〈Brief Note〉

Estimation of protein, total polyphenol, chlorogenic acid, caffeine, and caffeic acid contents in Indonesian palm civet coffee (*Kopi Luwak*)

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Summary

Kopi Luwak is made from coffee berries that have been eaten by the Indonesian palm civet (*Paradoxurus hermaphroditus*). We measured the protein, total polyphenol, chlorogenic acid, caffeine, and caffeic acid contents in *Kopi Luwak*, and compared them with those in Toraja Kalosi and Gayo Mountain coffee beans.

We developed a new, simple, and fast (<5.5 min) high-performance liquid chromatography (HPLC) method, which measures chlorogenic acid, caffeine, and caffeic acid levels using an ODS column (3 μm, 4.0 × 100 mm).

The concentration of caffeine in *Kopi Luwak* coffee beans was lower and that of chlorogenic acid was higher than those in other coffee beans. The low caffeine concentration in *Kopi Luwak* coffee beans may reduce the chances of causing stress on the central nervous system and developing cardiac diseases. Chlorogenic acid is known for its taste-modifying property, inhibition of the elevation of blood glucose level, sugar absorption, and anticancer and anti-aging properties. *Kopi Luwak* coffee beans may possess higher function than those of other coffee beans.

Key words: *Kopi Luwak*, chlorogenic acid, caffeine, high-performance liquid chromatography

Introduction

Coffee is one of the most popular beverages in the world, and it is appreciated for its characteristic taste and aroma; recently, it has been recognized for its potential beneficial effects on human health.

Several species of shrubs belonging to the genus *Coffea* produce berries from which coffee is extracted. Two main commercially cultivated species include *Coffea canephora* (predominantly known as "robusta") and *C. arabica.* The robusta strain contains approximately 40%–50% more caffeine than that of arabica.

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No other coffee may have a shorter supply and more distinct flavor and history than that of the Indonesian palm civet coffee (*Kopi Luwak*). With a price tag of 1300 Dollars per kilogram, it is undisputedly the rarest and most expensive coffee or beverage in the world. It is indeed rare and unique because it is processed in the digestive system of the indigenous palm civet (*Paradoxurus hermaphroditus*). *Kopi Luwak* (Indonesian words for coffee and civet) is obtained from the Indonesian islands of Java, Sumatra, and Sulawesi. The unique method of production, and not the origin, contributes to its mystique and high cost.

The components of *Kopi Luwak* have not been studied in detail[1-4]. We evaluated the protein, total polyphenol, caffeine, caffeic acid, and chlorogenic acid contents of coffee.

Materials and methods

Materials

The samples of *Kopi Luwak* (Robusta; PT. Lintas Jaya Ababi Co., Ltd.), Toraja Kalosi (Lemon Peel Co., Ltd.), and Gayo Mountain (Lemon Peel Co., Ltd.) coffee were used in this study.

Methods

Each sample (1.00 g) was extracted with 100 mL of water at 80°C for 30 min. Protein concentration was determined using the Bio-Rad protein assay kit I (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions[5]. Bovine serum albumin was used as a standard. Total polyphenol concentration was determined using the Folin–Ciocalteu method[6]. The high-performance liquid chromatography (HPLC) system comprised a Shimadzu LC-20 AD pump (Shimadzu, Kyoto, Japan) and Rheodyne 7125 sample injector with a 20-μL sample loop (Rheodyne, Cotati, CA, USA). An ODS-4 column (3 μm, 4.0 × 100 mm; GL Sciences, Tokyo, Japan) was used, and the flow rate of the mobile phase was 0.5 mL/min. The mobile phase components were methanol (A) and 5 mmol/L phosphate buffer (pH 2.5) (B). The solvent ratio of A and B was 3:7. Detection was carried out at a wavelength of 280 nm (4 absorbance/ 1000 mV).

Results and discussion

Figure. 1 shows chemical composition (dry weight %) in three kinds of coffee beans (*Kopi Luwak*, Toraja Kalosi, and Gayo Mountain). The concentrations of chlorogenic acid, caffeine, and caffeic acid in three different types of coffee beans were estimated. While determining the concentrations of chlorogenic acid, caffeine, and caffeic acid in coffee beans, the retention times on HPLC chromatograms were between 5 and 30 min[7-11]. Our method had a relatively rapid speed of analysis because we compared the length of HPLC column and the particle size. The chlorogenic acid, caffeine, and caffeic acid were separated within 5.5 min using an ODS column (3 μm, 4.0 × 100 mm).

The concentration of caffeine in *Kopi Luwak* coffee beans was lower than that in other coffee beans (Table 1). The civet might have digested the caffeine in coffee beans or might have eaten the coffee beans with relatively poor caffeine content. The concentration of chlorogenic acid in *Kopi Luwak* coffee beans was higher than that in other coffee beans. The low caffeine content of *Kopi Luwak* coffee beans may reduce the chances of imparting stress on the central nervous system and developing cardiac diseases. Chlorogenic acid is known for its taste-modifying nature, inhibition of the elevation of blood glucose level, sugar absorption, and anticancer and anti-aging properties. *Kopi Luwak* coffee beans may possess higher function than those of other coffee beans.

The amount of caffeic acid was low in all the tested coffee beans (0.00463%–0.0558%). Caffeic acid has been reported to induce chromosomal abnormalities. Thus, reduced levels of this compound in coffee beans are beneficial with respect to health.

The amounts of proteins and polyphenols were high in the coffee beans (0.85%–1.1% and 7.56%–7.94%, respectively). Proteins produce the flavor-enhancing volatile heterocyclic compounds during the browning reaction, which imparts the specific

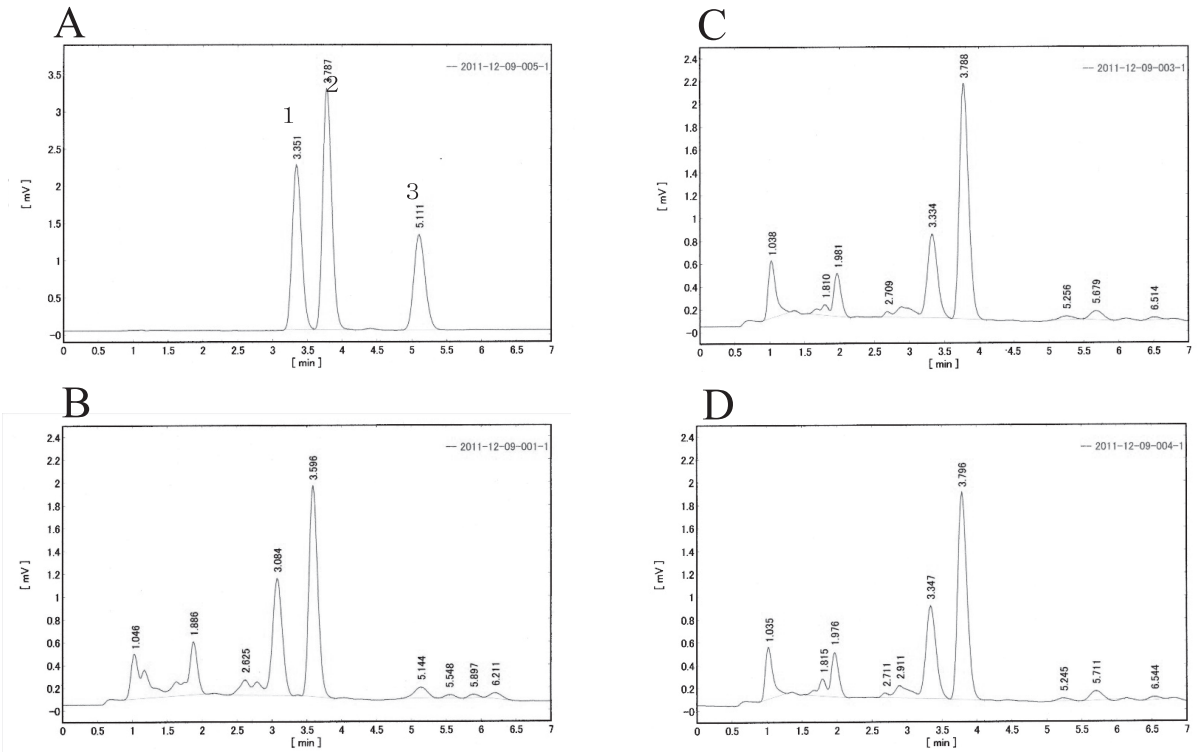

Fig. 1 The Chromatographic conditions are described under materials and methods. Column: ODS-4 (3 μm, 4.0 × 100 mm), Detection: 280 nm (4 absorbance/ 1000 mV), Flow rete: 0.5 mL/ min, mobile phase: methanol/ 5 mmol/L phosphate buffer (pH 2.5) = 3/ 7.
(A) Standard materials (1, chlorogenic acid; 2, caffeine; 3, caffeic acid); (B) Kopi Luwak; (C) Toraja Kalosi; (D) Gayo mountain.

flavor to these coffee beans. It is reported that polyphenols have diverse physiological properties; e.g., antimutagenicity, antitumor action, antioxidant behavior, and reduction of plasma cholesterol[12]. Coffee is considered as a good and healthy drink because of the presence of many polyphenols. Toraja Kalosi and Gayo Mountain are popular coffee beans. In this study, chemical composition of between these popular coffee beans and *Kopi Luwak* was examined, respectively. The species of coffee beans of Toraja Kalosi and Gayo Mountain is *C. arabica.* That of *Kopi Luwak* is *C. canephora. C. canephora* contains approximately 40%–50% more caffeine than that of *C. arabica.* The indigenous palm civet may digest caffeine in coffee beans. Investigation in the digestion system of the indigenous palm civet is now in progress.

Table1. Chemical composition (dry weight %) of coffee beans.

	Kopi Luwak	Toraja Kalosi	Gayo Mountain
Protein (%)	1.1 ± 0.01	1.1 ± 0.02	0.85 ± 0.03
Polyphenols (%)	7.56 ± 0.03	7.94 ± 0.01	7.76 ± 0.03
Caffeine (%)	0.361 ± 0.03	0.423 ± 0.02	0.372 ± 0.001
Caffeic acid (%)	0.0558 ± 0.0013	0.00463 ± 0.0002	0.0155 ± 0.0018
Chlorogenic acid (%)	0.293 ± 0.001	0.218 ± 0.001	0.242 ± 0.002

Data are presented as mean ± SEM from five infusions per each sample.

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