, and Protein Content of Local Indonesia Arabica Coffee Base on Its Cupping and Variety Variation

Misto[1]*, *Siti* Indarti[1], *Yustina* Safitri[1], *Mutmainnah*[1], *Tri* Mulyono[2], *Dela* Dwi Alawiyah[3]

[1]Department of Physics, Faculty of Mathematics and Natural Sciences, University of Jember, Jember, Indonesia.
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Jember, Jember, Indonesia
[3]Research Center for Bio-Molecule Engineering, Universitas Airlangga, Surabaya 60115, Indonesia

Abstract. The increased demand for high-quality coffee led to the need to evaluate coffee quality for market acceptance. Indonesia has Arabica, which has the potential for high selling value. Arabica coffee varieties, including Ateng, Lini S, and Sigararutang, affect the content of compounds in coffee beans, such as chlorogenic acid, caffeine, melanoidin, sucrose, and protein. The objective of this study was to analyze the impact of Arabica coffee varieties on the compound composition and flavor characteristics of coffee. The UV-Vis spectrophotometric methods were used to determine the quantity of chlorogenic acid, caffeine, melanoidin, sucrose, and protein in different Arabica coffee varietals. The study revealed that the content of these compounds differed among the varieties. The Lini S variety had the highest chlorogenic acid and caffeine content, while the Ateng variety had the highest melanoidin, sucrose, and protein content. The varying levels of chlorogenic acid and caffeine in coffee significantly impact its flavor, resulting in a unique sour and bitter taste in coffee brew. Melanoidin, a high-molecular-weight brown compound, contributes to the malty flavor of coffee, along with sweet, nutty, caramel, and spice notes. Sucrose adds a sweet hint to coffee brew, while protein has a relatively minimal influence on coffee flavor.

1 Introduction

Coffee is one of the plantation crops that have high economic value. In Indonesia, the most widely grown coffee comes from the Arabica type, *Coffea arabica* L., which has many varieties, such as Ateng, Lini S, and Sigararutang. Arabica coffee is recognized as the best and highest-grade coffee compared to other coffees. Brewed Arabica coffee produces different flavors depending on the variety (coffee genetics). Arabica coffee tends to show a slightly sour and bitter taste, while Robusta coffee has flavor characteristics that lean more

* Corresponding author: misto.fmipa@unej.ac.id

toward bitterness [1]. The flavor characteristics of Arabica coffee are very complex, with most Arabica coffees having aromas such as fruits, flowers, or nuts [2].

The diversity of coffee varieties is one of the factors in the content of compounds contained in different coffee beans. Research on the content of compounds such as caffeine, chlorogenic acid, melanoidin, trigonelline, carbohydrates, fat, and protein has been conducted using spectrophotometric methods. Therefore, research has been conducted to measure the compound content of chlorogenic acid, caffeine, melanoidin, sucrose, and protein of Indonesian local Arabica coffee of various varieties using a UV-Vis spectrophotometer as seen in Figure 1, the UV-Vis spectrophotometric method is based on the interaction of monochromatic rays with a light source. Absorbance is determined by measuring the amount of light that enters the sample. Log-absorbance, which can be expressed as a linear or logarithmic scale, represents the ratio of light intensity transmitted by the solution to light intensity originating from the source. The measurement method using a UV-Vis spectrophotometer is an alternative to analyzing the content of compounds in solution because of its ease and simplicity [3].

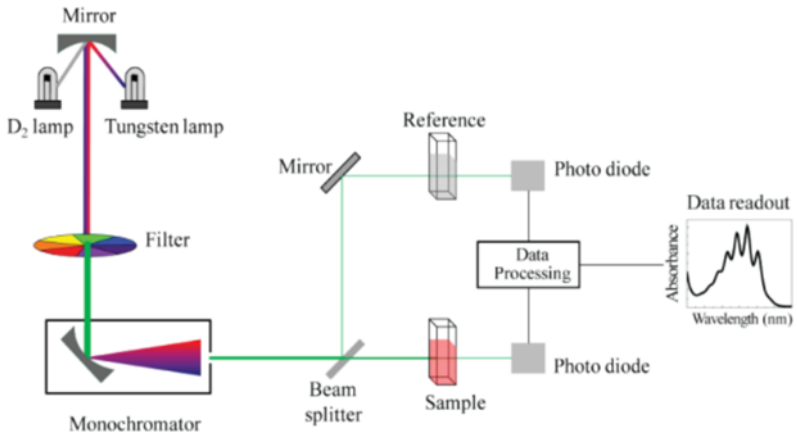

Fig. 1. Schematic illustration of the UV-Vis Spectrophotometer

The Lambert-Beer law is applied by the UV-Vis spectrophotometer. It states that the concentration of a substance or the thickness of the solution has an exponential relationship with the amount of visible light radiation absorbed or transmitted by the solution [4]. Arabica coffee with different varieties, like the Sigararutang, Ateng, and Lini S varieties, will create different flavors. The three varieties can produce diverse flavors. In general, the flavor of Sigararutang coffee presents a unique combination of Arabica's softness with Robusta's boldness, with a distinctive aroma of flowers and fruits and a touch of chocolate and caramel. Arabica coffee beans derived from the Ateng variety generally exhibit less acidity when compared to other Arabica varieties. Ateng coffee also has the potential to bring a touch of spice aroma or even herbal characteristics. Arabica coffee of the Lini S variety results from a natural cross between Arabica and Liberica coffee. Lini S coffee has a fruit-like sweet and sour flavor. Cupping notes of local Indonesian Arabica coffee with medium roasting (210°C) and semi-washed processing are shown in Table 1 [5].

Table 1. Cupping notes of three local Indonesian Arabica coffee varieties

Arabica Coffee Varieties	Cupping Notes
Sigararutang	Dark Caramel
	Brown Spices
	Lime
Ateng	Brown Sugar
	Orange Peel
	Ginger
	Nutmeg
Lini S	Pineapple
	Sweet Candy
	Orange
	Black Tea

2 Materials and Methods

2.1 Arabica coffee varieties

The materials used were local Indonesia Arabica coffee of three varieties (Lini S, Sigararutang, and Ateng), chlorogenic acid standard, caffeine standard, PA methanol, chloroform, calcium carbonate, glucose, glycine, dichloromethane, sucrose standard, bovine serum albumin (BSA), biuret, NaOH and distilled water. Arabica coffee samples came from Otten Coffee stores obtained from various regions. Based on information from the Arabica coffee bean provider, the coffee fruit is a semi-washed process often known as wet grinding. The semi-washed process involves a two-stage drying process. The harvested coffee cherries are removed from their outer shells with a depulper and dried until the coffee moisture content reaches 30-35%. Following the peeling of the horn skin, the coffee beans will go through a second drying process until the moisture percentage in the coffee beans reaches 10-12%. The dried coffee beans (green beans) are then roasted at a medium temperature of 210°C for 10 minutes. General standards for roasting levels of coffee beans can be seen in Table 2 [6].

Table 2. General roasting degree standard of coffee beans

Figure	Temperature (°C)	Roast Level	Excess
	180 - 205	*Light Roast*	Light brown color, no oil on the surface of the seeds, sweet, not too bitter, high acidity, high content of chlorogenic acid, caffeine, sucrose, and protein compounds, low melanoidin content
	210 - 220	*Medium Roast*	Brown color, oil is rarely visible on the surface of the beans, sweet, bitter, and sour flavors are balanced, lower content of chlorogenic acid, caffeine, sucrose, and protein compounds, and high melanoidin content compared to light roast
	240 - 250	*Dark Roast*	Dark brown color, visible oil on the surface of the seeds, a dominant bitter taste, low acidity, lower content of chlorogenic acid, caffeine, sucrose, and protein compounds, and high melanoidin content compared to medium roast

2.2 Chlorogenic acid and caffeine extraction, measurement, and calibration

In a beaker glass, each 2 g coffee sample was placed and dissolved in 150 ml of hot distilled water. Filter paper and a funnel were used to filter the coffee solution. After placing the coffee filtrate into a separatory funnel, 1.5 gram of calcium carbonate (CaCO3) was added in order to dissociate the caffeine compounds from the chlorogenic acid. The mixture was extracted three times with 25 ml chloroform [7]. Chloroform binds caffeine compounds to form caffeine extraction, forming two layers: the upper layer is chlorogenic acid extract, and the lower layer is caffeine extract bound by chloroform. Chlorogenic acid extract was filtered, heated to a solid, and diluted using 50 ml methanol. The caffeine extract bound by chloroform was evaporated with a hotplate. In a 100 ml volumetric flask, distilled water was used to dilute the powdered caffeine extract from coffee samples that were solvent-free.

Using methanol PA and 100 mg of pure chlorogenic acid powder, a 100 ml volumetric flask was filled to capacity in order to create a 1000 ppm concentration of chlorogenic acid standard solution. The mother solution was pipetted 1.0 mL, 2.0 mL, 3 mL, and 4 mL, and then each was diluted using methanol PA in a 10 mL volumetric flask. The calibration curve was obtained using four dilutions of 100 ppm, 200 ppm, 300 ppm, and 400 ppm of chlorogenic acid standard solution. The absorbance was determined at a 200-400 nm wavelength using a UV spectrophotometer [7].

In a 100 ml volumetric flask, 250 mg of the standard caffeine powder was weighed and dissolved in 90°C hot distilled water to produce a concentration of 2500 ppm. 50 milliliter volumetric flasks containing distilled water were pipetted and filled with standard caffeine solutions ranging from 0.2 to 0.8 milliliters, as well as 1 milliliter. The caffeine standard solution was diluted five times to yield a calibration curve: 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. Cahyono et al. used a UV-Vis spectrophotometer to measure the absorbance at a wavelength of 200–400 nm. [8].

A protein standard solution with a 500 ppm concentration was made by dissolving 50.0 mg of BSA powder in 100 milliliters of distilled water. Pipetted portions of the standard solution, namely 2.0 mL, 4.0 mL, 6.0 mL, and 8.0 mL, were combined with 1.0 mL of biuret reagent and diluted with DI water in a 10.0 mL volumetric flask [9]. Protein standard solution was diluted four times, to yield the calibration curve: 100 ppm, 200 ppm, 300 ppm, and 400 ppm. Protein standard solution concentration yields the following result. A UV spectrophotometer was employed to quantify the absorbance between 200 and 300 nm in wavelength.

2.3 Data analysis

By using linear regression to calculate the concentration of each compound's standard solution at its maximum absorbance, the compound content in parts per million was determined (ppm) [10]. Absorbance measurements were taken with three repetitions. The calculation of the compound content in Arabica coffee is shown in the following equation:

$$Content\ \left(\frac{mg}{g}\right) = \frac{x\left(\frac{mg}{l}\right) \times Vs\ (l) \times fp}{Ws(g)} \quad (1)$$

The variables x, Vs, fp, and Ws represent the concentration in milligrams per liter, the final volume of solution after extraction, and the initial mass of the coffee sample, respectively. The percentage (%) of Arabica coffee compounds content is shown using the following equation:

$$Content\ percentage\ (\%) = \frac{kadar\left(\frac{mg}{g}\right)}{100\ (mg)} \times 100\% \quad (2)$$

The compound content value of Indonesian local Arabica coffee was then statistically analyzed through the Anova test: Two-Factor with Replication using the variety variation factor to determine the factors that can affect the compound content value with the following hypothesis:
H_0: There is no difference in compound content influenced by coffee variety.
H_1: There is a difference in compound content influenced by coffee variety.
If P exceeds a, F is less than Fcrit, then H0 is accepted, and H1 is rejected. If P is less than Fcrit, then H0 is rejected, and H1 is accepted

3. Results and Discussion

3.1 Absorbance and linearity of standard chlorogenic acid

Chlorogenic acid is a compound in the phenolic component group, can dissolve in water, and is formed by combining caffeic acid and quinic acid [11]. In previous studies, the maximum absorbance of chlorogenic acid occurred at a wavelength of 290 nm. Figure 2 displays the absorbance of the standard solution of chlorogenic acid at a wavelength of 200–400 nm. According to Figure 2, the wavelength at which the chlorogenic acid standard solution absorbs the most is 325 nm. Figure 3 illustrates the strong linear relationship between absorbance and chlorogenic acid concentration that was found through analysis of the chlorogenic acid standard solution.

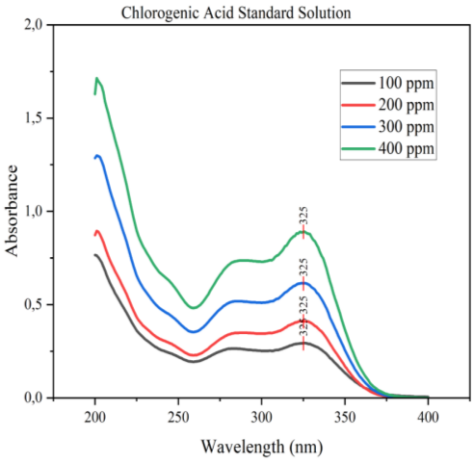

Fig. 2. The absorbance of chlorogenic acid standard solutions varies with the concentration within the 200-400 nm wavelength spectrum range.

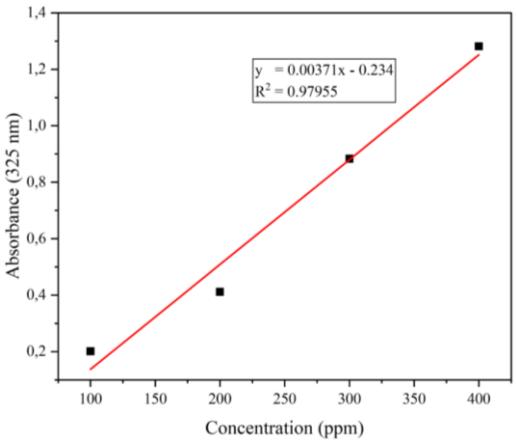

Fig. 3. A calibration curve can be obtained by measuring the absorbance of known concentrations of chlorogenic acid at 325 nm.

Light can be absorbed by chlorogenic acid compounds, especially at UV and visible light wavelengths. The light absorption can cause electrons from the compound's functional groups (such as phenolic, hydroxyl, and carboxyl) to jump to higher energy levels, causing the electrons to vibrate and spin faster. As the electrons descend back to the lower energy level, heat is released, and this energy can be measured as absorbance.

3.2 Absorbance and linearity of standard caffeine

Caffeine is an alkaloid compound in the form of a white powder, odorless, bitter taste, and somewhat tricky in water but readily soluble in chloroform [12]. Previous studies showed that the maximum absorbance of caffeine standard solution was at a wavelength of 273 nm [8]. Caffeine standard solution measured at wavelengths of 200-400 nm is shown in Figure 4. The maximum absorbance of the caffeine standard solution occurred at a wavelength of 271 nm. A linear relationship between absorbance and concentration was found through analysis of the caffeine standard solution, as Figure 5 illustrates.

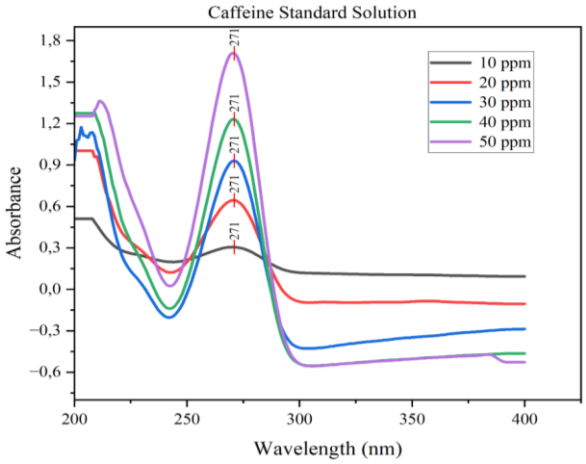

Fig. 4. The change in concentration of the caffeine standards solution's absorbance within the 200–400 nm wavelength range.

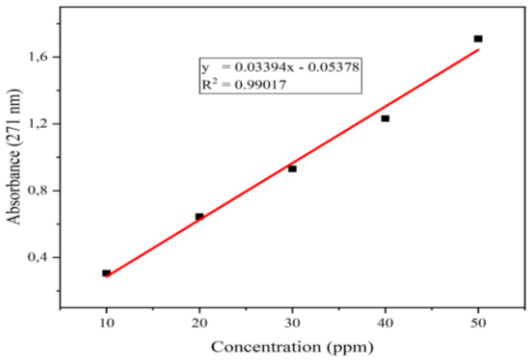

Fig. 5. A calibration curve is produced by measuring the absorbance of caffeine at 271 nm in known concentrations.

Caffeine is an aromatic heterocyclic organic compound with a pyrimidine ring and a bonded imidazole ring. One of the functional groups that can affect the light absorption of caffeine is the aromatic pyrimidine ring in its structure. The pyrimidine ring has conjugate double bonds that allow light absorption at specific wavelengths, especially in wavelengths, especially in the UV and visible light spectra.

3.3 Absorbance and linearity of standard melanoidin

After reducing sugars like glucose and lactose react with substances that contain free amino groups like amino acids and peptides, melanoidin is the result [13]. According to [14] earlier report, melanoidin exhibits absorption properties in the 320–350 nm region [13] and according to [15] the wavelength at which melanoidin absorbs the most is 325 nm. At a wavelength of 300–500 nm, Figure 6 shows the absorbance of the standard melanoidin solution. Melanoidin standard solution absorbance reaches its maximum at 336 nm wavelength. Analysis of melanoidin standards gave a robust linear relationship between absorbance and melanoidin concentration, shown in Figure 7.

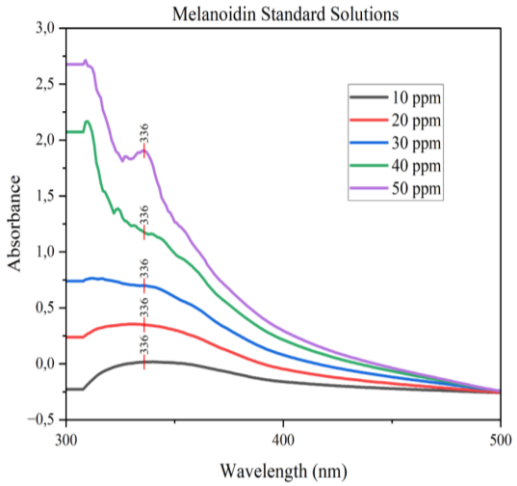

Fig. 6. The absorbance of a standard solution of melanoidin on the variation of concentration within the 300–500 nm wavelength range.

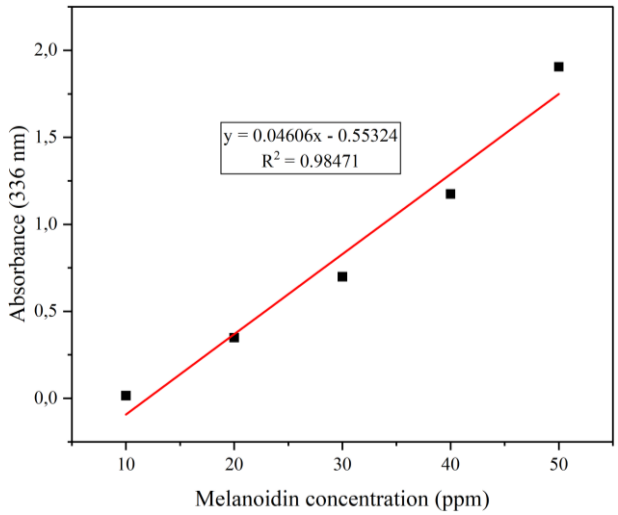

Fig. 7. The absorbance of known concentrations of melanoidin at 336 nm gives a calibration curve

The melanoidin molecule absorbs photon energy from light and raises its electron energy level when it comes into contact with light. Heat or light will be released, causing the electrons to return to their lower energy level. With a UV spectrophotometer, the released energy can be expressed as absorbance at a particular wavelength.

3.4 Absorbance and linearity of standard sucrose

Sucrose is a simple sugar comprising one glucose molecule and fructose. The range of 260–270 nm is where sucrose absorbs the most, and at 210 nm, it is essentially undetectable [16]. Figure 8 displays the sucrose standard solution's absorbance at a 200–300 nm wavelength. According to Figure 8, the wavelength at which the sucrose standard solution absorbs the most is 260 nm. Analysis of sucrose standards gave a robust linear relationship between absorbance and sucrose concentration, shown in Figure 9.

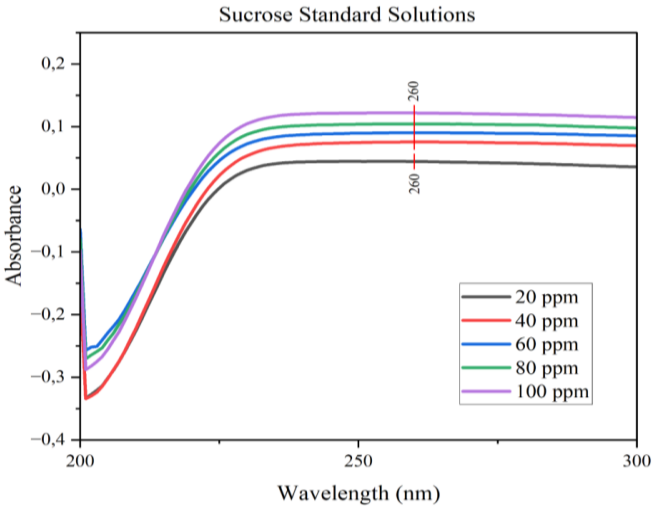

Fig. 8. The sucrose standard solution's absorbance in relation to concentration variations within the 200–300 nm wavelength range.

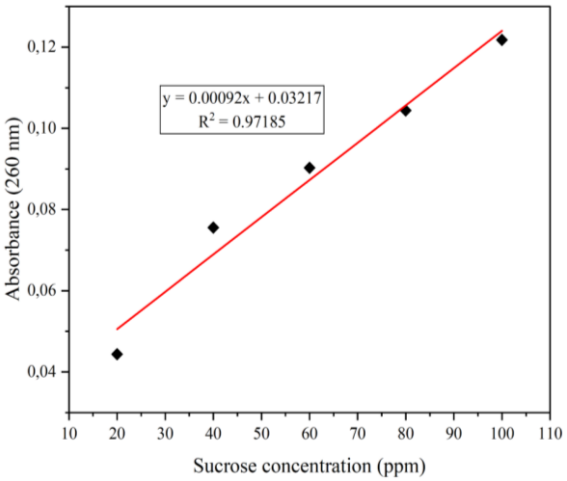

Fig. 9. The absorbance of known concentrations of sucrose at 260 nm gives a calibration curve.

The pi-electron group is the functional group that contributes to light absorption in the sucrose molecule. The pi-electron group consists of double bonds in the sucrose molecular structure, such as those in carbon-carbon (C=C) bonds. When the sucrose molecule is in contact with ultraviolet light, the electrons in the pi-electron groups can move to higher energy levels, causing light absorption at specific wavelengths. Some factors include other groups present in the sucrose molecule. Carbonyl (C=O) and hydroxyl (OH) groups can also contribute to the light absorption of the sucrose molecule [17].

3.5 Absorbance and linearity of the standard protein

Bovine serum albumin (BSA) has consistent and reliable chemical and biochemical properties, allowing BSA to serve as a standard protein in many types of analysis, including spectrophotometric spectrophotometry. BSA has an absorption peak at 230 nm as the backbone spectrum [18]. A protein's backbone is a continuous sequence of atoms made up of three atoms that repeat: carbon, nitrogen, and alpha-carbon. Each amino acid's major center in the protein structure is alpha-carbon (C) [19]. Figure 10 displays the protein standard solution's absorbance at a 200–300 nm wavelength. At a wavelength of 222 nm, the protein standard solution has its maximum absorbance. Analysis of protein standards gave a robust linear relationship between absorbance and sucrose concentration, shown in Figure 11.

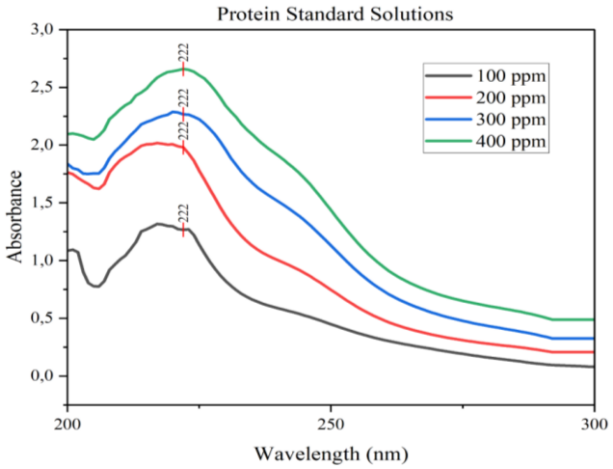

Fig. 10. The protein standards solution's absorbance in the 200–300 nm wavelength range as a function of concentration variation.

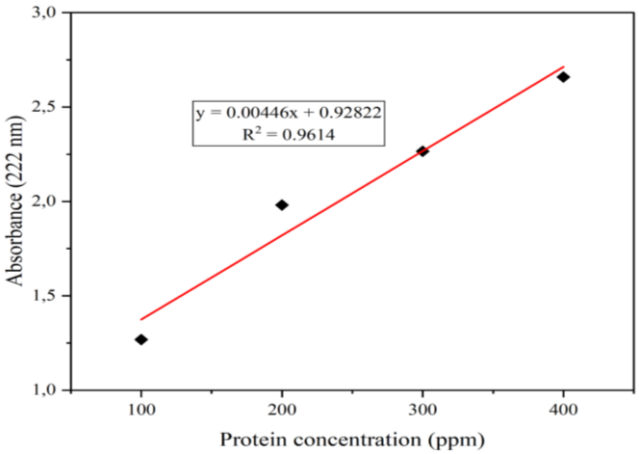

Fig. 11. The absorbance of known concentrations of protein at 222 nm gives a calibration curve.

Some pi-electron bonds in functional groups, such as peptide bonds, can absorb light. Light absorption by pi-electron bonds can stimulate electrons to move to higher energy levels. Other chromophore groups, such as tryptophan and tyrosine, may be helpful in light absorption.

3.6 Correlation of chlorogenic acid, caffeine, melanoidin, sucrose, and protein content with cupping notes and local Indonesian Arabica coffee varieties

Chlorogenic acid is a phenolic compound found in coffee [20]. The structure of chlorogenic acid includes a carboxylic acid group and a hydroxyl group, which causes chlorogenic acid to have strong antioxidant properties [21]. Chlorogenic acid gives coffee its characteristic bitterness and is acidic [22]. Based on Table 3, the chlorogenic acid content in the Sigararutang, Ateng, and Lini S coffee varieties was 14.91 mg/g, 12.23 mg/g, and 22.87 mg/g, respectively. In Sigararutang coffee, the flavor caused by chlorogenic acid is described as the acid of lime and the bitter taste of dark caramel. In comparison, Ateng coffee is described as acid from orange peel and a bitter taste from nutmeg. The flavor that may appear

due to chlorogenic acid in Lini S coffee is described as the acid of orange and the bitter taste of black tea. Although chlorogenic acid may contribute to acidity, many other factors, such as citric acid, can be more dominant in creating cupping notes or affecting coffee's orange, lime, and orange peel flavors.

According to Fajriana and Fajriati, caffeine is an alkaloid compound found in tea leaves, coffee beans, and chocolate. With a purine base known as xanthine, caffeine is a heterocyclic organic compound made up of an imidazole ring and a pyrimidine ring [5]. Pure caffeine has a white powder form that forms smooth hexagonal crystals, has no aroma, and gives a bitter taste sensation [22]. The caffeine content in Sigararutang, Ateng, and Lini S coffee varieties is 5.039 mg/g, 4.519 mg/g, and 5.721 mg/g, respectively. Caffeine in Sigararutang coffee contributes to the bitter taste described as a dark caramel flavor. The bitter taste in Ateng coffee is described as a nutmeg flavor, while in Lini S coffee, the bitter taste is described as black tea.

Melanoidins are a group of compounds formed during the roasting process of coffee beans. Melanoidins in roasted coffee contribute to its brownish color, fragrance, and unique taste. Melanoidins can impart a malty flavor, described as having a combination of sweet, nutty, and warm, complex caramel flavor nuances [13]. The highest melanoidin content was found in the Ateng variety, followed by Sigararutang and Lini S, with concentrations of 2.277 mg/g, 1.776 mg/g, and 1.581 mg/g, respectively. The melanoidin content in Arabica coffee with Sigararutang and Ateng varieties contributes to the character of the spice flavor in brewed coffee. The spice flavor in Sigararutang coffee is brown spices, while in Ateng coffee, the spice flavor is the spice flavor of nutmeg and ginger. In Lini S coffee, melanoidin contributes to providing a malty flavor described as the malty flavor of black tea.

Sucrose is a disaccharide made from a combination of two monosaccharides, glucose and fructose [23]. Sucrose is a sugar in coffee beans that can dissolve in water during coffee-making. Sucrose in coffee can give a soft and natural sweet touch produced after the brewing process. The Ateng, Lini S, and Sigararutang varieties' sucrose content was 54.69 mg/g, 48.69 mg/g, and 40.85 mg/g. The sweetness of Sigararutang, Ateng, and Lini S coffee is described as dark caramel, brown sugar, and sweet candy, respectively.

Proteins comprise one or more polypeptide chains, each composed of hundreds of amino acids [24]. The contribution of protein in producing flavor in food or beverages is less intense than some other compounds. The flavor contribution of protein depends on the type of amino acid that dominates in the polypeptide chain [25]. Protein content in the varieties Ateng, Lini S, and Sigararutang were 65.43 mg/g, 61.18 mg/g, and 57.95 mg/g. Protein in Arabica coffee does not contribute directly to coffee cupping notes. Protein has a minimal contribution to the flavor and aroma of the coffee. However, the flavor contribution from protein may vary depending on the type of amino acids contained in the protein. The fundamental components of proteins are amino acids, some of which can also contribute more pronounced flavors, like the sour taste of aspargine [25].

Table 3. The compound content of three Arabica coffee varieties and their cupping notes were processed by semi-washed and roasted at 210°C for 10 minutes

Arabica Coffee Varieties	Cupping Notes	Compound	Content (mg/g)	Content Percentage
Sigararutang	Lime	Chlorogenic Acid	14.91	1.49%
	Brown Spices	Melanoidin	1.776	0.18%
	Dark Caramel	Caffeine	5.039	0.50%
		Sucrose	40.85	4.09%
		Protein	57.95	5.80%
Ateng	Orange Peel	Chlorogenic Acid	12.23	1.22%
	Nutmeg	Caffeine	4.519	0.45%
		Melanoidin	2.277	0.23%
	Brown Sugar	Sucrose	54.69	5.47%
	Ginger	Protein	65.43	6.54%
Lini S	Orange	Chlorogenic Acid	22.87	2.29%
	Black Tea	Caffeine	5.721	0.57%
		Melanoidin	1.581	0.16%
	Sweet Candy	Sucrose	48.69	4.87%
	Pineapple	Protein	61.18	6.12%

Variety is one of the factors that can affect the content of compounds in coffee. Based on statistical tests using the Anova method: Two-Factor with Replication, the calculation results show that the P-value is 2.940E-17, which is less than the alpha value of 0.05, so it can be stated that H_0 is rejected and H_1 is accepted. The smaller the P-value, the greater the influence of the factor. Since H_1 is acceptable, it can be concluded that variety affects the compound content of local Indonesian Arabica coffee.

4 Conclusions

The conclusion obtained based on the research that has been done is that varieties can affect the content of compounds in local Indonesian Arabica coffee. It is also known that the content of compounds in coffee can affect the taste that appears in brewed coffee.

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