Metabolite Profile Evaluation of Indonesian Roasted Robusta Coffees by ^{1}H NMR Technique and Chemometrics

Nizar Happyana*, Elvira Hermawati, Yana Maolana Syah, and Euis Holisotan Hakim

Organic Chemistry Division, Chemistry Study Program, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, Jl. Ganesha No. 10, Bandung 40132, West Java, Indonesia

** Corresponding author:*

tel: +62-222502103
email: nizar@chem.itb.ac.id

Received: June 10, 2019
Accepted: August 2, 2019

DOI: 10.22146/ijc.46492

Abstract: *In this work, ^{1}H NMR analysis, along with a chemometrics approach, had been applied for investigating metabolite profiles of Indonesian roasted Robusta coffees obtained from Lampung and Aceh. In total, 24 compounds had been successfully detected in the ^{1}H NMR spectra of the Robusta coffee extracts. Concentrations of some identified metabolites present in the coffees were determined by the quantitative ^{1}H NMR technique. Orthogonal projection to latent structure-discriminant analysis (OPLSDA) was used as a primary method for the chemometric approach. OPLSDA had classified clearly the Robusta coffee samples corresponding to their origin. Loading plot and S-plot of the OPLSDA revealed characteristic metabolites for each Robusta coffee. The results indicated that quinic acid, mannose, arabinoses, and acetic acid were an important discriminant compound for Lampung Robusta coffees. Meanwhile, lipids, lactic acid, and 5-caffeoylquinic acid were found as characteristic metabolites for Aceh Robusta coffee. This report provided knowledge about the chemical composition of Lampung and Aceh Robusta coffees and shed more light on the diversity of Indonesian Robusta coffees. Furthermore, it confirmed that ^{1}H NMR analysis coupled with chemometrics was a powerful method for evaluating and classifying metabolite profiles of the roasted Robusta coffees.*

Keywords: *^{1}H NMR; chemometric; roasted Robusta coffee; Indonesia*

■ INTRODUCTION

Coffee is one of the most consumed nonalcoholic drinks in the world. The drink is well known for its unique flavors and remarkable aromas. Coffee also possesses physiological and psychological effects [1]. Arabica (*Coffea arabica* L.) and Robusta coffees (*Coffea canephora* P.) are the most consumed coffees worldwide. Arabica coffee is considered having a higher quality than Robusta since it possesses a better taste, an intense aroma, and lower caffeine content [2]. As the second most cultivated coffee after Arabica, Robusta has a more bitter taste and contains more caffeine and chlorogenic acids but fewer sugars [3]. However, Robusta coffee is easier to cultivate since it is more resistant to plant diseases, weather conditions, and able to grow at lower altitudes as well [3].

Literature studies show that many metabolomics and chemometric studies of Robusta coffee focused on the authentication and the differentiation of coffee species [4-12]. Several measurement methods have been used in these studies, including IR spectroscopies [6,12-13], Raman spectroscopy [7], UV-visible spectroscopy [8], GC [4-5], HPLC [9], GC-MS [11] and NMR [10]. Chemometrics combined with electronic nose and tongue had been used to analyze and classify 7 Chinese Robusta coffee cultivars with different roasting degrees [14]. Furthermore, chemometric approaches, along with GC-MS techniques, had been also applied to discriminate Chinese Robusta coffees based on their geographic origins [15]. Recently, this coupled method had been used to investigate the effects of chemical pre-treatment of Robusta coffee [16].

Indonesia is one of the biggest coffee producers in the world [17]. At least 70% of coffee plants cultivated in Indonesia are Robusta species. This coffee is cultivated in many Indonesian islands, including Sumatera, Java, Sulawesi, Papua, and Sumbawa. Aceh and Lampung that

located in Sumatra, are two popular regions producing Robusta coffee. The taste of Lampung Robusta is unique and different from the taste of Aceh Robusta. From the chemical point of view, the taste differences indicate the distinction of metabolite profiles since the taste of coffee is strongly related to its chemical components. However, the chemical information of Robusta coffees from Indonesia especially Lampung and Aceh, is very limited in the literature.

In this study, Robusta roasted coffees from Lampung and Aceh were analyzed with ^{1}H NMR technique for investigating their chemical profiles. Chemometric approaches were applied to classify the Robusta coffees based on their origins and to identify their characteristic metabolites. Furthermore, some identified metabolites in the Robusta coffees were successfully quantified by ^{1}H NMR method.

■ EXPERIMENTAL SECTION

Materials

All coffee samples used in this study were commercially roasted beans of Robusta coffees from Lampung (6 samples) and Aceh (6 samples) and obtained from some coffee companies/suppliers. The detail information of the coffee origins was depicted in Table 1. The extraction solvent used in this work, deuterated water (D_2O), was purchased from Merck (Germany). 3-(trimethyl silyl)-2,2,3,3-tetradeuteropropionic acid sodium salt (TSP) was bought from Merck (Germany). KH_2PO_4 and K_2HPO_4 that used for making a buffer solution were purchased from Merck (Germany).

Instrumentation

An Encore mill (Baratza, United States) was used to grind the roasted coffee beans. An ultrasonic bath (Krisbow, Indonesia) was used to sonicate the samples. An MC-12 High Speed Microcentrifuge (Benchmark Scientific, United States) were used to centrifuge the samples. A 500 MHz Varian Unity INOVA spectrometer (Agilent Technologies, United States) was used to record ^{1}H NMR spectra of the Robusta coffees.

Procedure

Sample preparation

The coffee sample preparation was carried out based on the reported works [18-19] with slight modification. The sample was prepared by mixing 200 g of ground Robusta coffee with 1 mL of D_2O containing TSP 1.00 mM in a 2 mL plastic tube. The sample was sonicated at room temperature for 20 min and incubated on a water bath at 90 °C for 30 min. Afterward, the sample was cooled on the water for 10 min, centrifuged for 5 min, and the supernatant was then separated from the precipitate. One hundred microliters of phosphate buffer (pH 5) were added into 400 µL of supernatant and then transferred into a 5 mm NMR tube.

^{1}H NMR measurement and processing

In the ^{1}H NMR measurement, the H_2O signals were suppressed by the presaturation method. One hundred

Table 1. Origins of Robusta coffees used in the present study

Sample code	Coffee origin	Company/supplier
A1	Blangkejeren, Gayo Lues, Aceh	Fry Roast
A2	Linge, Aceh Tengah, Aceh	Rebbe Coffee Takengon
A3	Pintu Rime Gayo, Bener Meriah, Aceh	Serenade
A4	Takengon, Aceh Tengah, Aceh	Tampah Kopi Gayo
A5	Takengon, Aceh Tengah, Aceh	Raja Kopi Aceh
A6	Pintu Rime Gayo, Bener Meriah, Aceh	Garasco
L1	Liwa, Lampung Barat, Lampung	Fry Roast
L2	Ulubelu, Tanggamus, Lampung	Hilbrew coffee
L3	Liwa, Lampung Barat, Lampung	Kafein
L4	Liwa, Lampung Barat, Lampung	AKL
L5	Liwa, Lampung Barat, Lampung	AKL
L6	Ulubelu, Tanggamus, Lampung	Halokoffihouse

twenty-eight scans of 64 K data points are recorded with a spectral width of 8012 Hz, the acquisition time of 2.72 s, and a relaxation delay of 2 sec. The free-induction decay (FID) NMR data were processed with ACD/Labs 12.0 software (Advanced Chemistry Development, Inc., Toronto, Canada). This software was also used for referencing, phasing, baseline correction of ^{1}H NMR spectra. The chemical shifts of ^{1}H NMR spectra were referenced to the TSP signal. The signal assignments of the components in Robusta coffees were conducted by recognizing the fingerprinting chemical shifts of identified metabolites and comparing the spectra with the reference spectra of corresponding metabolites and with the data in the literature [18].

Multivariate statistical analysis

Alignment and bucketing of the ^{1}H NMR spectra were performed using ACD/Labs 12.0 software (Advanced Chemistry Development, Inc., Toronto, Canada). Bucketing was carried out by integrating regions of equal width (0.02 ppm) within δ 0.50–10.00 ppm and performed with an intelligent bucketing option as well. The residual water signal at δ 4.73–5.22 ppm were excluded from the multivariate data analysis. The caffeine signals at δ 3.22–3.49 ppm and δ 3.82–3.88 ppm were also excluded for avoiding spurious principal components (PCs) as a consequence of signal shifting [20]. The buckets were normalized to a total integral to avoid dilution effects of the samples. The processed data sets extracted from the ^{1}H NMR spectra were imported into SIMCA-P version 12.0 (Umetrics, Umeå, Sweden) for the multivariate statistical analysis. The data were then scaled with the Pareto scaling type. The principal component analysis (PCA), an unsupervised pattern-recognition approach, was performed to check intrinsic variation in the data set. Orthogonal projection to latent structure-discriminant analysis (OPLSDA), a supervised pattern-recognition approach, was applied as primary methods for extracting maximum separation among samples. The data sets of the roasted Robusta coffee were divided into 2 groups based on their geographical origins (Lampung and Aceh) and then analyzed with OPLSDA method. The percent of the response variation explained by the models (R2X and R2Y), and the percent of the response variation predicted by the models according to cross validation (Q2) were computed. Hotelling's T2 regions, shown as an ellipse in the score plot, defined the 95% confidence interval of the modeled variation.

Quantitative ^{1}H NMR analysis

For evaluating metabolites quantitatively in Lampung and Aceh Robusta coffees, the obtained ^{1}H NMR data were further processed based on a previous report [21] with slight modifications. TSP signal (1 mM) was used as an internal standard. The quantification was conducted by calculating the relative ratio of the peak area of selected proton signals of the target metabolites to the singlet peak of the TSP signal. The statistical calculation of quantitative ^{1}H NMR analysis was performed using Microsoft Excel 2013.

■ RESULTS AND DISCUSSION

Identified Metabolites in the Roasted Robusta Coffees

In this work, metabolites in the roasted Robusta coffee samples (Lampung and Aceh) were recognized by identifying their fingerprint signals in the ^{1}H NMR spectra and comparing them with the spectra of corresponding reference compounds. The metabolite identification was further confirmed by comparing the spectra with the data reported in the literature [18-19,22]. In total, 24 metabolites were successfully identified in the Robusta coffees, as depicted in the ^{1}H NMR spectra of the Robusta coffee (Fig. 1). Some molecular structures of the identified metabolites were described in Fig. 2.

Caffeine, as one of the major compounds in the roasted coffee bean, was clearly identified in the ^{1}H NMR spectra. The strong singlet signals at δ 3.28, 3.45 and 3.88 ppm were assigned as the 3 *N*-methyl of caffeine. Meanwhile, the singlet signal at δ 7.83 ppm was designed as an aromatic proton of caffeine. The intense signals of caffeine in the ^{1}H NMR spectra of the roasted coffees indicated that the compound is thermally stable during the roasting. Thus, caffeine is an excellent quantitative marker for coffees as proposed by previous reports [18,23]. The proton signals belong to 3 dominant compounds of chlorogenic acids, namely 3-caffeoylquinic

acid, 4-caffeoylquinic acid, and 5-caffeoylquinic acid were also clearly visible in the aliphatic and aromatic regions of the ^{1}H NMR spectra as shown in Fig. 1. Chlorogenic acids, the ester form of caffeic acid and quinic acid, are major compounds in coffees. However, during the roasting, some chlorogenic acids degrade into quinic acid and γ-quinide since the compounds are unstable thermally [24]. The signals of quinic acid, another major compound in the roasted coffee, were recorded in the ^{1}H NMR spectra

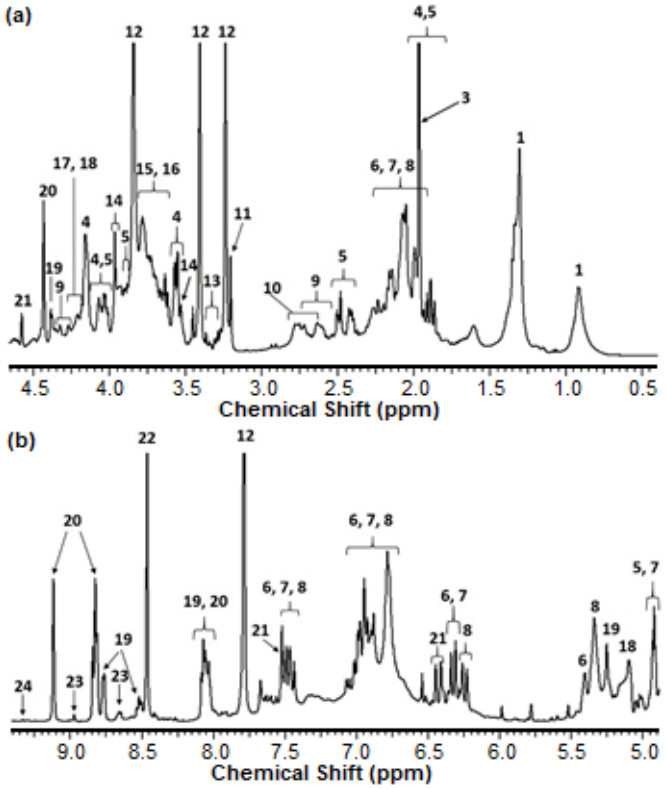

Fig 1. Characteristic signals of the metabolites identified in the ^{1}H NMR spectrum of the Robusta coffees. (a) Expansion of the ^{1}H NMR spectrum from 0.4 to 4.6 ppm. (b) Expansion of the ^{1}H NMR spectrum from 4.8 to 9.4 ppm. 1: lipids; 2: lactic acid; 3: acetic acid; 4:quinic acid; 5: γ-quinide; 6: 3-caffeoylquinic acid; 7: 4-caffeoylquinic acid; 8: 5-caffeoylquinic acid; 9: malic acid; 10: citric acid; 11: choline; 12: caffeine; 13: inositol; 14: β-(1-4)-D-mannopyranose unit; 15: β-(1-4)-D-galactopyranose unit; 16: β-(1-6)-D-galactopyranose unit; 17: α-(1-3)-L-arabinofuranose unit; 18: α-(1-5)-L-arabinofuranose unit; 19: *N*-methyl-pyridinium; 20: trigonelline; 21: 2-furyl-methanol; 22: formic acid; 23: nicotinic acid; 24: 5-(hydroxymethyl) furfural

at δ 4.16, 4.05, 3.57, and in the range 1.88–2.07 ppm. Proton signals belong to γ-quinide, an ester cyclic of quinic acid, were successfully detected at δ 4.91, 4.06, 3.89, and in the range 2.41–2.49 and 1.95–2.14 ppm.

Trigonelline is another major compound in the coffees. This compound was identified in the ^{1}H NMR spectra by detecting its proton signals at δ 4.44, 8.07, 8.82, 8.84, and 9.12 ppm. Trigonelline is degraded during the roasting process into some compounds including *N*-methyl-pyridinium and nicotinic acid [24]. Both degradation products were also successfully identified in the ^{1}H NMR spectra of the Robusta coffees. The signals belong to *N*-methyl-pyridinium were recorded at δ 4.37, 8.02, 8.51, and 8.75 ppm. Meanwhile, the proton signals of nicotinic acid were detected at δ 8.27, 8.66 and 8.97 ppm. Sucrose is a major component of green bean coffee. In this work, apparently sucrose had been degraded completely during the roasting; thus, it could not be detected in the ^{1}H NMR spectra of the roasted Robusta coffees. However, some products of sucrose degradation, including acetic acid, formic acid, lactic acid, 2-furylmethanol, and 5-hydroxymethylfurfural were successfully identified in the spectra. Proton resonances of acetic acid and formic acid were detected clearly as strong singlet signals in the spectra at δ 1.96 and 8.46 ppm, respectively. The fingerprint signal of lactic acid was recorded at δ 1.36 ppm and the proton resonances belong

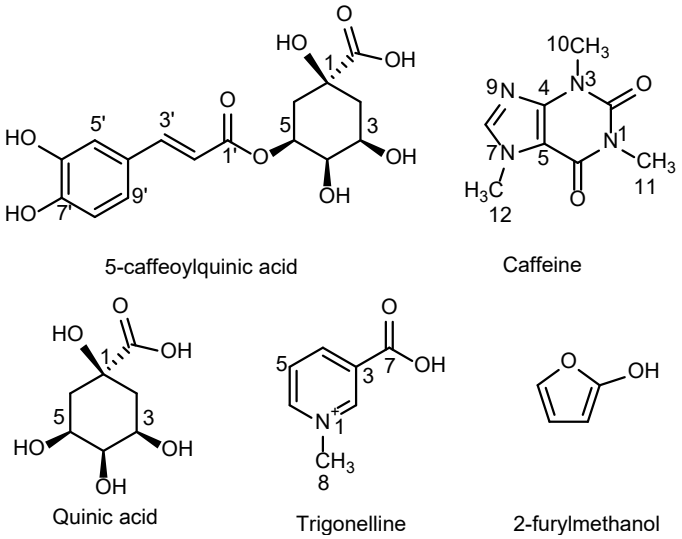

Fig 2. Some molecular structures of identified metabolites in the Robusta coffees

to 2-furylmethanol were recorded at δ 4.56, 6.43, and 7.50 ppm. Meanwhile, the proton resonance of 5-hydroxymethylfurfural has identified at δ 9.38 ppm as a weak signal.

Strong proton signals of lipids were recorded clearly at δ 0.92 and 1.30 ppm and assigned to methyl and methylene protons of fatty acids chains, respectively, as predicted by a previous report [22]. Other organic acids were also successfully identified in the aliphatic region of the ^{1}H NMR spectra, namely malic acid at δ 2.36 and 2.68 ppm, and then citric acid at δ 2.61 and 2.74 ppm. Further investigation of the aliphatic region revealed the presence of sugar compounds including α-(1-3)-L-arabinofuranose unit (3-arabinose), α-(1-5)-L-arabinofuranose unit (5-arabinose), β-(1-4)-D-mannopyranose unit (mannose), β-(1-4)-D-galactopyranose unit (4-galactose) and β-(1-6)-D-galactopyranose unit (6-galactose). The proton signals belong to the sugar compounds were depicted in Fig. 1. The sugar identification in the roasted Robusta coffees is in accordance with the literature [18-19,24]. Furthermore, a characteristic proton signal corresponding to inositol, sugar alcohol, was detected at δ 3.28 ppm, as shown in Fig. 1. The last identified metabolite found in the spectra was choline. The fingerprint signal of this compound was recorded at δ 3.22 ppm.

^{1}H NMR Quantitative Analysis

Some identified metabolites in the roasted Robusta coffees were analyzed semi-quantitatively using the ^{1}H NMR technique. The concentrations of choline, 2-furylmethanol, caffeine, formic acid, *N*-methyl pyridinium, nicotinic acid and trigonelline in Lampung and Aceh roasted Robusta coffees were successfully determined as shown in Table 2. Compared to the other quantified metabolites, caffeine was found as the most abundant metabolite either in the Aceh Robusta coffees or in the Lampung Robusta coffees. It confirmed that caffeine is the major compound found in the roasted Robusta coffee. The concentration of some quantified metabolites in the Lampung coffees was higher as compared to the Aceh coffees, e.g., the concentration of formic acid in the Lampung coffees was 10.5 mM, while that in the Aceh coffee was 5.4 mM. The opposite case was found for caffeine concentration in the samples. The concentration of caffeine in the Aceh coffees was 25.3 mM and higher as compared to its concentration in the Lampung coffees (22.8 mM). Choline concentration in the Lampung coffees (1.7 mM) was similar to its concentration in the Aceh coffees (1.8 mM).

Discrimination of Metabolite Profiles

The processed data sets obtained from the ^{1}H NMR spectra were evaluated with multivariate statistical analysis for classifying the roasted Robusta coffees (Lampung and Aceh) based on their geographical origin. In the initial step, the data were analyzed by PCA, an unsupervised pattern-recognition approach performed without using knowledge of the sample class. This approach resulted in a model with 3 principal components (PCs) explaining 80.5% of the total variability (R2X). However, PCA could not provide enough separations (data not shown); thus, the analysis was continued further with OPLSDA method, a supervised pattern-recognition approach. OPLSDA provides a better group separation model and reveals differences among groups since it combines the strengths

Table 2. Relative quantifications of roasted Robusta coffee metabolites

Compound	The concentration of roasted Robusta coffees (mM)	
	Aceh (± SD)	Lampung (± SD)
Choline (δ 3.19–3.22 ppm)	1.8 ± 0.1	1.7 ± 0.1
2-furylmethanol (δ 4.56–4.59 ppm)	3.5 ± 0.3	4.1 ± 0.4
Caffeine (δ 7.74–7.84 ppm)	25.3± 1.6	22.8 ± 0.9
Formic acid (δ 8.44–8.48 ppm)	5.4 ± 0.5	10.5 ± 1.2
N-methyl pyridinium (δ 8.49–8.55 ppm)	3.5 ± 0.1	2.6 ± 0.2
Nicotinic acid (δ 8.93–8.98 ppm)	0.45 ± 0.04	0.26 ± 0.02
Trigonelline (δ 9.09–9.14ppm)	4.41 ± 0.5	4.9 ± 0.3

of the partial least square discriminant analysis (PLSDA), and soft independent modeling of class analogy (SIMCA) classification [25].

The obtained OPLSDA model resulted in the good separation of the roasted Robusta coffee samples based on their geographical origin. This model possessed 5 components, 66.0% of cross validation coefficient (Q2), and explained 81.4% and 99.2% of total variations (R2X and R2Y, respectively). The OPLSDA model was validated further by Y scrambling validation on the corresponding PLSDA model (90.9% of R2Y and 53.3% of Q2), as demonstrated by a previous report [22]. This validation was carried out by performing 200 rounds of a random permutation of the Y variable resulting the regressions of Q2 lines intersected the y-axis at points below zero [Q2 = (0.00, -0.096); R2 = (0.00, 0.542)] and thus, confirming the statistical validity of the obtained model.

The score plot of the OPLSDA model successfully separated Lampung Robusta coffees from Aceh Robusta coffees, as described in Fig. 3(a). In order to identify the differentiate metabolites in the separation, the corresponding loading plot (Fig. 3(b)) was evaluated. The loading plot revealed some identified metabolites contributing importantly in the separation, including lipids (buckets at δ 0.89–0.94, 1.27–1.29, 1.29–1.35 ppm), lactic acid (bucket at δ 1.35–1.41 ppm), acetic acid (bucket at δ 1.94–1.98 ppm), quinic acid (buckets at δ 2.13–2.19, 3.54–3.60, 4.12–4.18, 4.00–4.05 ppm), mannose (bucket at δ 3.92–3.97 ppm), arabinoses (bucket at δ 4.18–4.24 ppm), 5-caffeoylquinic acid (bucket at δ 5.30–5.36 ppm), and formic acid (bucket at δ 8.44–8.49 ppm).

The S-plot of the OPLSDA model was generated to obtain a better evaluation of signals influencing the differentiation and reveals the discriminant metabolites

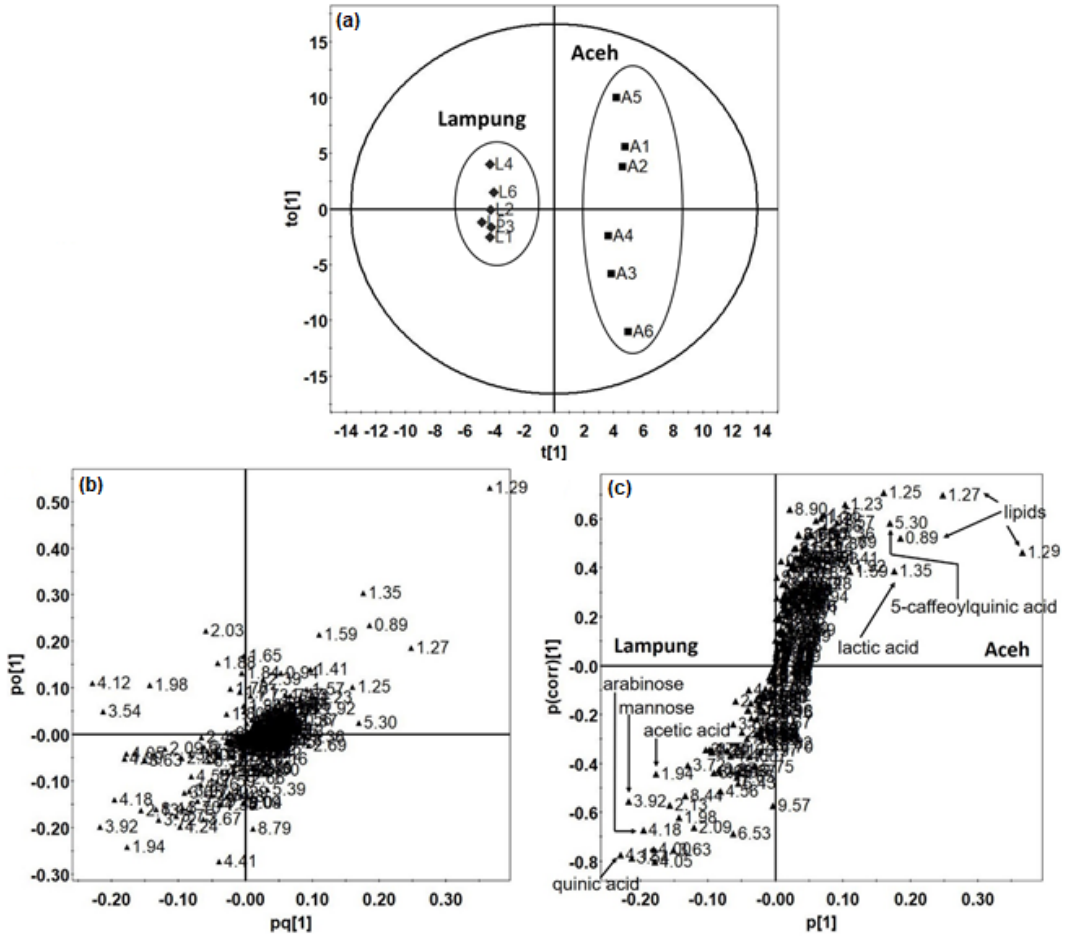

Fig 3. OPLSDA score (a) and loading (b) plots from ^{1}H NMR spectra of Lampung and Aceh roasted Robusta coffees. S-plot (c) revealed characteristic metabolites for each Robusta coffee

for each Robusta coffees. Based on the S-plot (Fig. 3(c)) evaluation, Lampung Robusta coffees were characterized by quinic acid (buckets at δ 3.54–3.60, 4.12–4.18, 4.00–4.05 ppm), mannose (bucket at δ 3.92–3.97 ppm), 3-arabinose (bucket at δ 4.18–4.24 ppm) and acetic acid (bucket at δ 1.94–1.98 ppm). Meanwhile, Aceh Robusta coffees were characterized by lipids (buckets at δ 0.89–0.94, 1.27–1.29, 1.29–1.35 ppm), lactic acid (bucket at δ 1.35–1.41 ppm) and 5-caffeoylquinic acid (bucket at δ 5.30–5.36 ppm).

As seen in the S-plot (Fig. 3(c)), some buckets corresponding to quinic acid were located at the edge of the S-plot of the Lampung Robusta coffee zone. It indicated that quinic acid is the most discriminant compound for Lampung Robusta coffees. Meanwhile, the bucket position of lipids at 1.29–1.35 ppm was at the edge of S-plot of Aceh Robusta coffee zone and far enough from the others indicating that lipids were the most discriminant metabolites for Aceh Robusta coffees. Literature reported that the coffee lipids consist of several fatty acids including palmitic, stearic, oleic, vaccenic, linoleic, linolenic and arachidic acids [26]. Furthermore, lipids are surface-active agents contributing to foam and emulsion formations of coffee brew [19] and correlated with the formation of the coffee body [27]. Thus, the high concentration of lipids in the coffees apparently is responsible for the strong body of Aceh Robusta coffees.

■ CONCLUSION

In this report, metabolite profiles of Indonesian roasted Robusta coffees from Lampung and Aceh had been evaluated by the ^{1}H NMR technique along with the chemometric approach. This technique had successfully identified metabolites present in the roasted Robusta coffees, and some of them had been analyzed semi-quantitatively. The roasted Robusta coffees were clearly differentiated based on their geographic origin by the chemometric approach. Moreover, quinic acid was found as the most discriminant compound for Lampung Robusta coffees, while lipids were discovered as the characteristic metabolites for Aceh Robusta coffees. The results of this study extended our understanding of Indonesian Robusta coffees.

■ ACKNOWLEDGMENTS

This study was funded by the Institute for Research and Community Services, Bandung Institute of Technology, via RISET ITB 2017 Grant under research contract No. 108q/I1.C01/PL/2017.

■ REFERENCES

[1] Haile, M., and Kang, W.H., 2019, The role of microbes in coffee fermentation and their impact on coffee quality, *J. Food Qual.*, 2019, 4836709.

[2] Lashermes, P., and Anthony, F., 2007, "Coffee" in *Technical Crops*, Eds. Kole, C., Springer, Berlin, 109–118.

[3] Farah, A., 2012, "Coffee constituents" in *Coffee: Emerging Health Effects and Disease Prevention*, Eds. Chu, Y.F., John Wiley and Sons Inc., New Jersey, 21–58.

[4] Alves, M.R., Casal, S., Oliveira, M.B.P.P., and Ferreira, M.A., 2003, Contribution of FA profile obtained by high-resolution GC/chemometric techniques to the authenticity of green and roasted coffee varieties, *J. Am. Oil Chem. Soc.*, 80 (6), 511–517.

[5] Cossignani, L., Montesano, D., Simonetti, M.S., and Blasi, F., 2016, Authentication of *Coffea arabica* according to triacylglycerol stereospecific composition, *J. Anal. Methods Chem.*, 2016, 7482620.

[6] Downey, G., and Spengler, B., 1996, Compositional analysis of coffee blends by near infrared spectroscopy, *Ir. J. Agric. Food Res.*, 35 (2), 179–188.

[7] El-Abassy, R.M., Donfack, P., and Materny, A., 2011, Discrimination between Arabica and Robusta green coffee using visible micro-Raman spectroscopy and chemometric analysis, *Food Chem.*, 126 (3), 1443–1448.

[8] Suhandy, D., and Yulia, M., 2017, Peaberry coffee discrimination using UV-visible spectroscopy combined with SIMCA and PLS-DA, *Int. J. Food Prop.*, 20, S331–S339.

[9] Alonso-Salces, R.M., Serra, F., Reniero, F., and Héberger, K., 2009, Botanical and geographical characterization of green coffee (*Coffea arabica* and

Coffea canephora): Chemometric evaluation of phenolic and methylxanthine contents, *J. Agric. Food Chem.*, 57 (10), 4224–4235.

[10] Wei, F., Furihata, K., Koda, M., Hu, F., Kato, R., Miyakawa, T., and Tanokura, M., 2012, ^{13}C NMR-based metabolomics for the classification of green coffee beans according to variety and origin, *J. Agric. Food Chem.*, 60 (40), 10118–10125.

[11] Zambonin, C.G., Balest, L., De Benedetto, G.E., and Palmisano, F., 2005, Solid-phase microextraction–gas chromatography mass spectrometry and multivariate analysis for the characterization of roasted coffees, *Talanta*, 66 (1), 261–265.

[12] Okubo, N., and Kurata, Y., 2019, Nondestructive classification analysis of green coffee beans by using Near-Infrared spectroscopy, *Foods*, 8 (2), 82.

[13] Downey, G., Briandet, R., Wilson, R.H., and Kemsley, E.K., 1997, Near- and mid-infrared spectroscopies in food authentication: Coffee varietal identification, *J. Agric. Food Chem.*, 45 (11), 4357–4361.

[14] Dong, W., Zhao, J., Hu, R., Dong, Y., and Tan, L., 2017, Differentiation of Chinese Robusta coffees according to species, using a combined electronic nose and tongue, with the aid of chemometrics, *Food Chem.*, 229, 743–751.

[15] Dong, W., Tan, L., Zhao, J., Hu, R., and Lu, M., 2015, Characterization of fatty acid, amino acid and volatile compound compositions and bioactive components of seven coffee (*Coffea robusta*) cultivars grown in Hainan province, China, *Molecules*, 20 (9), 16687–16708.

[16] Liu, C., Yang, Q., Linforth, R., Fisk, I.D., and Yang, N., 2019, Modifying Robusta coffee aroma by green bean chemical pre-treatment, *Food Chem.*, 272, 251–257.

[17] ICO (International Coffee Organization), 2019, *Coffee Report Market April 2019*, http://www.ico.org/documents/cy2018-19/cmr-0419-e.pdf, accessed on 17 May 2019.

[18] Wei, F., Furihata, K., Hu, F., Miyakawa, T., and Tanokura, M., 2011, Two-dimensional ^{1}H-^{13}C nuclear magnetic resonance (NMR)-based comprehensive analysis of roasted coffee bean extra, *J. Agric. Food Chem.*, 59 (17), 9065–9073.

[19] Wei, F., Furihata, K., Miyakawa, T., and Tanokura, M., 2014, A pilot study of NMR-based sensory prediction of roasted coffee bean extracts, *Food Chem.*, 152, 363–369.

[20] D'Amelio, N., Fontanive, L., Uggeri, F., Suggi-Liverani, F., and Navarini, L., 2009, NMR reinvestigation of the caffeine–chlorogenate complex in aqueous solution and in coffee brews, *Food Biophys.*, 4 (4), 321–330.

[21] Cagliani, L.R., Pellegrino, G., Giugno, G., and Consonni, R., 2013, Quantification of *Coffea arabica* and *Coffea canephora* var. Robusta in roasted and ground coffee blends, *Talanta*, 106, 169–173.

[22] Consonni, R., Cagliani, L.R., and Cogliati, C., 2012, NMR based geographical characterization of roasted coffee, *Talanta*, 88, 420–426.

[23] Tavares, L., and Ferreira, A.G., 2006, Quali- and quantitative analysis of commercial coffee by NMR, *Quím. Nova*, 29 (5), 911–915.

[24] Wei, F., Furihata, K., Koda, M., Hu, F., Miyakawa, T., and Tanokura, M., 2012, Roasting process of coffee beans as studied by nuclear magnetic resonance: Time course of changes in composition, *J. Agric. Food Chem.*, 60 (4), 1005–1012.

[25] Bylesjö, M., Rantalainen, M., Cloarec, O., Nicholson, J.K., Holmes, E., and Trygg, J., 2006, OPLS discriminant analysis: Combining the strengths of PLS-DA and SIMCA classification, *J. Chemom.*, 20, 341–351.

[26] Villarreal, D., Laffargue, A., Posada, H., Bertrand, B., Lashermes, P., and Dussert, S., 2009, Genotypic and environmental effects on coffee (*Coffea arabica* L.) bean fatty acid profile: Impact on variety and origin chemometric determination, *J. Agric. Food Chem.*, 57 (23), 11321–11327.

[27] Buffo, R.A., and Cardelli-Freire, C., 2004, Coffee flavour: An overview, *Flavour Fragr. J.*, 19 (2), 99–104.