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Chlorogenic Acid Isolation from Coffee as Affected by the Homogeneity of Cherry Maturity

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Abstract

Chlorogenic acid is a polyphenol compound which has an antioxidant properties. The objectives of this research are to understand and compare the characteristics of caffeine, chlorogenic acid, and polyphenolic content in Robusta coffee treated differently. These are necessary to find out effective postharvest method for chlorogenic acid extraction. There were two group of samples with the different treatment in process and post harvests. The coffee was treated differently during the sortation and classified into selected coffee of red cherry (fully mature cherry) and unselected coffee (partly mature cherry). The sortation was to maximize the chlorogenic acid content in green coffee bean sample from red cherry bean as the raw material. This research found that the contents of chlorogenic acid extract of green coffee bean from the selected red cherry was higher than the unselected. The HPLC analysis for chlorogenic acid contents of green coffee bean from the selected red cherry was 4.41%, it was higher than other samples tested.

Keywords: Cholorogenic acid, coffee bean, sortation, Robusta

INTRODUCTION

Coffee distributors in Indonesia are interested to buy all quality of coffee. There is no additional price or incentives for good quality coffee (Handayani, 2015). The price of coffee without selection and classification at the farm level is low (Ramanda *et al.*, 2016). Farmers tend to do harvest not only during the harvest season. The unselected coffee is produced by farmers without selection and classification, while the selected coffee is coffee which is selected based on red cherry color. The reason of unselected harvest is saving cost and energy (Sudaryanto *et al.*, 2016).

The post-harvest treatment has an effect on coffee quality. Therefore, it is necessary to select the appropriate post-harvest process in producing coffee powder or extract based on the product characteristics to be made. The harvest is done in several stages. The farmers pick the coffee based on fruit ripeness, the harvest periods are vary from 2-4 times a year depend on the fruit ripeness (Ramanda *et al.*, 2016). The first stage is preliminary picking for pre-riped fruits (Teniro *et al.*, 2018). The coffee should be harvested when the color is red, the red color indicates the level of ripeness. However, farmers pick coffee fruit on various condition, they pick the unselected (various colors) and red (selected) picking. Although farmers apply red picking, the picking method is not optimal because the percentage of red fruit is only about 70% (Aklimawati *et al.*, 2014).

The purpose of selection is to separate between the ripen cherries and the defective cherries, broken, not uniform in size or attacked by pests and diseases. Coffee are carefully sorted based on quality (defective, black, broken, hollow, and attacked by pests (Ramanda *et al.*, 2016).

One of active compound in coffee is chloregenic acid. It is produced from coffee by extraction (Sukohar & Muhartono, 2015). It has antioxidant and anticancer benefits (Kajikawa *et al*., 2018; Liang & Kitts, 2016; Maalik *et al*., 2016; Naveed, 2018; Tajik *et al.*, 2017; Sukohar & Muhartono, 2015). It is not only good for health, but also affects the taste and aroma (Taveira *et al.*, 2015). Chlorogenic acid in green coffee is higher than in roasted coffee (Cuong *et al*., 2014), however decrease due to the heating process (Jeszka-Skowron *et al.*, 2016). The postharvest period also has effect on variation on chlorogenic acids contents (Kouadio *et al*., 2014).

MATERIALS AND METHODS

Red coffee cherries (selected) were obtained from smallholder applying organic fertilizer in Pesawaran, Lampung and supplied by PT. Ghaly Rolies. Unselected coffee is harvested from farmers in the Ulubelu area of Tanggamus from a commercial coffee supplyer. All the coffee trees are classified as Robusta coffee.

This research compares different samples of coffee from different maturity level and then analyze the caffeine and chlorogenic acid contents of each sample. The wet process of green coffee bean production are hulling of fresh coffee cherry using huller, washing of pulp using a washer machine, then, the green beans were dried using oven at 40-50^{O}C to obtain green coffee with a constant weight or a constant moisture content of 10-12% using the 110 AMB Moisture balance tool. After the water content was reached, grinding process was done using Philips type HR2157.

Green coffee beans are processed further into roasting by a 20 kg capacity roaster with a temperature of 200-300^{O}C for approximately 1 hour. Organics selected roasted coffee obtained from Ghalkoff while unselected roasted coffee is obtained from Coffee 49.

Chlorogenic acid testing using the LAAN-A-LC-E008 manual Shimadzu Application News No. L.306 and Agilent Application Note 2016, caffeine tested according to SNI 01-3542-2004 (6.6.A.2) for coffee powder and total phenolic content tested by spectrophotometer using Folin Ciocalteu reagents.

Testing and isolation of chlorogenic acid using a modification of the LAAN-A-LC-E008 manual method Shimadzu Application News No. L.306 and Agilent Application Note 2016. The 0.2 g samples and 50 mL of deionized purified water from Milli-Q system (Millipore). The sample was placed in erlenmeyer and stored in the 100^{O}C incubator for 10 min. The sample was adjusted with aqua pure to 100 mL in a flask. The sample which is used for quantitative determination of chlorogenic acid and caffeine using HPLC was filtered with a syringe filter (pore size 0.45 μm, diameter 13 mm). Each extraction and testing was carried out twice with less than 5% difference. Chlorogenic acid and caffeine were tested by HPLC. Chlorogenic acid and caffeine were tested based on an external standard method with a concentration range of 5.10, 20, 40, and 60 ppm. The standard calibration curve was used to determine the correlation between signals measured from the area of the peak area and the concentration of the calculated sample. Retention time of UV absorption at maximum wavelength 273 nm for caffeine and l max 327 nm for chlorogenic acid.

A 10 mL of both the standard solution and the sample test were injected into the HPLC (Shimadzu LC 20 AD) using a 50 mL syringe with the following conditions: column VP ODS C-18, 250x4.6 mm, mobile phase aquadest filter: acetonitrile: phosphate buffer (60:20:20), flow rate of 1 mL/minute, column temperature of $30^{O}C$, PDA detector, running a mixture of standard caffeine and chlorogenic acid.

The polyphenol content was determined using the Folin Ciocalteu spectrophotometric method. Standard solution of gallic acid (10-100 ppm) was used to obtain a calibration curve. The content of polyphenol compounds is measured as a percentage of gram gallic acid equivalent per 100 g extract. The were two replications for each analysis.

RESULTS AND DISCUSSION

Based on the chromatogram analysis (Figure 1), the maximum wavelength (l) for 40 ppm caffeine standard solution was on 273 and 204 nm (a, b). In chromatogram and UV spectrum of caffein on 273 nm, there were peaks which consisted of caffeine peak and chlorogenic acid peak. The appearance of chlorogenic acid peak looks similar to 40 ppm chlorogenic acid standard solution chromatogram on maximum wave-length (l) (c). It indicates that the absorp-tion area of chlorogenic acid is wide and appear on the wave length for detection of caffeine. While in reading the standard chlorogenic acid on lmax 372 nm peak from caffeine does not appear on retention time = 5.159 (c and d).

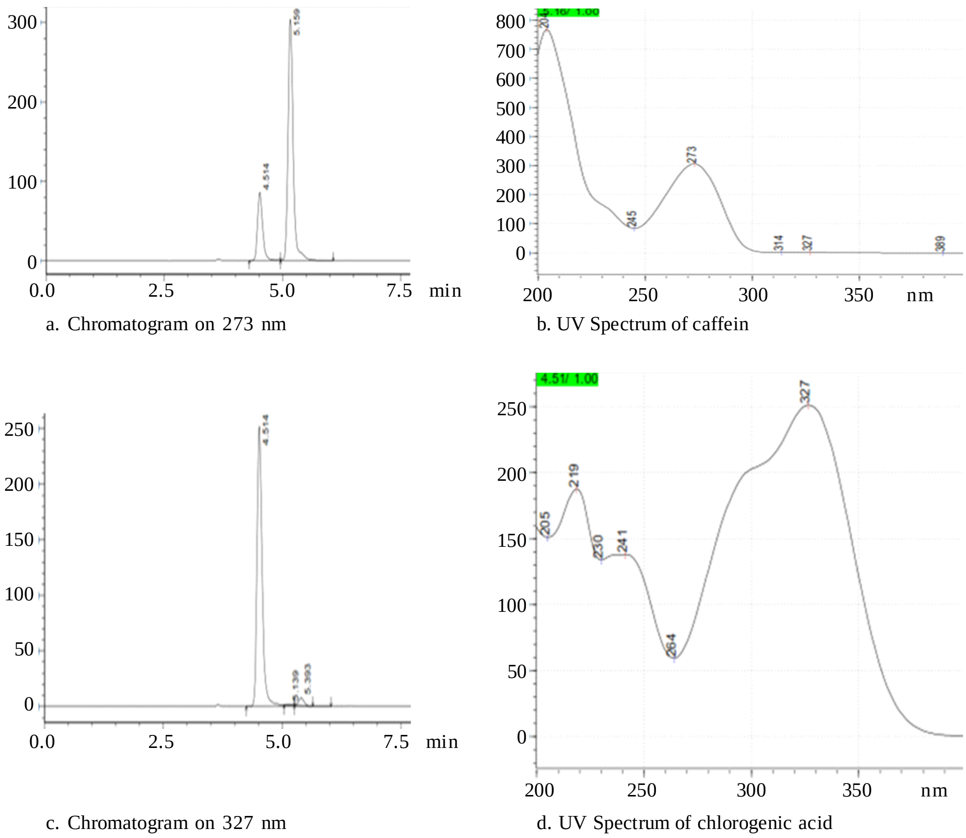

Figure 1. HPLC Chromatogram (a and c) and UV spectrum and retention time (b and d) from chlorogenic acid and caffeine

The main group of phenolic compounds is hydroxycinamic acid which is found in almost all plants. Phenolic compounds which are in the hydroxycinamic acid compounds group are insoluble compounds and bind to plant cell wall components. That cinnamic acid derivatives were identified as two intense bands as chlorogenic and caffeine acids with maximum absorption in regions 219 and 325 nm (Lee, 2000).

Chlorogenic acid content in selected coffee is higher than the unselected. There is no large difference of caffeine content in selected and unselected coffee. The effect of roasting decreases the chlorogenic acid substance both in selected and unselected coffee. The roasting changes the bean color become dark by Mailard reaction (Priftis *et al*., 2015). The roasting does not effect significantly on caffeine content. This is due to the effect of temperature on chlorogenic acid substance. Most of the chlorogenic acid become caffeine acid and quinic acid during roasting (Clifford, 1985; Aklimawati, 2014).

The caffeine and chlorogenic acid contents in coffee bean are higher than in parchment coffee. This finding may be used as the basic consideration for future research about coffee parchment utilization.

The potential antioxidant activity of plant materials depends on the content of phenolic compounds in the plants or extracts (Amarowicz & Pegg, 2019). Table 1 shows the total phenolic content, which calculated by linear regression method based on gallic acid standard solution (10-100 ppm). X represents the gallic acid standard solution, and Y is the absorbance. The linear regression, the statistical calculations as a straight line equation $Y = 0.0107x + 0.0037$ ($R^2 = 0.9999$). The calculation of phenolic content is based on that equation. The total phenolic content of each sample before and after roasting of the two types of coffee shows differences as shown in Table 1. Total phenolic of unselected coffee content is higher than in selected coffee. It is assumed that phenolic compound in unselected coffee is due to the presence of other phenolic compounds besides chlorogenic acid. Chlorogenic acid is recoqnized classified an antioxidant while caffeine is classified as alkaloid that correlated with the quality of coffee roasted beverage (Jeszka-Skowron *et al*., 2015; Patriche *et al*., 2015; Rothwell *et al*., 2014).

Roasting process does not have effect on the total of polyphenolic contents. The effect of temperature on green bean only decreases the chlorogenic acid content and is followed by an increase in caffeine content as if there is no effect on the total phenolic value. There are some process of chlorogenic acid during roasting, such as: isomerization, hydrolysis, and degradation into small molecular weight compounds (Amarowicz & Pegg, 2019).

Table 1 also describes the total polyphenolic contents in coffee green bean is higher than in parchment coffee. Extraction of chlorogenic acid and caffeine from the parchment coffee indicates the low content of active compounds which is inefficient to be extracted.

Table 1. Caffeine, chlorogenic acid, and total phenolic content

Sample	Caffeine (anhydrous), %	Chlorogenic acid, %	Total phenolic content, %
Roasted selected coffee	2.32 ± 0.02	0.026 ± 0.00	3.54 ± 0.00
Green bean selected coffee	1.70 ± 0.03	4.41 ± 0.27	3.94 ± 0.04
Parchment bean selected coffee	0.71 ± 0.00	0.40 ± 0.01	1.83 ± 0.01
Roasted unselected coffee	2.42 ± 0.02	0.03 ± 0.01	4.74 ± 0.00
Green bean unselected coffee	2.02 ± 0.02	3.60 ± 0.06	5.21 ± 0.06

Notes: Figures are means ± standard deviation.

CONCLUSIONS

The selection of coffee in harvest based on maturity have an effect on chlorogenic acid contents. The green bean selected coffee contains chlorogenic acid of 4.41%, higher than the green bean unselected coffee. For the purpose of extracting chlorogenic acid, it is better to select the coffee, which means harvest only the ripe coffee or red cherry coffee. Caffeine contents in unselected coffee both in green bean and roasted (2.02–2.42%) are higher then selected coffee, while total phenolic contents (3.94–5.21%) tend to be lower if the coffee are roasted both in selected and unselected. The roasting decreases chlorogenic acid contents (about 0.026–0.3%).

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