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Mineral composition and antioxidant activity of partially digested coffee "Kopi Luwak"

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ABSTRACT

Background: Kopi Luwak is a rare type of coffee, that is partially digested in the Asian palm civet's digestive tract before roasting. It therefore possesses a distinctive flavor and unique properties, shaped by the digestive enzymes in the civet's intestine.

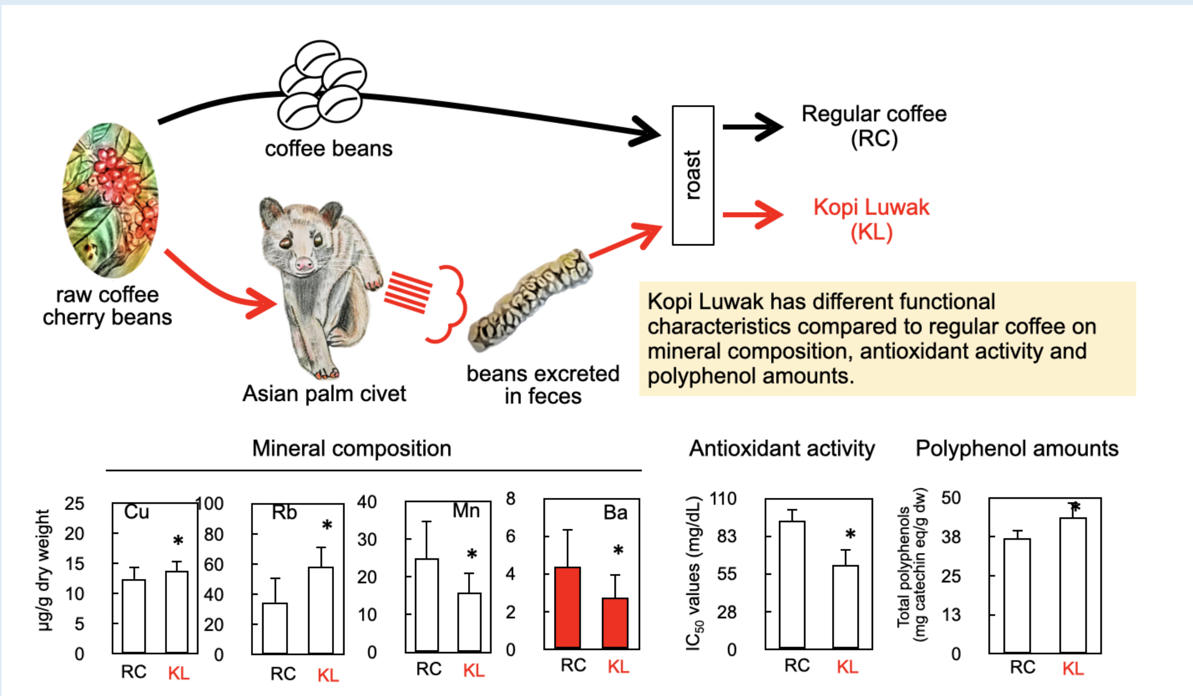
Objective: In this study, the differences of mineral composition, antioxidant potency and total polyphenol amount between commercially available Kopi Luwak and regular coffees (without digestive fermentation) were evaluated.

Methods: Twenty-one regular type of coffee bean and 17 of Kopi Luwak bean were purchased from local markets or Kopi Luwak production company in Indonesia. The samples were then analyzed for the levels of 23 minerals (Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, V and Zn) using ICP-MS system. The total polyphenol amount and antioxidant activity were evaluated using Folin-Ciocalteu assay and DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay, respectively.

Results: Of these 23 minerals, the concentrations of Mn and Ba were significantly lower in Kopi Luwak than in regular coffee. Otherwise, the concentrations of Cu and Rb in Kopi Luwak were significantly higher than those in regular coffee. The IC_{50} value on DPPH radical scavenging assay of Kopi Luwak (61.4±2.8 mg/dL) was significantly lower than that of regular coffee (93.6±1.9 mg/dL). Total polyphenol amount for Kopi Luwak was significantly higher than regular coffee. The correlation factor between these factors was $r = -0.5910$ and $p < 0.001$.

Conclusion: Kopi Luwak had lower concentrations of Mn and Ba, but higher levels of Cu and Rb compared to regular coffee. Additionally, Kopi Luwak exerted strong antioxidant activity and contained higher polyphenol amounts than regular coffee. Thus, our findings imply that Kopi Luwak is an important candidate for functional foods.

Keywords: antioxidant potency, ICP-MS, Kopi Luwak, mineral composition, polyphenols



INTRODUCTION

Coffee is widely recognized as one of the most popular beverages in the world. The global market for "ready-to-drink beverages," such as coffee and tea was estimated to be worth 103.62 billion USD in 2022 and is projected to grow at an annual growth rate of 6.2% until 2030 [1]. Undoubtedly, one reason for the popularity of coffee for daily consumption is its various functional properties, such as anti-obesity, anti-cardiovascular disease, and anti-diabetes activities [2]. It is also widely known that coffee is rich in biological active ingredients, such as polyphenols, and shows strong antioxidant activity [3]. Therefore, the shift towards healthier and more convenient beverage choices is a major force on market growth [1].

Unroasted green coffee has recently aroused consumer interest because of its nutritional potential [4]. On the other hand, the roasting process affects the taste of the coffee beverage by physically and chemically changing the flavor and bitterness of the coffee beans [5]. For example, temperature causes changes in bioactive compounds, such as polyphenols and the appearance of Maillard reaction compounds, which affect the sensory and antioxidant properties of the product [3]. Therefore, coffee brewed from roasted coffee is highly popular. In the "ready-to-drink market", consumers are also attracted to the wide range of varieties that cater to diverse tastes in the coffee beverages [1]. Therefore, in recent years, coffee with flavors such as cinnamon, chocolate, and hazelnut have become widely accepted.

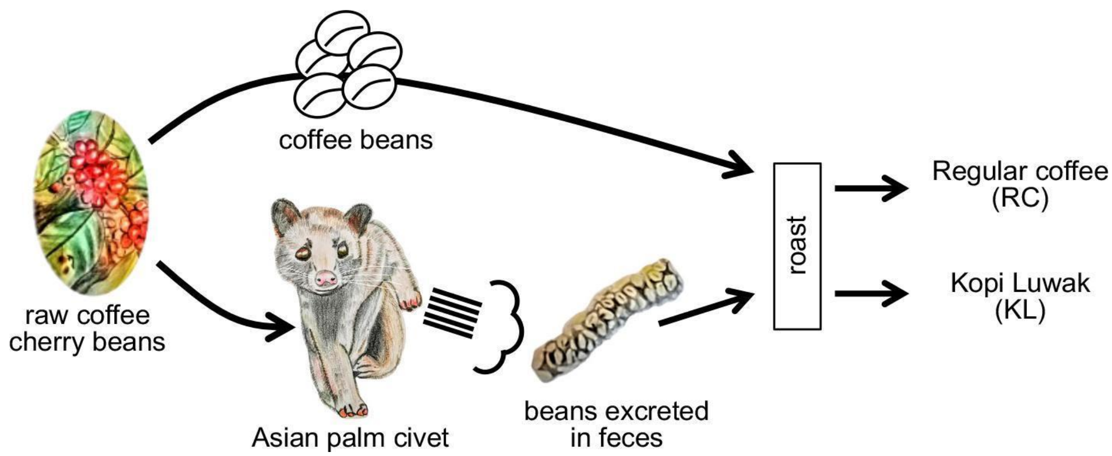


Figure 1. Conceptual diagram of the processing method for Kopi Luwak (KL).

Kopi Luwak is one of Indonesia's most popular coffees. The wild Asian palm civet (*Paradoxurus hermaphroditus*) likes to eat raw coffee cherry beans [6], which it partially digests then excretes in its feces. The excreted beans are then collected, washed, dried, and roasted to make Kopi Luwak (Figure 1). The annual production of Kopi Luwak is estimated to be 127 kg, but this is recognized to be a remarkable underestimation. With prices reaching 200-400 USD per kg, Kopi Luwak is known as the most rare and expensive coffee [7]. On the other hand, the potential threat to both the welfare and conservation of wildlife posed by civet farms came to the attention of the media following an undercover investigation carried out by the BBC in Indonesia in September 2013 [7]. The World Society for the Protection of Animals examined the footage and found that civets are usually kept in inappropriate conditions and estimated that thousands of wild civets are poached each year to maintain these breeding farms. In contrast, traditional production methods of Kopi Luwak coffee do not pose a threat to the welfare and conservation of civets as these methods do not involve the removal of civets from their natural environments (See, the report by D'Cruze et al. [7]).

Several reports have explained how Kopi Luwak differs from regular coffee, which has not been digested and fermented in the Asian palm civet. Kopi Luwak is generally darker in color, appearing a darker red than regular coffee beans. The beans are also affected by digestive enzymes as they pass through the digestive tract of the Asian palm civet, causing the breakdown of the proteins in the beans [8]. Hence, the protein amount in Kopi Luwak is lower than in regular coffee beans [9]. The contents of alkaloids, such as caffeine, trigonelline and xanthine, and the pH are lower, while the lipid content is higher than those of regular coffee [9]. The chlorogenic acid content of Kopi Luwak is lower than regular coffee, presumably because it is metabolized by the bacterial flora in the colon of the Asian palm civet [10]. On the other hand, no difference in the contents of amines, such as theanine has been reported [10]. Metabolite profiling of Kopi Luwak and regular coffee using gas chromatography equipped with mass spectrometer (GC/MS) analysis has also shown that some metabolic parameters, such as citric acid and inositol/pyroglutamic acid ratio are different [11]. Watanabe et al. (2020) also compared the fecal microbiome of Asian palm civet with the other 14 animal species, finding that the *Gluconobacter* species were specifically present in Asian palm civet, thus indicating that it may contribute to the fermentation of coffee beans in its gastrointestinal tract [12]. These changes make the components, taste and flavors of Kopi Luwak very distinctive.

Other important components that can affect taste are minerals, such as calcium, copper, iron, magnesium and zinc, with one example, magnesium in drinking water, being perceived as a positive taste [13]. Interestingly, it has been reported that when vegetables are fermented, the concentrations of minerals, such as iron, magnesium, potassium and zinc decrease during the fermentation process [14]. This report suggests that Kopi Luwak, which is produced through digestion and fermentation in the digestive tract, has a different mineral composition compared with the regular coffee, which may affect its taste and flavor. However, no studies have comprehensively compared the mineral concentrations of Kopi Luwak with those of regular coffee. In addition, there are few reports comprehensively discussing differences in the functional properties of commercially available Kopi Luwak and regular coffees, particularly under the viewpoints of antioxidant activity and polyphenol content.

Therefore, in this study, we firstly compared the amounts of 23 mineral components in 17 Kopi Luwak coffees and 21 regular coffees, which were purchased from local markets in Indonesia or Kopi Luwak production company in Malang Regency and Batu municipality, East Java, Indonesia using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) system. In addition, differences in antioxidant activity and polyphenol content were compared as functional effects between Kopi Luwak and regular coffees.

METHODS

Materials: Twenty-one regular type of coffee beans and 17 of Kopi Luwak beans were purchased from local markets in Indonesia or Kopi Luwak production company in Malang Regency and Batu municipality, East Java, Indonesia independently. All coffee beans obtained in this study were roasted, and the effects of differences in the roasting process were not dealt with in this study. Information about the samples is summarized in Table 1. Standard solutions containing 23 minerals (Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, V and Zn), phenol reagent and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were obtained from Fuji Film Wako Pure Chemical Co. (Osaka, Japan). Nitric acid (HNO_3) for metal analysis was from Kanto Chemical Co., Inc. (Tokyo, Japan). The other reagents used were the highest grade available.

Microwave digestion*:* One of the digestion methods used in mineral analysis is the heating treatment using microwaves. In which methods, highly productivity is obtained in a sealed container with acid reagents. Nitric acid (HNO_3), which is a highly oxidizing reagent, is used for acid decomposition of all organic compounds composed of hydrocarbon groups, and widely used as the methods for mineral analysis [15]. This chemical reaction follows formula (1).

$$(CH_2)x + 2HNO_3 \rightarrow xCO_2 + 2NO + 2H_2O \quad (1)$$

In this study, we employed microwave digestion using HNO_3 as described in a previous report with some modifications [15]. In brief, the individual sample was powdered using a Grindomix GM200 knife mill (Retsch GmbH, Haan, Germany) then oven-dried at 90°C for 24 h.

Two mL of 61% HNO_3 were added on the dried powders (0.100 g) in PTFE sample container (Model PT-70, Sanai-Kagaku Co., Aichi, Japan), and then digested in a microwave oven with 200 W at 30 min. After cooling, the digested samples were filtered with the filter paper (5C, Advantec Inc., Tokyo, Japan). The filtrates were diluted ca. 250 times with ultra-pure water, and then weighed to determine the dilution factor. The microwave digestion process was repeated independently three times on individual samples listed in Table 1.

Table 1. Regular coffee and Kopi Luwak samples used in this study.

Regular coffees (RC)			Kopi Luwak coffees (KL)	
No.	Memo		No.	Memo
RC-1	Bali Kintamani Arabica Coffee		KL-1	Dian Kopi Luwak
RC-2	Piltik coffee Arabika		KL-2	Semekar Luwak Coffee
RC-3	Excelso Sumatra Madheling		KL-3	Excelso Kopi Luwak Tana Toraja
RC-4	Kopi Manglayang Bumi Sinamukti		KL-4	Otten Luwak Premium
RC-5	Papua Arabika		KL-5	Caffine Dope Arabika Luwak
RC-6	Often Sindora		KL-6	Kopi Keranjang Luwak
RC-7	Bali Robusta		KL-7	Worcas Bali King
RC-8	Java Singa Coffee Robusta		KL-8	Worcas Sumatra King Group
RC-9	Java Singa Arabika		KL-9	Arutala Luwak Liara Gaya
RC-10	Often Sidikalang		KL-10	Awi Coffee Kopi Luwak
RC-11	Java Dampit Coffee		KL-11	Luce Coffee Luwak Ijen
RC-12	Often Coffee kerinci		KL-12	Kompeni Kopi Luwak Robusta
RC-13	Excelso Robusta Gold		KL-13	Loewak d'coffee Roasted
RC-14	Often Robusta Flores		KL-14	Luwak Gunung Kawi Roasted Robusta
RC-15	Excelso Classic		KL-15	Wild Luwak Roasted
RC-16	Aceh Gayo Arabica		KL-16	Dian Coffee Yellow Roasted
RC-17	Often Coffee Garut		KL-17	Luwak Gunung Kawi Arabika
RC-18	often Coffee Gayo			
RC-19	often Robusta Lampung			
RC-20	Often Latimojong			
RC-21	Excelso Kalosi Toraja			

Individual regular coffee bean (RC) and Kopi Luwak bean (KL) were purchased from local markets in Indonesia or Kopi Luwak production company in Malang Regency and Batu municipality, East Java, Indonesia independently.

ICP-MS analysis: The amounts of the 23 minerals listed above were analyzed using Agilent 7850 ICP-MS system (Agilent Technologies Inc., Santa Clara, CA, USA) using the parameters on Table 2 according to our previous study with some modifications [15]. When the concentration of the analyzed minerals was under the detection limit, the value of the methodological detection limit was used for convenience as shown in Table 3.

Table 2. Operational parameters of ICP-MS system (Agilent 7850)

Parameters	Setting
RF power	1600 watt
Nebulizer flow rate (carrier gas)	0.68 liter/minute
Plasma gas flow	15 liter/minute
Sweeps	100
Reading	1
Number of replicates	3
Scanning mode	Peak hopping
Sampling cone orifice (Ni)	1.1 millimeter
Skimmer cone orifice (Ni)	0.4 millimeter
Isotopes of selection	^{7}Li, ^{24}Mg, ^{27}Al, ^{43}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{85}Rb, ^{88}Sr, ^{95}Mo, ^{111}Cd, ^{118}Sn, ^{133}Cs, ^{137}Ba, ^{208}Pb, and ^{209}Bi

Table 3. The method detection limit (MDL) in this study using ICP-MS (Agilent 7850)

Mineral name	Li	Mg	Al	Ca	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
MDL (ng/g dry weight)	0.3	3.0	3.0	13	0.1	0.2	0.3	5.0	0.1	0.1	1.0	6.0
Mineral name	As	Se	Rb	Sr	Mo	Cd	Sn	Cs	Ba	Pb	Bi	
MDL (ng/g dry weight)	0.8	0.7	0.1	0.2	0.2	0.1	0.3	0.1	0.2	0.3	0.2	

Extraction: Fifty milligrams of coffee fine powders were extracted using 2 mL of 90% methanol solution containing 0.5% phosphoric acid according to our previous method [16]. After standing in an ultrasonic bath for 1 min, the reaction mixture was centrifugated at 2,000 g for 10 min. The supernatant was dried with a CC-105 centrifugal concentrator (TOMY Seiko Co., Tokyo, Japan). The residue was dissolved in 0.5 mL of dimethyl sulfoxide, and then moved to the following analysis.

DPPH radical scavenging assay: The extracts described above, and its dilutions were added to 250 nmol DPPH reaction mixture composed with 50% ethanol in 50 mM acetate buffer (pH 5.5), and the signal was monitored at 517 nm using microplate reader (Multiskan GO basic, Thermo Fisher Scientific K.K., Tokyo, Japan) [17]. The half maximal inhibitory concentration (IC_{50}) value was then calculated as the concentration of coffee sample solution required for scavenging half of DPPH radicals (125 nmol) within 30 min.

Folin-Ciocalteu Assay: Total polyphenol amounts existed in the Kopi Luwak and regular coffees were evaluated using the Folin-Ciocalteuu method with some modifications [18]. Eighty microliters of catechin as the standard compound or the extracts were mixed with phenol reagent (100 µL), and then 80 µL of 10% Na_2CO_3 solution was added. After incubation at room temperature for 1 h under dark conditions, absorbance at 760 nm was recorded.

Statistical analysis: The data are indicated as mean value ± standard deviation (S.D.). The statistical significance was determined using the unpaired Student's t-test (JMP Pro version 16.0, SAS Institute Japan Inc., Tokyo, Japan). Correlation analysis was performed with Excel Tokei (Tokyo, Japan). The alpha value for making comparisons was set at 0.05.

RESULTS

Comparison of mineral concentrations of Kopi Luwak and regular coffees*:* The present study used the ICP-MS system to analyze and compare the concentrations of 23 minerals in Kopi Luwak and regular coffees. Of these, the concentrations of 7 minerals (Ca, Cr, Cu, Fe, Mg, Mn and Zn) are shown in Figure 2. The results for the other 7 minerals (Al, Ba, Co, Cs, Rb, Sr and V) are shown in Figure 3. The following minerals were below the methodological detection limits: As, Bi, Cd, Li, Mo, Ni, Pb, Se and Sn. The concentrations of two of the minerals measured in this study, Mn and Ba were significantly lower in Kopi Luwak than in regular coffee ($p < 0.05$) (Figures 2 and 3). The concentrations of Zn, Cr, Co and V were also remarkably lower in Kopi Luwak than in regular coffee, but the differences were not significant ($p \geq 0.05$). Otherwise, Cu and Rb in Kopi Luwak were significantly higher than those in regular coffee ($p < 0.05$) (Figures 2 and 3).

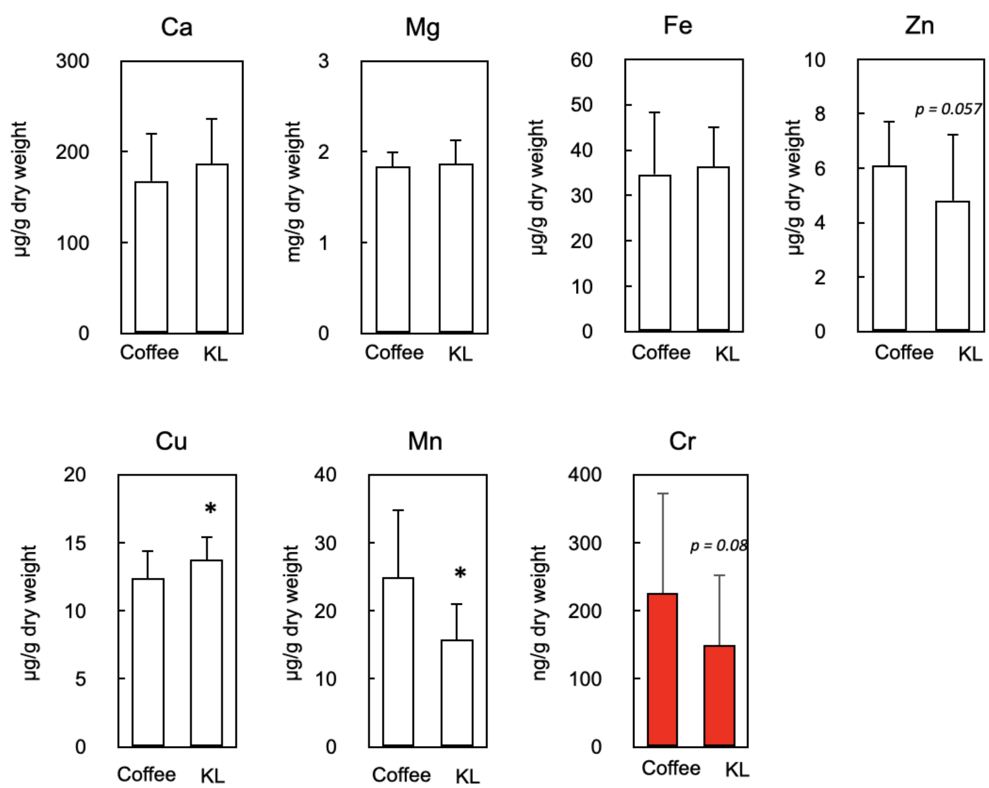


Figure 2. Comparison of 8 essential mineral concentrations between Kopi Luwak (KL) and regular coffee. Data is expressed as mean ± S.D. (regular coffee, n = 21; Kopi Luwak, n = 17). *Significantly different *vs* regular coffee ($p < 0.05$).

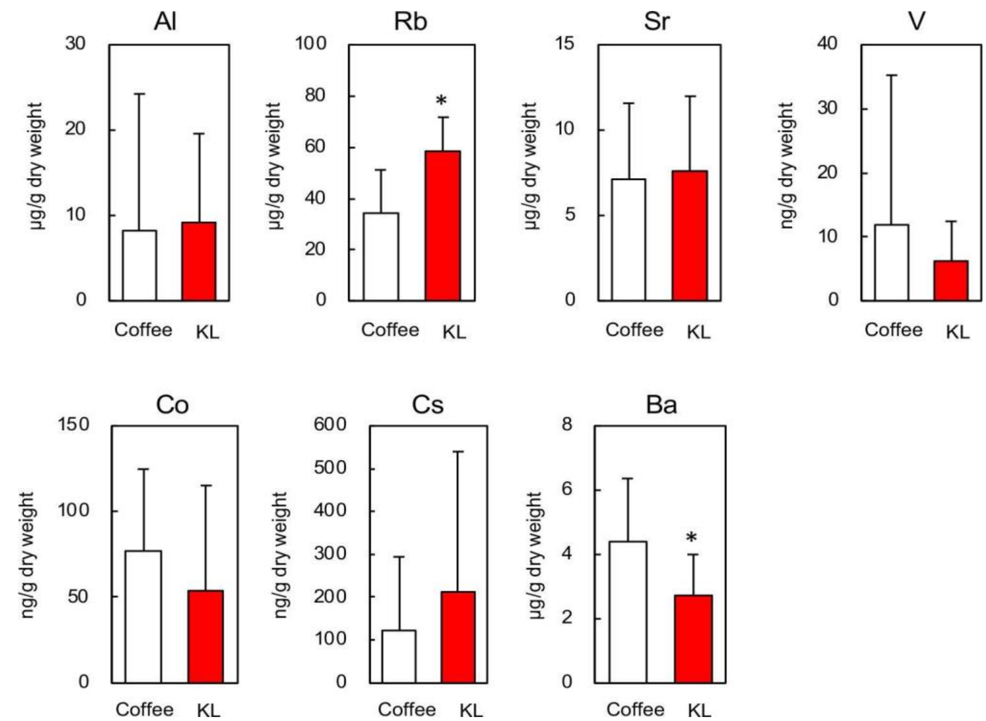


Figure 3. Comparison of 7 mineral concentrations between Kopi Luwak (KL) and regular coffee. Data is expressed as mean ± S.D. (regular coffee, n = 21; Kopi Luwak, n = 17). *Significantly different *vs* regular coffee group (p < 0.05).

Antioxidant activity and total polyphenols: The antioxidant activity of Kopi Luwak and regular coffee were indicated as the IC_{50} value, which was the amount required to scavenge 50% of DPPH radicals (Figure 4A). The IC_{50} value of Kopi Luwak (61.4±2.8 mg/dL) was significantly lower than that of regular coffee (93.6±1.9 mg/dL). Total polyphenol amount for Kopi Luwak (43.8±1.2 mg catechin equivalent/g dry weight) was significantly higher than that of regular coffee (37.1±0.6 mg catechin equivalent/g dry weight) as shown in Figure 4B. Correlation factor was calculated using individual data obtained from DPPH radical scavenging activity and total polyphenol amounts in both Kopi Luwak and regular coffee samples (Figure 4C), and found the negative correlation on both factors (r = -0.5910; p = 9.34E-05).

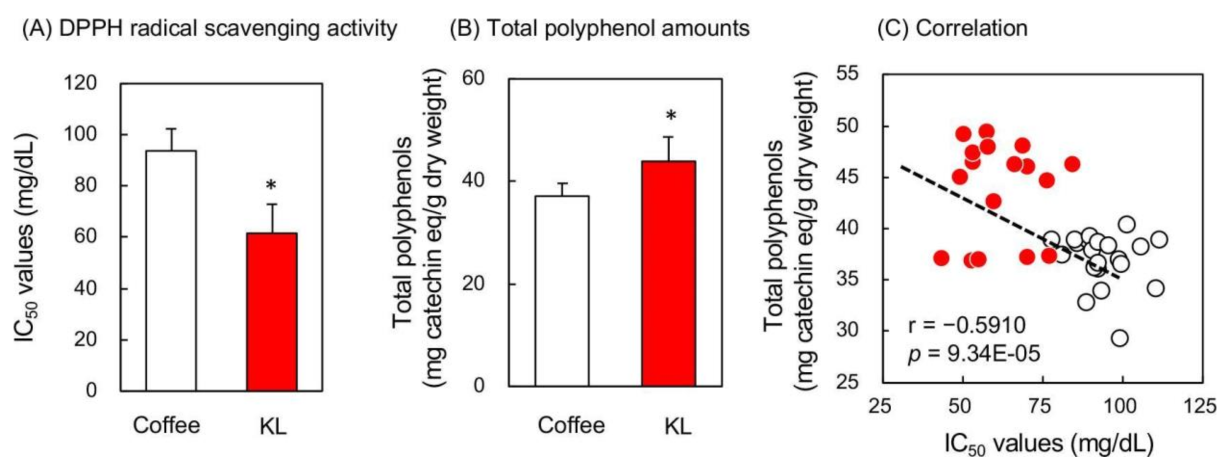


Figure 4. Antioxidant activity and total polyphenol amounts Kopi Luwak (KL) and regular coffee. (A) antioxidant activity as the amount required to scavenge 50 % of DPPH radicals (125 nmol) during 30-min (IC_{50} value). (B) total polyphenol amounts as catechin equivalent. (C) Correlation between DPPH radical scavenging activity and total polyphenols of Kopi Luwak (●) and regular coffee (○). Data is expressed as mean ± SD (regular coffee, n = 21; Kopi Luwak, n = 17). *Significantly different *vs* regular coffee group (p < 0.05).

DISCUSSION

The use of ICP-MS, which can simultaneously measure the concentrations of multiple minerals, has enabled comparisons across various food products [19]. Therefore, the present study used the ICP-MS system to analyze and compare the concentrations of 23 minerals in Kopi Luwak and regular coffees. In Japan, 13 minerals (Ca, Cr, Cu, Fe, I, K, Mg, Mn, Mo, Na, P, Se and Zn) are covered by the Dietary Reference Intakes for Japanese established by the Ministry of Health, Labor and Welfare [20]. Of these, the amounts of 7 minerals (Ca, Cr, Cu, Fe, Mg, Mn and Zn) and the other 7 minerals (Al, Ba, Co, Cs, Rb, Sr and V) in regular coffee summarized in Figures 2 and 3 were comparable with previous reports analyzed using ICP-MS [21], thus confirming the validity of the results obtained in this study.

The concentrations of two of the minerals measured in this study, Ba and Mn were significantly lower in Kopi Luwak than in regular coffee ($p < 0.05$) (Figures 2 and 3). The concentrations of Co, Cr, V and Zn were lower in Kopi Luwak than in regular coffee, but the differences were not significant ($p \geq 0.05$). Kopi Luwak is produced by a fermentation process involving microorganisms in the intestine of the Asian palm civet. In general, microorganisms found in the mammalian digestion system, including the Asian palm civet, are those derived from different types of lactic acid bacteria, mainly *Lactobacillus* and *Bifidobacterium* [22]. A literature review revealed no reports that had tracked changes in mineral composition before and after fermentation in the gastrointestinal tracts of mammals, such as the Asian palm civet. On the other hand, several reports have tracked the changes in minerals when vegetables were fermented *in vitro* with lactic acid bacteria, including the orders, *Lactobacillales*, *Bacillales*, and *Bifidobacteriales*. Kiczorowski et al. reported that plant-derived foods, such as carrots, broccoli, and bell peppers that have undergone lactic acid fermentation contain less Co, K, Mg, Zn, and other minerals than those that have not been fermented [14]. They suggested that such a phenomenon might be the result of metal binding to the cell walls on the fermentation of fungi and bacteria. Thus, the reduction in the concentrations of these minerals in Kopi Luwak observed in the present study might have been caused during fermentation by the intestinal microflora in the Asian palm civet's digestive tract.

The concentrations of Cu and Rb in Kopi Luwak were significantly higher than those in regular coffee ($p < 0.05$) (Figures 2 and 3). It is speculated that the increase in these two minerals may be related to their eating habits, in which is predominantly frugivorous (fruits and plants comprising 97% of the diet). Additionally, their diets have also high intra-annual and inter-annual variations [23]. Future comparisons of the mineral composition of coffee beans before and after consumption by the civet, will need to take account of information on their diets to provide more definitive results. Cu is an essential mineral for humans, because of its involvement in numerous biological processes including iron metabolism, neuropeptide synthesis and antioxidant defense [24]. Additionally, it has been reported that marginal copper deficits may contribute to the development and progression of several disease states, including cardiovascular disease and diabetes [25]. According to the report by Pennington and Young [26], daily Cu intakes are low (<80%) compared with the intakes recommended by the National Academy of Sciences. Therefore, our results imply that Kopi Luwak can be an important source of Cu. Although Rb can cause health problems when ingested in large amounts, there is no health concern with Rb in Kopi Luwak because its levels are low [27].

Differences in mineral concentration can affect the taste and palatability of food. For example, the same dashi broth prepared using soft water (ca. 30 mg/L as hardness) was preferred to that prepared using very hard water (ca. 1,500 mg/L as hardness) [28]. In the present study, there were no significant differences between Kopi Luwak and regular coffee in the concentrations of minerals, such as Ca and Mg ($p \geq 0.05$), which are known to affect taste [13], as shown in Figure 2. However, Kopi Luwak was characterized by lower concentrations of Cr and Mn, and higher concentrations of Cu and Rb compared with regular coffee (Figures 2 and 3). Currently, no information is available on the effects of these four minerals on taste and palatability. However, it is unlikely that such small differences observed in Kopi Luwak affect taste or palatability.

In this study, we further evaluated the antioxidant activity and total polyphenol amount of Kopi Luwak and regular coffee. Both samples gave IC_{50} values, indicating clearly that they exert dose-dependently antioxidant activity (Figure 4A). This is consistent with the results reported for roasted regular coffee [29]. And the IC_{50} value of Kopi Luwak was significantly lower, indicating that the antioxidant activity of Kopi Luwak is higher than that of regular coffee. The total polyphenol amount in the regular coffee was about 40 mg/g (Figure 4B), a level comparable to that in previous reported amount [30]. The clear negative correlation between antioxidant activity (IC_{50} value) and total polyphenol amount was obtained (Figure 4C). In general, polyphenols are well known to possess strong antioxidant activity [31]. Therefore, the increased polyphenol content may be partly responsible for the higher antioxidant activity in Kopi Luwak than in regular coffee. The result, in which total polyphenol amount in the Kopi Luwak was significantly higher, indicated that polyphenols in coffee cherry may have increased during the digestion process in the small intestine of Asian palm civetIt is known that polyphenols are affected by the structural changes that occur during gastrointestinal digestion. For example, Correa et al. reported that the total amount of phenolics in rosemary and green tea extracts decreased after gastrointestinal digestion, whereas the polyphenols in Yerba Mate extracts were unaffected by digestion when using human microbiota [32]. It should be noted here, however, that these effects obtained using human gut microbiota are not observed in systems with pig or rat gut microbiota [32]. In other words, the effects of the intestinal microflora differ according to the animal species. Yang et al. reported that non-extractable bound polyphenols derived from Fu brick tea are released through the action of colonic microflora, as demonstrated in a mouse model of dextran sulfate sodium-induced ulcerative colitis [33]. The results of this previous study suggest that during passage through the wild Asian palm civet, the non-extractable bound polyphenols in coffee cherry may be more easily released by the action of their gut microbiota. As described above, microorganisms found in the mammalian digestion system, including the Asian palm civet, are those derived from different types of lactic acid bacteria, mainly *Lactobacillus* and *Bifidobacterium* [22]. Although not fermented by the gut microbiota, in vitro studies have shown a 2.5-fold increase in 2-hydroxy-3-(4-hydroxyphenyl) propanoic acid content in mulberry juice, and an increase in polyphenols in rosemary and thyme extracts has been increased during the fermentation process by *Lactobacillus plantarum* [34-35]. Therefore, although there are no reports on the effects of polyphenols on the gut microbiota of wild Asian palm civet, it is possible that the polyphenols in coffee beans are increased during their passage through their gastrointestinal tract.

CONCLUSIONS

In this study, we compared Kopi Luwak, a rare and expensive coffee produced through digestion and fermentation in the digestive tract of the wild Asian palm civet, with regular coffee. Using ICP-MS to analyze 23 minerals, we found for the first time that Kopi Luwak contained lower concentrations of Ba, Cr, Mn, and Zn, but higher levels of Cu and Rb compared to regular coffee. . Additionally, we also found that the antioxidant activity of Kopi Luwak was higher than regular coffee. The main reason for this was suggested to be the increase in polyphenol content during passage through the digestive tract of the wild Asian palm civet. Thus, our findings imply that Kopi Luwak is an important candidate for functional foods. However, there is still no consensus on the definition of "functional foods," and as a result, many institutions lack a comprehensive process for their classification [36-37]. It is recognized that "functional foods" are not a substitute for conventional medicines, but they can be used in conjunction with Western medicine to support the optimization of health in people with chronic diseases and to prioritize the management of symptoms related to those diseases [38].

List of Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; KL, Kopi Luwak; RC, regular coffee

Authors' contributions: ADI, DY, IW, HS designed the study. ADI, DY, RG, YO carried out the laboratory work. ADI, DY, YO, SH analyzed the data. ADI, DY, HS wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: None of the authors have any financial interests or conflict of interests to report.

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