Brewing characterization for optimum functional properties of Dampit Robusta (Coffea canephora) and Liberica (Coffea Liberica) coffee leaves tea

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Abstract. Coffee leaf is rich of bioactive compound such as phenolic acids, flavonoids, alkaloids, tannin, and caffeine. This study aims to determine optimal brewing conditions for Dampit Robusta and Liberica coffee leaf tea with total phenolic content, tannin content and antioxidant activity as response, also to determine its sensory characteristics. The results suggest that the optimum brewing condition of Dampit Robusta coffee leaves tea was obtained at 95.30°C for 5.14 minutes with total phenolic compound of 468.26 ± 4.16 mg GAE/g, tannin content 303.95 ± 8.17 µg TAE/g, and IC₅₀ antioxidant activity was 14.91 ± 2.48 ppm. Meanwhile the brewing of Dampit Liberica coffee leaves tea was optimum at 95.52°C for 6.03 minutes, with the total phenolic content, tannin content, and antioxidant activity were 562.72 ± 20.21 mg GAE/g, 434.86 ± 34.05 µg TAE/g and 12.68 ± 1.89 ppm consecutively. It was also found that Robusta tea leaves have more dominant floral flavour while Liberica tea leaves were dominated by burnt flavour, bitter taste and astringent mouth-feel.

Keywords: Dampit Coffee Leaf Tea, Brewing, Functional, Sensory

1. Introduction
Coffee is a commodity that has been widely planted in Indonesia and has a high economic value. In 2017, Indonesia exported 467.800 tons with an export value of USD 1187.2 million [1]. Dampit (300-460 asl) is one of coffee producers in East Java Province, Indonesia. There are several types of coffee developed such as Robusta, Arabica and Liberica.

Conventionally, the commercial use of coffee plants is limited to processing coffee beans into brewed drinks and food additives. Even though it is not as popular as coffee beans, coffee leaf tea has been consumed since a long time ago. In Ethiopia, the tea is called as “Kutti” and has been consumed for more than 200 years. In West Sumatra, the tea is popular as “Aia Kawa” and it is believed to refresh the body and prevent disease, as well as to increase stamina. The health benefits may be related to the bioactive compounds in coffee leaves, such as phenolic acids, flavonoids, alkaloids, tannin, and caffeine [2].
It has been reported that several factors that influence the extraction results such as the brewing process are the type of solvent, solvent concentration, particle size, temperature, pH, and extraction time [3]. Thus, it was expected that by optimizing the brewing/extraction conditions, it may also optimize its functional properties.

The scope of this current study is limited to observe Liberica coffee leaves tea as well as the Robusta. Total phenolic compound, tannin content, antioxidant activity of both coffee leaves tea were recorded for sets of temperature.

2. Methods

2.1 Tools and Materials
The old Robusta and Liberica coffee leaves were obtained from Dampit region, Malang. Several reagents related to chemical analysis used in the study were included 1,1-diphenyl-2-pycrilhidrazil (DPPH) 0.2 mM in methanol, folin ciocalteu, Na$_2$CO$_3$, gallic acid, tannic acid, aquadest, methanol, mineral water, and cracker. The equipments needed were included electric oven, analytic scale, spectrophotometer, spatula, glass stirrer, aluminium foil, glassware and plastic cups.

2.2 Research Design
Optimization was conducted by using Response Surface Method (RSM) Central Composite Design (CCD) in Design Expert 9.0 software with 5 center point resulting 13 runs. The lower and upper limit for temperature were set for 91°C and 99°C, while the brewing time were set for 3 and 7 minutes. The sensory characterization for optimum samples were conducted by applying Rate-All-That-Apply Method which were involving 110 untrained panelists.

2.3 Sample Preparation
Robusta and Liberica coffee leaves collected from Dampit district were washed with flowing water and cut into small size ±2 x 1 cm. The leaves then dried using electric oven at 95°C for 3 hours. Dried coffee leaves then ground into powder then stored in closed container. Coffee leaf tea beverage was prepared by decoction technique from the mixture of 1 g coffee leaf tea powder and 100 ml of mineral water. The decoction temperature and time varied from 89.34°C to 100.66°C for 2.17 to 7.83 minutes. The total phenolic compound, tannin content, and antioxidant activity of each tea was then measured. After finding a solution, verification process was conducted to confirm. The optimum sample was then sensory evaluated by RATA method.

2.4 Total Phenolic Content
Total phenolic content was determined using Folin-Ciocalteu modified method [4]. Standard solution stock was made from the mixture of 0.01 g gallic acid with 50 ml aquadest. The standard with concentration 0, 20, 40, 60, 80, and 100 ppm was made from the stock by diluting with methanol. 1 ml of sample was diluted with methanol in 10 ml volumetric flask. 0.4 ml of the solution was taken and added with 2 ml 10% folin-ciocalteu reagent, vortexed thoroughly and let it still for 5 minutes. After that 1.6 ml of 7.5% Na$_2$CO$_3$ was added into the solution, vortexed, then incubated in dark place for 30 minutes. Then the absorbance was measured at wavelength of 751.5 nm. Concentration was calculated based on the standard curve and total phenolic content was calculated according to the formula below. The total phenolic content was expressed in mg Gallic Acid Equivalent (GAE)/g sample.

2.5 Tannin Content
Measurement of tannin content were done using Folin-Ciocalteau modified method [5]. Standard solution stock was made from the mixture of tannic acid with aquadest. The standard with concentration of 0, 10, 20, 30, 40, and 50 ppm were made from the stock by diluting it with methanol. 1 ml of sample was diluted with methanol in 10 ml volumetric flask. 0.4 ml of the solution was taken and added with 2 ml 10% folin-ciocalteu reagent, vortexed thoroughly and let it still for 5 minutes. After that 1.6 ml of 7.5% Na$_2$CO$_3$ was added into the solution, vortexed, then incubated in dark place.
for 30 minutes. Then the absorbance was measured at wavelength of 755 nm. Concentration was calculated based on the standard curve and tannin content was calculated according to the formula below. The total phenolic content was expressed in µg Tannic Acid Equivalent (TAE)/g sample.

2.6 Antioxidant Activity
Antioxidant activity was determined using the DPPH method modified from Molyneux [6]. 0.4 ml; 0.8 ml; 1.2 ml; 1.6 ml; and 2 ml sample were added with 3.6 ml; 3.2 ml; 2.8 ml; 2.4 ml; and 2 ml methanol, and then vortexed. 1 ml of 0.2 mM DPPH was added into each sample, vortexed, and then incubated in dark place for 30 minutes. After that the absorbance was measured at a wavelength of 517 nm. The Inhibitory Concentration (IC₅₀) is the concentration of sample that is able to inhibit 50% of DPPH radicals [7].

2.7 Sensory Evaluation
Descriptive test was conducted to determine the characteristic of coffee leaf tea beverage. The method used was Rate-All-That-Apply (RATA) involved 110 untrained panelists. The optimum Robusta and liberica coffee leaf tea from chemical optimization then brewed with the same ratio (1:100) at optimum temperature and duration of brewing. The 20 ml of tea was served to the panelists for evaluating the attributes at room temperature (22°C).

3. Results and Discussions
Figure 1 and 2 showed that higher temperature and longer brewing time increase total phenolic compound and tannin content, but reduce IC₅₀ of Robusta and liberica coffee leaf tea until it reach the optimum point (Robusta: 95.30°C for 5.14 minutes; Liberica: 95.52°C for 6.03 minutes). The stability of phenolic compound is an important aspect to be considered in food processing. Factors affecting the stability are exposure to high temperature, light, oxygen, amount of solvent, the presence of enzyme, protein, ion, metal, or association of phenol with other food constituent [8].The use of high temperature in brewing can help the release of phenolic compound from cell walls thus increasing the phenolic content in tea [9]. But overly high temperature is not recommended because it can make the phenolic compound undergo degradation.

It was reported that, Robusta coffee leaves have higher phenolic levels [10] in which, the old coffee leaves had a total phenolic compound value higher than the young one [11]. It was also previously reported that brewing avocado herbal tea at 100°C for 5 minutes had the lowest phenolic content [12]. This may be affected by evaporation of volatile phenolic compounds, decomposition of phenolic compounds, and the complex formation between phenol and other compounds. The longer brewing time can increase the total phenolic content in tea. But brewing for too long is not recommended as brewing it for more than 5 minutes would not provide any significant difference to the phenolic content [13].

Figure 1. (a) 3D surface of total phenol of Robusta tea
Figure 1. (b) 3D surface of tannin content of Robusta tea
Similar trend was also observed for tannin content. All types of tannins can dissolve in water, methanol, ethanol, acetone and have great solubility particularly when dissolved in hot water [14]. Tannins will break down into pyrogallol, pyrocatechol and phloroglucinol when heated to temperatures of 2100 F-2150 F (98,890°-101,670° C). The longer duration for brewing also affect the amount of tannin content [15]. But tannin has an optimal point where increasing brewing time will tend to reduce tannin content. At the beginning of the brewing process all the compounds in the leaves especially the tannins will be extracted out and mixed with solvents (water), and after reaching the optimum point some of the compounds contained in the material will decrease [16]. The decrease in tannins may also be affected by the hydrolysis process during the process (extraction) and continuous heating, in which tannins can be hydrolyzed into glucose and tannic acid.

Coffee leaf can be considered as a strong antioxidant because the IC50 is lower than 50 [7]. The higher temperature and longer brewing time increase the ability of tea beverage to scavenge free radicals. This is caused by increasing compounds that contribute to antioxidant activity, such as phenolic compounds and flavonoid. However overly high temperature can cause degradation of phenolic compound. On the other hand, the shorter brewing time is considered to be less effective as the compound may not be completely extracted. It was reported that the optimal brewing time to obtain the highest antioxidant capacity of green tea is 5 minutes [17].
Out of 26 sensory attributes that were in the questionnaire, there were 4 attributes that are significantly affected by the differences between Robusta and Liberica coffee leaf tea. Those include, floral aroma, burnt flavor, bitter taste, and astringent mouthfeel. Floral aroma shown to be more intense in Robusta than that in Liberica tea. Meanwhile, the burnt flavor, bitter taste, and astringent mouthfeel shown to be more intense in Liberica than those of Robusta tea. This may be attributed by higher total phenolic and tannin content in the Liberica compared to the Robusta. Flavonoid, which is a phenolic compound, and caffeine contribute a bitter taste in tea. Phenolic compound may also contribute to the astringent mouthfeel, as it may form a complex with salivary protein which is holding water thus produce a mouth-drying sensation [18].

Table 1. Sensory Characterization of coffee leaf tea

| Attributes         | P-Value | Leaf Type | Mean    |
|--------------------|---------|-----------|---------|
| Floral Aroma       | 0.033   | Robusta   | 1.3 ± 1.6 |
|                    |         | Liberica  | 0.9 ± 1.2 |
| Burnt Flavor       | 0.046   | Robusta   | 0.4 ± 0.9 |
|                    |         | Liberica  | 0.7 ± 1.3 |
| Bitter Taste       | 0.000   | Robusta   | 1.1 ± 1.3 |
|                    |         | Liberica  | 1.8 ± 1.5 |
| Astringent Mouthfeel| 0.019  | Robusta   | 1.3 ± 1.3 |
|                    |         | Liberica  | 1.8 ± 1.5 |

4. Conclusions

The brewing temperature and time for the optimum functionality considering total phenolic compound, tannin content, antioxidant activity of Dampit Robusta coffee leaf tea was observed at 95.30°C for 5.14 minutes with total phenolic content of 468.26 ± 4.16 mg GAE/g, tannin content of 303.95 ± 8.17 µg TAE / g, and antioxidant activity (IC50) of 14.91 ± 2.48 ppm. Meanwhile, the optimal conditions for Dampit Liberica coffee tea leaves was observed at 95.52°C for 6.03 minutes. The optimum conditions for the Liberica were related to the total phenolic content of 562.72 ± 20.21 mg GAE / g, tannin content of 434.86 ± 34.05 µg TAE / g, antioxidant activity of 12.68 ± 1.89 ppm. Sensorially, Liberica coffee leaf tea was more intense in burnt flavor, bitter taste and astringent mouthfeel attributes while Robusta coffee leaf tea was more intense in floral aroma attribute.
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