The Effect of Roasting Temperature on Ferulic Acid Levels of Robusta Coffee Bean with Thin Layer Chromatography (TLC)-Densitometry

Sugiyanta Sugiyanta[1,2], Harianto Notopuro[3], Jusak Nugraha[4], Soetjipto Soetjipto[3], Retno Handajani[3]

[1]Doctoral Student, Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Lecturer, Department of Biochemistry, Faculty of Medicine, Universitas Jember, Jember, Indonesia, [3]Professor, Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [4]Professor, Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia

Abstract

The purpose of this study was to quantitatively analyze ferulic acid levels of Robusta coffee bean extracts from two samples from different regions (Argopuro and Kaliwining, Jember District), with different roasting temperatures (180 °C and 200 °C for 10 minutes) using Thin Layer Chromatography (TLC)-Densitometry method. The analytical method used was the TLC-Densitometry, which utilized methanol solvent p.a; stationary phase: Merck silica Gel 60 F254 plate; chloroform mobile phase: methanol: formic acid (v/v/v) = (85:15:1) in a saturated chromatographic chamber; the test concentration of 50 ppm and the plates were analyzed with the Densitometry winCATS Camag scanner using a UV-Vis detector at 254 nm wavelength and blue fluorescence at 366 nm wavelength. The results showed that the standard correlation coefficient (R) of ferulic acid was 0.9947. Quantitative analysis of ferulic acid levels obtained on the average results of Argupuro coffee bean extract at roasting temperatures 180 °C and 200 °C amounted to 1.935598±0.020671 mg/g and 1.419963±0.051633 mg/g, while Robusta Kaliwining coffee bean extract at 180°C and 200°C roasting temperature were 3.18087±0.06738 mg/g and 2.420237±0.1989 mg/g. Statistical analysis with One-Way Anova obtained *p* value <0.005. Least Significant Different Test (LSD) obtained *p* value <0.005 in each group. Based on the statistical analysis it was concluded that there was an effect of roasting temperature on ferulic acid levels in Robusta coffee bean extract, with the highest ferulic acid content obtained in Robusta Kaliwining coffee bean extract in Jember Regency at roasting temperature of 180 °C was 3.180875±0.020671 mg/g.

Keywords: *ferulic acid, Robusta coffee bean, roasting, TLC-Densitometry.*

Introduction

Coffee is one of the most consumed drinks in the whole world. Robusta coffee comes from the equator forests in Africa, from the west coast to Uganda. Coffee plants can start to produce coffee fruit after the age of 4-5 years depending on local maintenance and climate condition. Since 1900, Robusta coffee has been widespread throughout the tropical island. Robusta coffee can grow better in areas with an altitude of 0-1000 meters asl, where it is not suitable for Arabica coffee that requires a height of more than 1000 meters above sea level to avoid pest Hemelia vastatrix (HV) attacks. This causes more robusta coffee to be cultivated in Indonesia where the area is dominated by lowlands[1].

Jember Regency is one of the biggest coffee manufacturer regions in East Java. The total coffee plantations in Jember are 16,882 ha with the production of 4,911 ha of community coffee spread across 27 sub-districts in Jember. Furthermore, there are 14 gardens managed by PT. Perkebunan XII (6,009 ha), 7 estates of

Corresponding author:
Harianto Notopuro
Email: hariantonotopuroprof@gmail.com

2,22267 ha (PDP) and 10 estates of 3,695 are managed by the other private sector[2]. Kaliwining Plantation (45 masl) and Durjo Plantation Sumber Kembang Argopuro village (450-650 masl) are plantation areas in Jember with commodities such as Robusta coffee.

Coffee is rich in compounds with high antioxidant activity such as polyphenols, especially chlorogenic acid and its degradation products (ferulic, cucumber, and caffeic acid), and melanoidin compounds derived from the Maillard reaction[3]. Coffee bean extract is one of the most popular drinks in the whole world. Only a small proportion of coffee consumers drink coffee extracts made from green coffee beans. Most of them prepare a coffee drinks from roasted and ground coffee beans or from instant coffee, form of coffee that has been obtained in a high temperature process. Roasting causes changes in the coffee bean content through modification or degradation. During the roasting process, high temperatures will result in degradation of polyphenols[4]. Chlorogenic acid, malic and citric acid levels decrease, while ferulic acid and quinic acid increase due to degradation of chlorogenic acid[5]. The roasting temperature used, ranges from 160 °C to 240 °C for 8 to 24 minutes[6]. The optimal roasting time for Robusta coffee is around 10 minutes[7].

One of coffee polyphenols degradation is ferulic acid[8]. Ferulic acid is a type of phenolic acid found in components of cell walls, leaves and coffee beans. Ferulic acid is produced from the metabolism of phenylalanine and tyrosine. This acid has an important role for plants because of its ability to inhibit free radicals. Recent evidence shows that ferulic acid has anti-inflammatory, anti-diabetic, anticancer, and cardioprotective properties. Some investigations have also shown that foods rich in ferulic acid can prevent hypertension[9].

Ferulic acid can be quantified by many analytical methods, such as High-Performance Liquid Chromatography (HPLC) combined with photodiode array (PDA) wavelength detectors or UV-Vis detectors[10,11], UV-Vis spectroscopy[12,13], TLC[14], thin layer chromatography high performance (HP-TLC) [15], gas chromatography[16], chemiluminescence[17], and capillary electrophoresis[18]. Rapid quantitative and qualitative analysis with TLC of crude extracts will give practical and simple results[14]. The purpose of this study was to quantitatively analyze ferulic acid levels of Robusta coffee bean extracts from two samples with different regions namely Robusta Argopuro coffee and Robusta Kaliwining coffee from Jember Regency with different roasting roasting temperatures (180 °C and 200 °C for 10 minutes) using valid TLC-Densitometry method.

Materials and Methods

Chemical and Reagen

Coffee roaster, Coffee grinder, Coffee filter, Aquadest, Freeze dryer, Analytical scales, measuring flask, TLC silica gel 60 F254 plate (20 cm×20 cm), chloroform (Merck), methanol (Merck), formic acid (Merck), standard ferulic acid (Sigma-Aldrich), Camag's TLC Scanner, winCAT software.

Robusta Coffee Bean Sample

Dried green coffee beans obtained from two places, Robusta Argopuro green coffee beans Jember obtained at the State Polytechnic of Jember, Department of Agricultural Technology, whereas Robusta Kaliwining coffee beans obtained from the Central Java Coffee and Cocoa Kaliwining Research Center Jember East Java.

Roasting Procedures

A 500 gram sample was roasted using a drum coffee roaster. The initial roasting temperature is 180 °C, after that the roasting temperature is divided into two namely 180 °C and 200 °C for 10 minutes[19]. All roasting samples are ground using an electric grinder and ground coffee is filtered using a standard filter to remove large particles 440 grams of ground coffee are produced.

Extraction and freeze Dryer Procedures

Samples of 5 grams of ground coffee were transferred to 100 ml flasks and 60 mL of distilled water was added at 100 °C, and then stirred until the solution became homogenous[20]. After it reach homogeneous condition, the extracted water was filtered and the freeze drying of the filtered supernatant was carried out. The supernatant was put in a freeze dryer at -72 °C for 17 hours.

Ferulic Acid Analysis with TLC-Densitometry

Thin Layer Chromatography (TLC) method is used

to determine the level of ferulic acid. The stationary phase used is the 60 F254 silica gel plate (20 cm × 20 cm). The mobile phase in the system is consisted of chloroform: methanol: formic acid (85: 15: 1). Standard 1 µL ferulic acid is bottled using Camag's linomat at 15 mm from the bottom of the plate, the spot is dried. The plate is then inserted into the Wheaton chamber which is saturated with the mobile phase. Elution of the mobile phase is carried out along the 100 mm. The plate is then dried until all the mobile phases evaporate at room temperature and scanning is done using Camag's TLC Scanner which has been equipped with winCAT software. Detection was carried out by modify of the method[21]. Ferulic acid absorbs strongly at 254 nm wavelength while the blue fluorescence at 366 nm. Quantification of ferulic acid was obtained using a standard curve (Figure 1).

Quantitative analysis of ferulic acid in Robusta coffee bean extract.

Argopuro and Kaliwing Robusta coffee bean extract solution was prepared in methanol solution. The sample was applied to a 60 F254 silica gel plate, developed and dried as described above. Plate/plate TLC that has been eluted with mobile phase is observed under UV lamps. Standard color stains and coffee samples were observed under UV light which is dark blue fluorescence. Then the TLC plate that has been eluted is paid with a densitometer. Ferulic acid levels in Robusta coffee bean extract samples can be calculated based on standard curric acid curves (Table 1).

Statistical Analysis

All values are expressed as mean±SD. Data were analyzed using analysis of variance for each group (One-Way ANOVA). When a significant F value (p <0.05) is obtained, an LSD test is performed for post hoc analysis. Previously, Shapiro-Wilk Normality Test was carried out with a significance level of 5% to determine the normality distribution of data. In addition, the normality test is carried out as a condition in determining parametric or non-parametric tests. Furthermore, the Levene homogeneity test is performed to determine whether the data is homogeneously distributed or not.

Results

Linearity was obtained using five standard concentrations of ferulic acid. Concentration is 100; 200; 300; 400; and 500 ng. The results of linearity are shown in Fig. 1. Based on the results obtained by calculate the equation of linear regression of the calibration curve y = 4.171x+66.56 and the correlation coefficient (R2) = 0.9947. This correlation coefficient shows a linear result, because it fulfill the acceptance criteria, namely the value of the correlation coefficient (r) ≤ 1[22]. The equation of the line will then be used to determine the ferric acid content of the sample (Table 1).

Table 1 showed the results of Robusta Arguporo and Kaliwining coffee bean extracts with roasting temperatures of 180 °C and 200 °C, where the average of ferulic acid level from Robusta Argopuro coffee bean extract with 180 °C and 200 °C roasting temperatures are 1.935598±0.020671 mg/g and 1.419963±0.051633 mg/g.

The data obtained were then tested for Normality and Homogeneity. The Shapiro-Wilk normality test showed p >0.05, as of the data distribution was normally distributed and continued with the parametric test. The Levene homogeneity test showed p >0.05. This shows that the data was homogeneous. Data were then analyzed using analysis of variance for each group (One-Way ANOVA) and continued by LSD test. The p value <0.05, as of it can be concluded that there is a significant difference between the roasting temperature and ferulic acid levels. LSD test found there were significant differences between groups of Robusta coffee extracts against ferulic acid levels.

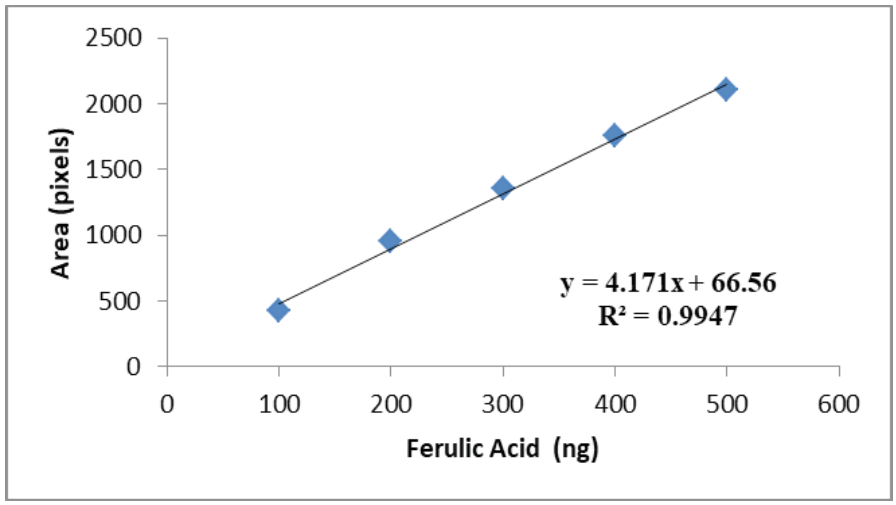

Figure 1. Standard curve of ferulic acid level.

Table 1. Ferulic Acid Level of Robusta Coffee Bean Extract.

Sample	Area	vol (µL)	Level (ng)	Level (ng/1000 µL)	Level (ng/g)	Level (mg/g)	Mean	SD
A	1210.1	14	274.1645	19583.17635	1958317.635	1.958317635	1.935598	0.020671
	1193.9	14	270.2805	19305.75059	1930575.059	1.930575059		
	1186.5	14	268.5064	19179.02524	1917902.524	1.917902524		
B	908.5	14	201.8557	14418.26215	1441826.215	1.441826215	1.419963	0.051633
	917.4	14	203.9895	14570.67507	1457067.507	1.457067507		
	861.3	14	190.5394	13609.95993	1360995.993	1.360995993		
C	1885.8	14	436.164	31154.57068	3115457.068	3.115457068	3.180875	0.06738
	1921.8	14	444.795	31771.07237	3177107.237	3.177107237		
	1964.4	14	455.0084	32500.59938	3250059.938	3.250059938		
D	1376.8	14	314.1309	22437.9217	2243792.17	2.24379217	2.420237	0.1989
	1457	14	333.3589	23811.35048	2381135.048	2.381135048		
	1605.7	14	369.0098	26357.84498	2635784.498	2.635784498		

Note: A) Robusta Argopuro coffee bean extract 180 °C roasting temperature; B) Robusta Argopuro coffee bean extract roasting temperature 200 °C; C) Robusta coffee bean extract Kaliwining roasting temperature 180 °C; D) extract Robusta Kaliwining coffee beans roasting temperature 200 °C.

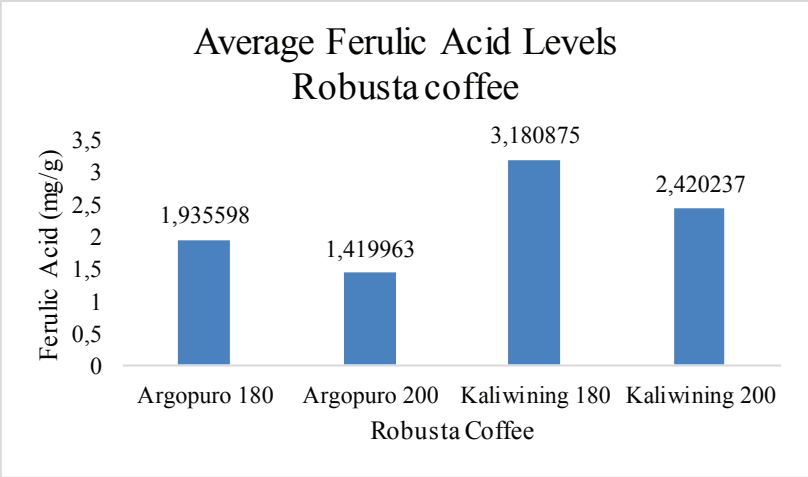

Figure 2. Average of Argopuro and Kaliwining Robusta Coffee's Ferulic Acid Levels (mg/g) at 180 ºC and 200 ºC roasting temperatures.

Discussion

Roasting is an important processing step for developing the unique chemical, physical and sensory characteristics of roasted coffee[23]. During the roasting process, coffee beans experience high temperatures, resulting in physical-chemical changes in their structure. Roasting from 180 °C, coffee beans begin to form compounds that are responsible for the color, taste and aroma of roasted coffee beans[24].

Ferulic acid is defined as the result of the coffee chlorogenic acid degradation due to the roasting process. Ferulic acid is also found in food, especially its free form, has an important function to protect human health[25]. Roasting will increase the amount of free ferulic acid and reduce the ferric acid bounding form. It is estimated that the increase in the amount of free ferulic acid due to some part of the ferulic acid which is bound in food will be in its free form due to the roasting effect[26].

In Argopuro and Kaliwining Robusta coffee beans, ferulic acid levels reach a peak at roasting temperatures of 180 °C and decrease at 200 °C. This is because of ferulic acid experiencing degradation in the temperature above of 180 °C[27].

Ferulic acid levels of Kaliwining Robusta coffee bean extract is greater than in Argopuro Robusta coffee bean extract. Robusta coffee grows optimally in the lowlands, hence, the ferric acid levels is greater in Robusta coffee in the Kaliwining area which is lower than the Argopuro area.

Conclusion

In sum, there is an effect of roasting temperature on ferulic acid level in Robusta coffee bean extract with the highest ferulic acid level obtained in Kaliwining Robusta coffee bean extract at Jember Regency at 180 °C roasting temperature is 3.18087±0.06738 mg/g.

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

1. Rahardjo P. *Panduan Budidaya dan Pengolahan Kopi Arabika dan Robusta*. Jakarta: Penebar Swadaya; 2012.
2. Kompas. *Eksportir Kopi Jawa Timur dihimbau Penuhi Syarat Jepang*, Koran Kompas, October 2nd 2004; 2004.

3. Vignoli JA, Viegas MC, Bassoli DG, Benassi MT. Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. *Food Research International*. 2014; 61: 279-285.
4. Hecimovic I, Belscak-Cvitanovic A, Horzic D, Komes D. Comparative study of polyphenols and caffeine in different coffee varietes affected by the degree of roasting. *Food Chemistry*. 2011; 129(3): 991-1000.
5. Ginz M, Balzer HH, Bradbury AGW, Maier HG. Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. *European Food Research and Technology*. 2000; 211(6): 404-410.
6. Hingse SS, Digole SB, Annapure US. Method development for simultaneous detection of ferulic acid and vanillin using high-performance thin-layer chromatography. *Journal of Analytical Science and Technology*. 2014; 5: 1-9.
7. Dharmawan A, Cahyo F, Widyotomo S. Determining optimum point of robusta coffee bean roasting process for taste consistency. *Pelita Perkebunan*. 2018; 34(1): 59-65,
8. Mahatmal R, Sangeetha M. Studies on the growth, characterization, physicochemical properties and anti-bacterial activity of ferulic acid crystals. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2016; 8: 121-126.
9. Alam MA. Anti-hypertensive Effect of Cereal Antioxidant Ferulic Acid and Its Mechanism of Action. *Frontiers in Nutrition*. 2019; 6: 121.
10. Przybysz MA, Widła G, Dłużewska E. Consumer preferences on coffee drinking. The influence of temperature and roasting time of coffee bean on espresso aroma and taste. *Zeszyty Problemowe Postępów Nauk Rolniczych*. 2013; 572: 65-79.
11. Shaikh HA, Vandana J. A novel, simple, rapid RP-HPLC method for simultaneous estimation of ferulic acid, quercetin, piperine and thymol in the ayurvedic formulation. *International Journal of Applied Pharmaceutics*. 2018; 6: 303-308.
12. Lima E, Flores J, Cruz AS, Leyva Gomez G, Krotzsch E. Controlled release of ferulic acid from a hybrid hydrotalcite and its application as an antioxidant for human fibroblasts. *Microporous and Mesoporous Materials.* 2013; 181: 1-7.
13. Merlin JPJ, Rajendra Prasad N, Shibli SMA, Sebeela M. Ferulic acid loaded poly-d,l-lactide-coglycolide nanoparticles: A systematic study of particle size, drug encapsulation efficiency and anticancer effect in nonsmall cell lung carcinoma cell line *in vitro*. *Biomedicine & Preventive Nutrition.* 2012; 2: 69-76.
14. Mabinya LV, Mafunga T, Brand JM. Determination of ferulic acid and related compounds by thin-layer chromatography. *African Journal of Biotechnology*. 2006; 5: 1271-1273.
15. Attimarad M, Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. *Pharmaceutical Methods*. 2011; 2(2): 71-75.
16. Olthof MR, Hollman PCH, Buijsman MNCP, Van Amelsvoort JMM, Katan MB. Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. *Journal of Nutrition*. 2003; 133: 1806-1814.
17. Shen G, Jia X, Jin J, Pang L, Chen Z, Du B. Determination of ferulic acid by flow injection chemiluminescence analysis based on an enhancement of the N-bromobutanimide–eosin-CrCl3 system in alkaline solution. *Luminescence*. 2013; 28: 536-541.
18. Lima DLD, Duarte AC, Esteves VI. Optimization of phenolic compounds analysis by capillary electrophoresis. *Talanta*. 2007; 72: 1404-1409.
19. Dong W, Hu R, Long Y, Li H, Zhang Y, Zhu K, Chu Z. Comparative evaluation of the volatile profiles and taste properties of roasted coffee beans as affected by drying method and detected by electronic nose, electronic tongue, and HS-SPME-GC-MS. *Food Chemistry*. 2019; 272: 723-731.
20. Dong W, Hu R, Chu Z, Zhao J, Tan L. Effect of different drying techniques on bioactive components, fatty acid composition, and volatile profile of robusta coffee beans. *Food Chemistry*. 2017; 234: 121-130.
21. Huang M, Liu D. Thin layer chromatography densitometri determination of soybean isoflavones in wild soybean (*Glycine soja*) Seeds. *Asian Journal of Chemistry*. 2013; 24: 1322.
22. Gandjar IG, Rohman A. *Kimia Farmasi Analisis*. Edisi XI. Yogyakarta: Pustaka Pelajar; 2013.
23. Wang X, Lim LT. Physicochemical characteristics of roasted coffee. In: *Coffee in Health and Disease*

Prevention. 247-254; 2015.

24. Ebrahimi-Najafabadi H, Leardi R, Oliveri P, Chiara Casolino M, Jalali-Heravi M, Lanteri S. Detection of addition of barley to coffee using near infrared spectroscopy and chemometric techniques. *Talanta*. 2012; 99: 175.
25. Guo W, Beta T. Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. *Food Research International*. 2013; 51(2): 518-525.
26. Abdel-Aal ESM, Rabalski I. Effect of baking on free and bound phenolic acids in wholegrain bakery products. *Journal of Cereal Science*. 2013; 57: 312-318.
27. Wu P, Li L, Du G-H. Ferulic Acid. In: *Natural Small Molecule Drugs from Plants*. 75-80; 2018.