Analysis of Luwak coffee volatile by using solid phase microextraction and gas chromatography

Analisis senyawa volatil kopi Luwak dengan menggunakan mikroekstraksi fase padat dan kromatografi gas

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Summary

Approach to authenticate Luwak coffee is made through analysis of volatile compounds of luwak coffee. Luwak coffee bean from type of Arabica obtained from Andungsari Plantation in Bondowoso district, East Java Province Indonesia, was wet processed and sundried prior to roasting step. As many as 120 g green bean was roasted at 170-220°C for 8-12 minutes until color is light brown (Agtron scale 65) and was ground prior to extraction. Volatile compounds of roasted Luwak Arabica coffee bean were extracted by using solid phase microextraction (SPME) at 60°C for 30 min. The extracted analyte was subsequently transferred to GC-FID system by splitless injection at 260°C with 5 min sampling time, continued with separation through 50% phenyl 50% dimethylpolysiloxane capillary column and oven temperature programmed from 60°C to 180°C with rate of 5°C/min. Resulted chromatogram shows major peaks mainly in Rt 8.36-9.981, and Rt 9.705-14.778, and minor peaks identified before peak Rt 10 and after Rt 24. Varied sample quantity ranged within 0.5-2.5 g produced chromatograms which were not significantly different ($p = 0.08$). This research also investigated the use of γ-picoline (4-methylpyridine) as internal standard. It was showed that γ-picoline appeared at Rt 8.6 ~ without overlaying other peaks originated from sample. Concentration of γ-picoline at 0.05 μL/g, resulted separable peak thus indicates its use for quantification.

Key words: Luwak, coffee, SPME, volatile, γ-picoline.

Ringkasan

Salah satu pendekatan untuk membuktikan keaslian kopi Luwak, dilakukan dengan menganalisa senyawa volatilnya. Kopi Luwak dari jenis arabika diperoleh dari Kebun Andungsari, Kabupaten Bondowoso, Jawa Timur. Kopi diproses dengan metode olah basah dan dikeringkan secara alami. Sebanyak 120 g kopi Luwak disangrai pada suhu 170-220°C selama 8-12 menit hingga diperoleh warna biji cokelat muda (skala Agtron no. 65) dan dihaluskan sebelum proses ekstraksi. Senyawa volatil kopi Luwak diperoleh dengan metode mikroekstraksi fase padat pada suhu 60°C selama 30 menit. Analit yang terekstrak diinjeksikan ke instrumen kromatografi gas secara splitless *pada suhu 260°C dengan waktu sampling 5 menit, dan dilanjutkan dengan pemisahan melalui kolom kapiler 50% fenil 50% dimetilpolisiloksan dengan pengaturan suhu awal 60°C dan dinaikkan 5°C/min* mencapai 180°C kemudian

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dipertahankan selama tiga menit. Kromatogram yang dihasilkan menunjukkan beberapa puncak dominan khususnya pada waktu retensi 8.36-9.981 menit dan 9.705-14.778 menit, dan beberapa puncak kecil sebelum waktu retensi 10 menit dan setelah 24 menit. Jumlah sampel yang digunakan bervariasi antara 0.5-2.5 g dan kromatogram yang terbentuk tidak berbeda signifikan. Penelitian ini juga mempelajari penggunaan γ-pikolin (4-metilpiridin) sebagai internal standard dan diperoleh data bahwa puncak γ-pikolin muncul pada waktu retensi 8.6 menit tanpa menutupi kemunculan puncak lainnya yang berasal dari sampel. Konsentrasi γ-pikolin sebesar 0.05 μL/g menghasilkan puncak yang terpisah dengan baik dan memungkinkan untuk digunakan dalam proses kuantifikasi.

Kata kunci: *Luwak, kopi, SPME, volatil, γ-pikolin*

INTRODUCTION

Luwak coffee bean is produced from droppings of Luwak (*Paradoxurus hermaphrodites*), a nocturnal cat, member of family viverridae (Krishnakumar, 2002). This type of coffee wins market interest, indicated by its phenomenal price. To date, roasted authentic Luwak coffee is offered about USD 195/lb (www.indocivet-coffee.com).

The high appreciation earned by this type of coffee is contrast to limited study of its properties and characters. Study of its physical aspect has been initiated by Marcone (2004), reported Luwak coffee bean is attributed with darker color and harder yet brittle texture. The research was utilizing SDS-PAGE to assess authenticity of Luwak coffee bean based on protein changes due to breakdown by proteolitic enzyme during Luwak digestation.

Another aspect that requests for characterization is volatile compounds. Volatile compound is important part of coffee since it configures coffee specific aroma and latterly contributes to coffee quality determination. Aided by gas chromatography (GC), works in coffee volatiles resulted list of up to 80 compounds (Buffo and Cardelli-Freire, 2004) which is widely used for identification of key-aroma compounds (Blank, *et al.* 1992), off-flavor indicators (Marin *et al.*, 2008; Toci and Farah, 2008) even discovery of antioxidant (Yanagimoto *et al.*, 2002).

Prior to GC analysis, volatile analyte should be extracted from solid sample. Extraction of volatile compounds traditionally is done with solvent distillation (Stoffelsma, *et al.,* 1968, Blank *et al.,* 1992). This method requires high temperature which allows compound decomposition due to thermal reaction. An illustration of how distillation could affect volatile analysis is in pyrazine quantification. Pyrazine, major constituent of coffee volatiles, is product of maillard reaction which reacts sugar and amino acid with presence of heat. The maillard reaction mainly occures in coffee roasting process, however it could also occure during distillation where heat is involved. Distillation yields maillard products and eventually adulterates pyrazine concentration in sample. This condition indicates solvent distillation potentially produces ambiguous quantification of volatile compounds.

Nowadays, practical and solventless Solid Phase Microextraction (SPME) has been a common practice (Akiyama *et al.*,2003, Akiyama *et al.,* 2005, Mondello *et al.*, 2005). Principle of this technique is trapping of volatile compounds by polymeric material. Selective extraction could be

made by variation in material, polarity or thickness of polymer. Volatile compound is best extracted by polydimethylsiloxane (PDMS) polymer. Furthermore, PDMS combined with carbowax/divinylbenzene polymer is oftenly suggested in flavor analysis (Supelco, 2007). Adulteration of non-volatile material could be avoided with headspace extraction. Headspace extraction performs exposure of polymeric tip onto atmospheric region of sample, while avoiding direct contact between polymer and sample. This technique is frequently used in flavor extraction.

This research is an initial step in studying Luwak coffee volatile compound. Technique of solid phase microextraction in headspace region obtained volatile analyte for GC-FID analysis. Chromatogram profile is investigated to identify major and minor peaks. Various sample amount is involved to observe its influence in chromatogram. This research also investigated the use of γ-picoline (4-methylpyridine) as internal standard. Study of volatile compounds is aimed to obtain more information of Luwak coffee bean, as preliminary effort to indicate Luwak coffee authenticity.

MATERIAL AND METHOD

Arabica Luwak coffee bean, were obtained from Indonesian Coffee and Cocoa Research Institute. Coffee plantation is located in Andungsari, Bondowoso District, East Java Province, Indonesia. Luwak coffee produced from dropping of domesticated luwak cat, fed with ripe arabica coffee cherry. Beans were wet processed and sundried prior to roasting step. As many as 120 g green bean were roasted at 170-220°C for 8-12 minutes until color is light brown (Agtron scale 65).

Extraction of coffee volatiles

Roasted coffee beans were ground by using dry miller for home use, until average ground coffee fineness. Ground sample (0.5-2 g)was transferred into 20 ml-sized glass vial and covered with plastic lid. Extraction of coffee volatiles was using Solid Phase Microextraction apparatus (Supelco 57330-U) coated by divinylbenzene/ carboxen/polydimethylsiloxane polymer (Supelco 57328-U). SPME fiber was introduced to sample headspace region at 60°C for 30 minute.

Analysis of volatile analytes

Volatile analysis was performed by Gas Chromatographic system (Shimadzu GC 2010). SPME fiber was injected to GC system in splitless mode at 260°C and allowed for 5 min sampling period before being removed. Volatile compound is separated by capillary column of 50% phenyl 50% dimethylpolysiloxane (Restec, Rtx-50) with dimention of 30 m x 0.25 mm ID x 0.25 μm thickness. Oven temperature program was set at 60°C for 3 min, then raised 5°C/min to reach 180°C for 3 min. Detector was using Flame Ionization Detector (FID) which was set at 200°C. Total analysis time required 30 min.

RESULT AND DISCUSSION

Chromatogram of roasted Luwak coffee

Principle of Gas Chromatography is separating sample extract (analyte) that naturally comes in complex mixture of chemical molecule by passing it through narrow column. Similar to other chromatography method, mobile phase (which in Gas Chroma-

tography, an inert gas is used) is required to bring analyte through column at different rates. Flow rate of each molecule depends on its chemical and physical properties and interaction with a specific column filling, called the stationary phase. In this condition, molecules are lined up based on their interaction with stationary phase, and reach the end of the column at different retention time. As molecule exit the column, they are detected and identified electronically.

One of the most versatile detector is Flame Ionization Detector (FID), where molecules coming out from column is mixed with hydrogen and air, and is directed into a flame, which breaks down organic molecules and produces ions. A voltage potential is applied just above the flame, resulting current that is subsequently measured and is proportional to the concentration of the components present (Hajslova and Cajka, 2008). Measured current is then transformed into a visual output named chromatogram. A chromatogram comprises of peaks, where one peak represents one specific molecule. Optimal separation in GC system is indicated by different peaks or patterns on the chromatogram correspond to different components of the separated mixture.

Figure 1 shows chromatogram of roasted Luwak coffee. Several peaks appear predominantly while the rests appear in moderate and minor size. Nine-teen peaks were identified as major peak with area more than one million. Major peak starts from RT 2.804; 3.865; 5.292; 7.335. Most of major peak were found in Rt 8.36-9.981, and continued in Rt 9.705-14.778. Subsequently two major peak are found separately in Rt 18.435-23.484.

Minor peak was identified when area is less then 10,000, eight-teen peaks were found at the beginning and the end of analysis. Those peaks started from Rt 5.692-9.498 and appeared again when analysis time reached 24.299-29.796 min, none of them were found within Rt 10-24. Minor peak was also found as early as Rt 1.877, however the subtlety of this peak should be considered as noise.

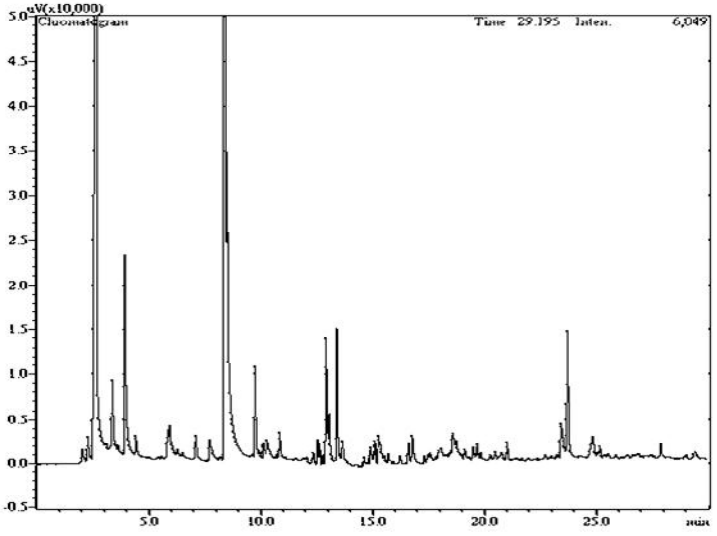

Figure 1. Chromatogram of Luwak coffee volatile.

Gambar 1. Kromatogram senyawa volatil kopi Luwak.

Effect of sample amount

Studies evident SPME extraction requires minimal amount of ground roasted coffee bean (Bicchi *et al*.,1997, Mondello *et al.,*2004, Zambonin *et al.,*2005). This research evaluated the use of sample amount ranges from 2g, to 1g and 0.5g. As respons to sample reduction, decrease occured in peak Rt 2.039; 2.275; 4.41; 7.085; 7.669; 8.481; 10.092; 10.245; 10.836; 12,911; 13.387; 13.646; 23.706; 28.215, on the other hand increasing peaks were found in Rt 2.648; 3.379; 3.922; 5.859; 5.928; 8.387; 9.734; 12.62; 13.05; 15.103; 15.331; 16. 767; 18.587; 18.732; 23.435.

Despite of fluctuating peaks due to sample variations, peak area of each sample amount were not significantly different (P = 0.08). This indicates that extraction could be performed in 0.5-2 g ground roasted sample. However, when using internal standard substance, sample amount should be considered to obtain required concentration. Fewer sample carries internal standard in higher concentration, affect SPME extractability and GC detection.

The use of γ-picoline (4-methylpyridine) as internal standard

Pyridine is a basic heterocyclic organic compound with the chemical formula C_5H_5N. It is structurally related to benzene, with one C-H group replaced by a nitrogen atom. The pyridine ring occurs in many important compounds, including azines and the vitamins niacin and pyridoxal. Pyridine is not abundant in nature, except for the leaves and roots of belladonna (*Atropa belladonna*) (Burdock, 1995) and in marshmallow (*Althaea officinalis*) (Taufel *et al*., 2005). However, pyridine is oftenly found in processed food, either thermally or microbiologically processed. The occurence in food brought significant organoleptic characteristics such as green, bitter, astringent and burnt properties (Maga, 1981). The bitterness of pyridine is strongly associated to its source, trigo-

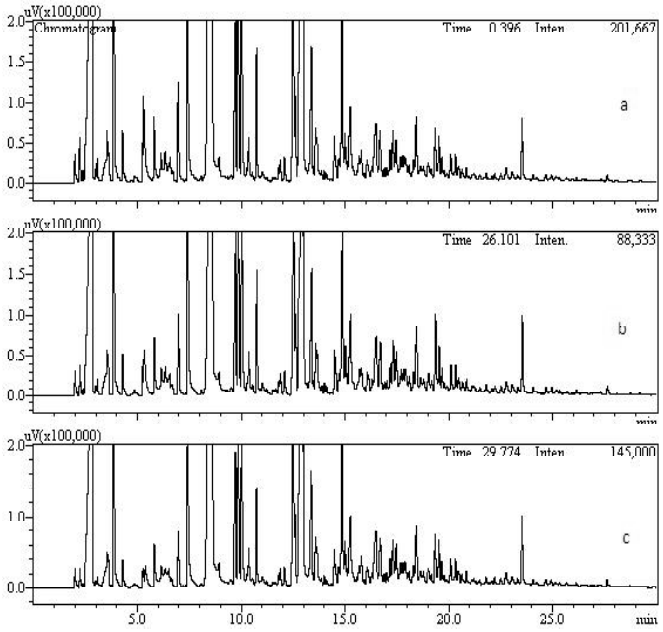

Figure 2. Chromatogram of volatiles from various sample amount; (a) 2.5 g; (b) 1 g; and (c) 0.5 g.

Gambar 2. Kromatogram senyawa volatil dari berbagai jumlah sampel; (a) 2.5 g; (b) 1 g; and (c) 0.5 g

nelline, where pyridine is resulted from degradation of trigonelline under roasting condition (McCamey *et al*.,1990).

Methylpyridine has three isomers consisted of 2-methylpyridine (α-picoline) and 3-methylpyridine (β-picoline). Both of these compounds are associated to green aroma (Flament, 2002). Another isomer; 4-methylpyridine (γ-picoline) was rarely reported in coffee, thus is selected as internal standard candidate. Although it was mentioned by Viani and Horman in 1974, it was not listed in their publication (Flament, 2002). Recent research reported γ-picoline in Brazilian roasted coffee (Oliveira *et al*.,2005), however its very rare occurence supports reason for selecting γ-picoline as internal standard.

Addition of 1 μL γ-picoline (Fluka 80240) into 0.5 g sample (2 μL/g), resulted inseparable peak and alteration of chromatogram profile. Since introducing substance in low concentration is difficult by using 10 μL-sized microsyringe, 5 g sample was homogenized with 0.1 μL γ-picoline then subjected 0.5 g for SPME extraction, expecting to put 0.02 μL/g γ-picoline in sample. The result showed distinguished peak without considerable interference to other peaks (Figure 4).

γ-picoline was identified at Rt 8.569-8.674. When γ-picoline concentration of 2 μL/g (Figure 4b) was introduced, the peak was merged with peak Rt 8.505. γ-picoline was distinguished when concentration was lowered to 0.4 μL/g (Figure 4c), however in this concentration the peak was not reaching the baseline. Clearly separated peak was obtained when concentration was reduced to 0.05 μL/g (Figure 4d). This research showed that γ-picoline is potential to be utilized as internal standard in concentration of 0.05 μL/g.

CONCLUSION

Analysis of Luwak coffee volatile is could be done by using head space Solid Phase Microextraction (SPME) and gas chromatography system. General purpose

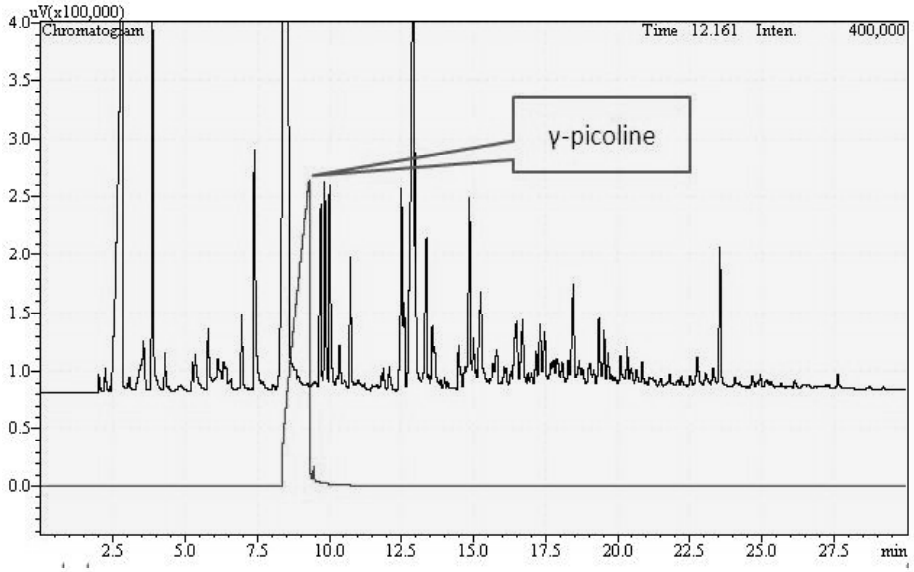

Figure 3. Position of γ-picoline (4-methylpyridine) within peaks originated from sample.

Gambar 3. Posisi γ-picoline *(4-metilpiridin) di antara puncak-puncak yang berasal dari sampel.*

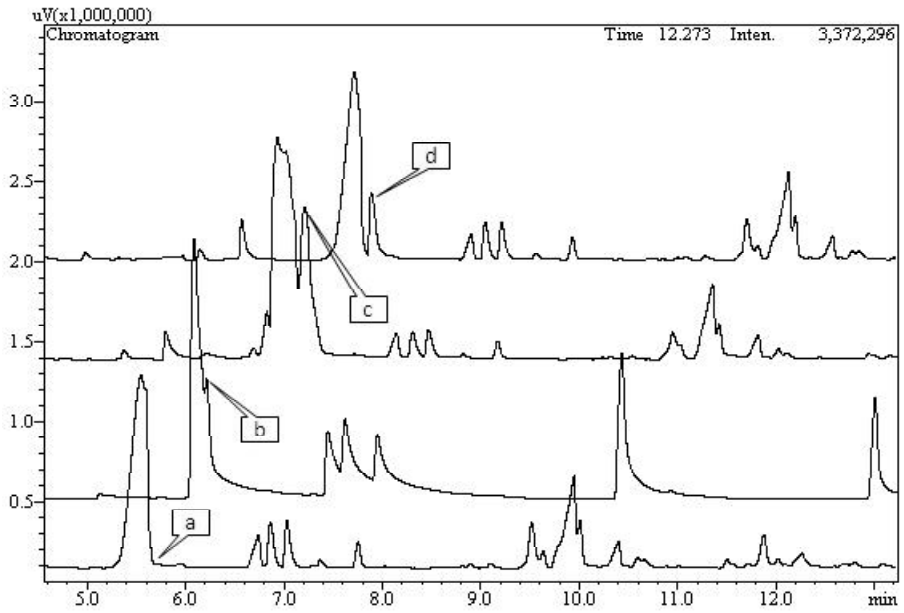

Figure 4. (a) original sample compared to sample with addition of γ-picoline; (b) 2 μL/g, (c) 0.4 μL/g, (d) 0.05 μL/g.

Gambar 4. (a) sampel sebelum penambahan γ-pikolin dan dibandingkan dengan sampel yang ditambahkan γ-pikolin pada konsentrasi (b) 2 μL/g, (c) 0.4 μL/g, (d) 0.05 μL/g.

Flame Ionization Detector (FID) is adequate to produce detailed chromatogram. SPME has performed simple volatile extraction in relatively short time with moderate heat, which prevents unexpected thermal reaction products. Internal standard for further quantification process, is proposed by using γ-picoline which is suggested to be introduced in low concentration.

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