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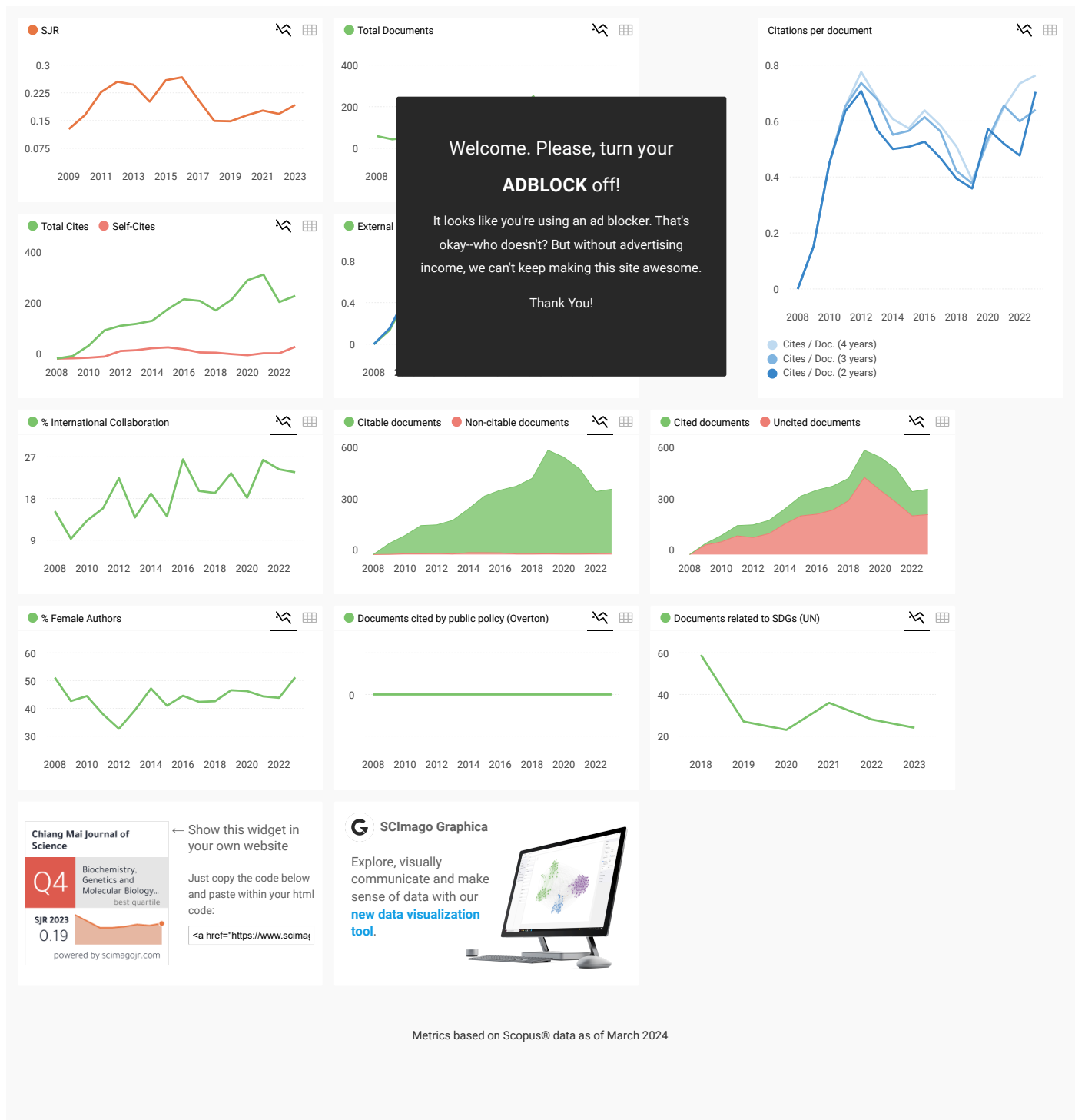
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Dr. Kriengkri Timsorn, Wilasinee Deepanya:

We have reached a decision regarding your submission to Chiang Mai Journal of Science, "Volatile Analysis of Coffee Flowers without and with Pollination Using a Portable Electronic Nose Coupled with Gas Chromatography".

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JOURNAL DETAIL

Volatile Analysis of Coffee Flowers before and after Pollination Using a Portable Electronic Nose and Gas Chromatography

Paper Type	Contributed Paper
Title	Volatile Analysis of Coffee Flowers before and after Pollination Using a Portable Electronic Nose and Gas Chromatography
Author	Khanittha Srinual, Kwanjit Anukulwattana, Kriengkri Timsorn and Wilasinee Deepanya
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Abstract:

Development of a portable electronic nose based on eight metal oxide gas sensors and gas chromatography (GC) analysis for classification of a difference in coffee flower odors are reported. Volatile organic compounds (VOCs) of arabica coffee (*Coffea arabica Linnaeus*) flowers before and after pollination were investigated. Principal component analysis (PCA) was performed for pattern recognition. The results showed that all metal oxide gas sensors exhibited sensitivity to odors of the coffee flower samples. PCA result showed good classification of coffee flower odors correctly corresponding to the samples before and after pollination. Based on GC analysis, it was found that pyrazine may be one of impact volatile organic compounds in arabica coffee flowers before pollination and amount of 2-methyl-1-butanol largely decreased after pollination. The pyrazine and 2-methyl-1-butanol may be used as a key compound for qualitative measurement using the electronics nose system with several advantages including rapid and accurate measurement, low cost and easy to use. We hope that this work will be useful for guidance on further studies of production of tea-like beverages using arabica coffee flowers.

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Research Article

Volatile Analysis of Coffee Flowers before and after Pollination Using a Portable Electronic Nose and Gas Chromatography

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ABSTRACT

Development of a portable electronic nose based on eight metal oxide gas sensors and gas chromatography (GC) analysis for classification of a difference in coffee flower odors are reported. Volatile organic compounds (VOCs) of arabica coffee (*Coffea arabica* Linnaeus) flowers before and after pollination were investigated. Principal component analysis (PCA) was performed for pattern recognition. The results showed that all metal oxide gas sensors exhibited sensitivity to odors of the coffee flower samples. PCA result showed good classification of coffee flower odors correctly corresponding to the samples before and after pollination. Based on GC analysis, it was found that pyrazine may be one of impact volatile organic compounds in arabica coffee flowers before pollination and amount of 2-methyl-1-butanol largely decreased after pollination. The pyrazine and 2-methyl-1-butanol may be used as a key compound for qualitative measurement using the electronics nose system with several advantages including rapid and accurate measurement, low cost and easy to use. We hope that this work will be useful for guidance on further studies of production of tea-like beverages using arabica coffee flowers.

Keywords: electronic nose, arabica coffee flower, pollination, VOC detection

1. INTRODUCTION

Coffee is one of the most popular beverages being consumed in many countries around the world [1]. Most commercial coffee production mainly involves two species of coffee including Arabica coffee (*Coffea arabica* Linnaeus) and Robusta coffee (*Coffea canephora* Pierre) [2, 3]. Arabica coffee is widely consumed representing about 80% of the global coffee market [4]. There are approximately 1000 volatile organic compounds

(VOCs) identified in roasted coffee beans [5, 6]. VOCs emitted by coffee provide the uniqueness of coffee species. Different VOCs are present in coffee such as hydrocarbon, aldehyde, alcohol, alkane, ketone, ester, phenol, acids and others [7, 8]. Regarding to coffee flowers (blossoms), they are a by-product and own the short flowering period after pollination by wind or bees [3, 9]. The coffee flowers can be used for production of healthy

food and beverages, e.g., tea-like beverages [10]. Moreover, high amounts of bioactive compounds like phenolics etc. are found in coffee flowers, which play a crucial role as antioxidants to prevent cancers, cardiovascular etc. [11]. Research on VOCs identification of coffee beans and coffee flowers has been reported [5-7, 12, 13]. The most research was focused on VOCs of roasted coffee beans.

For identification and quantification of VOCs in various odors, many analytical methods including gas chromatography/mass spectrometry (GC-MS) [12, 14-18], nuclear magnetic resonance (NMR) spectroscopy [19, 20] etc. have been successfully performed. Unfortunately, their limitation is related to complex, expensive and time-consuming process and requirements of well-trained technicians. An analytical instrument which offers low-cost, rapid-accurate operation and non-destructive measurement is still needed. In recent decades, electronic noses (E-noses) based on metal oxide sensors have attracted much interest for applications in qualitative analysis of food [13, 21-23]. E-nose can be used for quality assessment, grading and classification of food etc. For coffee, the E-nose can help to assess VOCs profiles of coffee, which are crucial for determination of coffee quality. The E-nose working relies on a change in resistances of metal oxide sensors when they react with VOCs and data analysis via pattern recognition methods [24, 25].

To the best of our knowledge, reports on E-nose applications for qualitative classification of VOCs emitted by coffee flowers before and after pollination are less available. In the present work, we are focused on detection of the differences in VOCs between coffee flowers before and after pollination, which will provide basic information on coffee flower selection for tea-like beverage production using an E-nose. We have developed a portable E-nose based on eight metal oxide gas sensors used for odor detection and our developed E-nose offers several distinct advantages over some other E-nose systems including simplicity, rapid, low cost, high

accuracy. Moreover, our developed E-nose can be used without environmental restrictions (no need nitrogen or air zero as a carrier gas). Principal component analysis (PCA) was used to analyze values of sensor responses obtained from eight metal oxide gas sensors after odor detection of coffee flower samples for data classification. GC analysis of VOCs was performed for parallel study.

2. MATERIALS AND METHODS

2.1 Coffee Flower Samples

Two different samples of coffee flowers, *coffee arabica*, before and after pollination as shown in Figure 1 were collected from a production area in Phetchabun, Thailand. It should be noted that the sample before pollination (symbol: AB) was obtained in bloom stage before four days of pollination. For the sample after pollination (symbol: AA) was obtained after three days of pollination. The samples were immediately kept in vials without cleaning. For E-nose measurement, the total weight of 3.0 ± 0.1 g for each sample was put in sample glass bottles. All samples were directly used and measured without heating treatments.

2.2 E-nose System and Measurement

Our developed (lab-made) portable E-nose system consists of three main parts; (I) air flow system, (II) odor sensing part and (III) system control & processing as presented in Figure 2. For air flow system, it was composed of an air pump, four solenoid valves and a flow meter which were connected to each other by Teflon tubes. In the odor sensing part, eight metal oxide gas sensors were installed in the sensor chamber made of a Teflon material, which was specially designed to equally flow coffee flower odors into each gas sensor. Table 1 shows a list of gas sensor array with their sensing types and detection ranges. The eight gas sensors were chosen in order to cover VOCs emitted by coffee flowers [5, 12]. Sample and reference glass bottles were connected to the chamber. In addition, the E-nose measurement relied on switching between the reference (clean



Figure 1. Coffee flowers before (AB) (a) and after (AA) pollination (b).

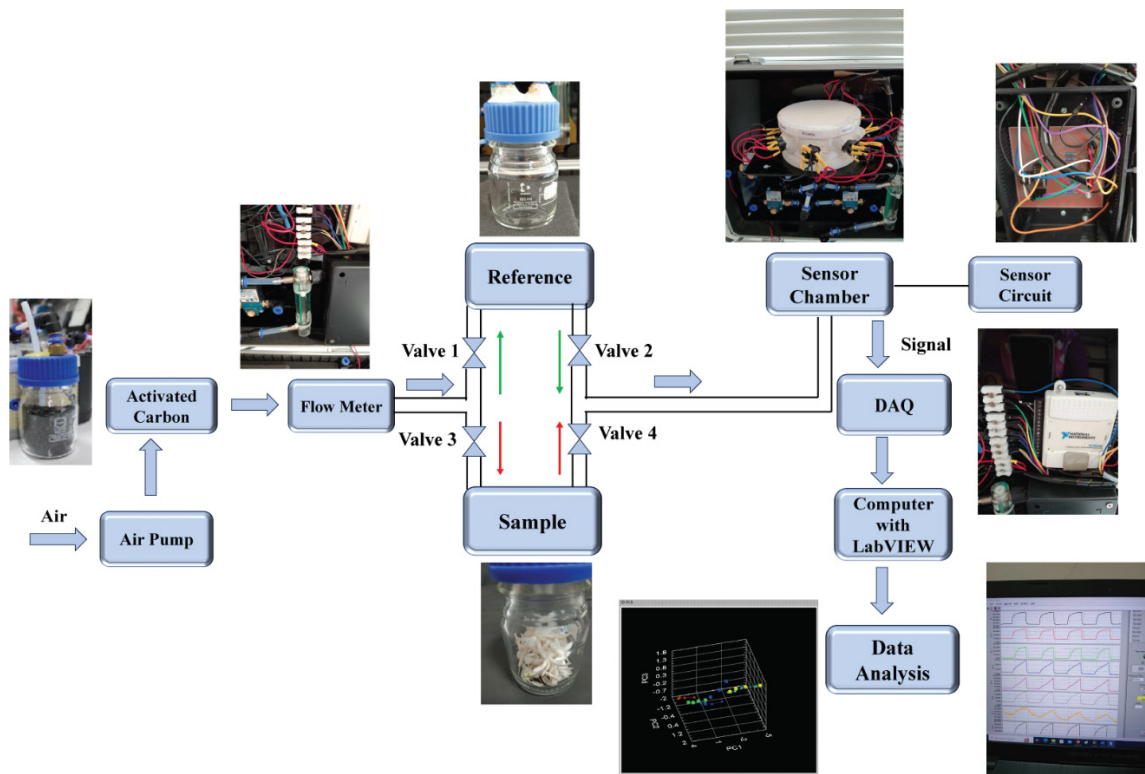


Figure 2. Schematic diagram of E-nose system used in this work.

Table 1. Sensing types and detection ranges of gas sensors used in E-nose system.

Sensor name	Sensing type (specificity from Figaro Inc., Japan)	Detection ranges (ppm)
TGS 821	Hydrogen	10-5,000
TGS 822	Organic solvent vapors	50-5,000
TGS 826	Ammonia	30-300
TGS 2600	Air contaminants	1-100
TGS 2602	VOCs and odor gases	1-30
TGS 2610	LP gas	300-10,000
TGS 2611	Methane	300-10,000
TGS 2620	Alcohol and solvent vapors	50-5,000

air) and the sample (coffee flower odors) glass bottles under control of the solenoid valves to avoid mixing of clean air and coffee flower odors. For system control & processing, DAQ (NI USB 6008) was used to collect signals generated from the eight gas sensors during measurement. All parts were controlled by LabVIEW software.

Starting measurement, sensor array was heated for an hour to activate sensing elements. In addition, metal oxide semiconductor, e.g. SnO_2 , is operated at high temperature (typical > 350 °C) [26]. Then, air was pumped passing an activated carbon bottle into a flow meter which was set at 3 L/min. The valves 1 and 2 were opened at the same time while the valves 3 and 4 were closed. Clean air in the reference bottle was carried into the sensor chamber for 2 min. In this step, gas sensor array was cleaned in order to obtain stable baseline resistances of all sensors. It should be noted that 2 min was enough time for the stable baseline resistances [26]. After 2 min, air flow path was switched from the reference to sample bottles. Coffee flower odors in the sample bottle were carried into the sensor chamber for 2 min. A change in gas sensor resistances during measurement of coffee flower odors was recorded every 1 s as a function of time. Each sample was repeated twelve times at room temperature (25 ± 1 °C) under air conditioner.

2.3 Sensor Response

Sensor response explains how sensor ability is sensitive to odors. Percentages of the sensor response can be calculated as the following equation:

$$\text{Sensor response (\%)} = \frac{R_0 - R_{\text{Sens}}}{R_0} \times 100 \quad (1)$$

where R_0 and R_{Sens} are a resistance value of a gas sensor in clean air and a resistance value of a gas sensor in coffee flower odors, respectively.

2.4 Principal Component Analysis (PCA)

PCA is a statistical technique and used to reduce data dimensionality with preserving most of information features [27, 28]. Based on an orthogonal transformation, PCA converts a data set of correlated variables into a set of uncorrelated variables called principal components (PCs.) Each principal component is ordered based on the total variance. The first component (PC1) contains the largest percentage of the total variance. The second largest percentage is on the second component (PC2), and so on. PC1 and PC2 are usually plotted as 2-D visualization to investigate similarities and differences of samples [29]. PCA result can be calculated as follows [30]:

$$\text{PCA} = \left(\left(\overline{\text{Cov}(X_{M \times N})} \right)_{\max \rightarrow \min} \otimes \text{Norm}(X_{M \times N}) \right)^T \quad (2)$$

where $\text{Cov}(X_{M \times N})$ is the covariance matrix of original dataset in matrix form ($X_{M \times N}$). M and N represent repetitions of measurement and the number of independent gas sensors, respectively. Norm is normalization of the data matrix and T is transpose.

In this work, input data for PCA analysis is sensor responses in a form of a matrix ($X_{M \times N}$). 192 sensor responses ($X_{24 \times 8}$) were input in PCA process. The PCA output was used to classify characteristics of sample data related to coffee flower odors.

2.5 VOCs Analysis of Coffee Flowers by Gas Chromatography (GC)

VOCs of coffee flower samples (AB and AA) were analyzed by GC. A headspace autosampler (PAL-RSI, Alpha M.O.S., Toulouse, France) was used to extract VOCs of coffee flower samples. Briefly, 0.15 g of coffee flowers were added into 10 mL specialized vials for headspace extraction. To saturate odors, the vials were incubated at 60 °C for 10 min. The headspace was directly injected into a gas chromatography with injection temperature of 200 °C and injection speed of 125

$\mu\text{L/s}$. The VOCs information was collected by a Heracles NEO (Alpha M.O.S., Toulouse, France). The VOCs were detected by flame ionization detectors (FID) at 260°C, and separated on columns (MXT-5: a low-polar column, 10 m x 0.18 mm x 0.4 x m). Hydrogen was used as a carrier gas at a constant flow rate of 1.0 mL/min. Each sample was repeatedly measured three times. Relevance index (RI) values of each unknown component were calculated according to MXT-5 columns. The VOCs were identified by comparing the measured RI values with Kovats relative retention index in the Arochem database. In addition, RI is a numerical value used to characterize the retention time of a compound relative to a reference compound. Quantitative determination of the VOCs was based on peak area.

3. RESULTS AND DISCUSSION

3.1 Sensor Response

Figure 3 shows an example of a sensor signal (TGS2602) obtained from measurement of coffee flower odors. The signal was recorded as resistance values versus a function of time. One loop represents one measurement. After odor on, the

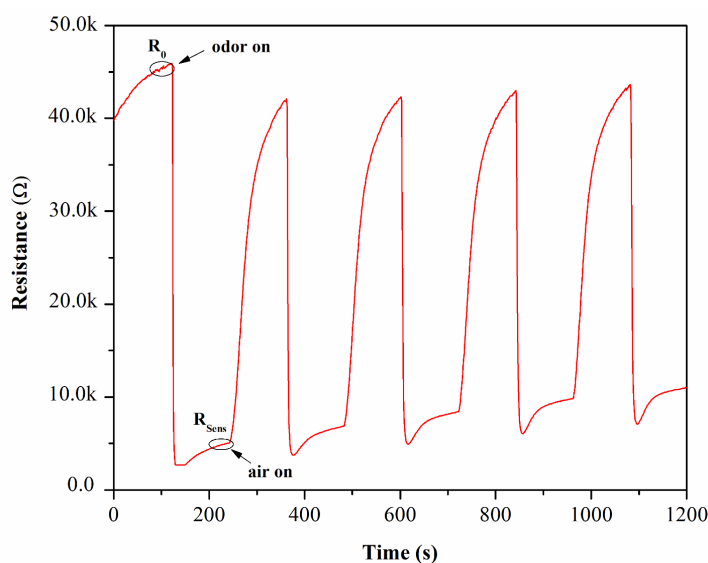


Figure 3. An example of a sensor signal (TGS2602) recorded as resistance values versus time obtained from measurement.

resistance values quickly decreased within 7-10 s in presence of coffee flower odors. After 2 min (air on), the resistance values returned to baseline resistance values within 120 s. The decrease in sensor resistance values is due to interaction between VOCs emitted by coffee flowers and adsorbed oxygen ions which form a potential barrier at grain boundaries on metal oxide surface [26, 31]. This interaction leads to decreased barrier height and sensor resistance values decrease. It is revealed that most VOCs emitted by coffee flowers are reducing gases and TGS gas sensors are n-type semiconductor devices [28].

All sensor responses to the odors of AB and AA samples are presented in Figure 4. It can be obviously seen that all sensor responses to AB sample odors were higher than sensor responses to AA sample odors. It indicates that coffee flower odors before pollination contain more amounts of VOCs than coffee flower odors after pollination. Based on GC analysis (Table 2), some identified VOCs after pollination decreased or vanished. All VOCs in the alkane and acid groups

were found to be decreased. Interestingly, it was found that pyrazine vanished after pollination (more details explained in GC analysis section). Also, we found a decrease in amounts of most VOCs in the alcohol group. It is attributed that these decreased or vanished VOCs cause lower sensor responses. To analyze sensor responses of each gas sensor, it was found that each gas sensor was differently sensitive to coffee flower odors. TGS 826 and 2602 exhibited the highest response and the lowest response was TGS 821 for both of before and after pollination. From Figaro product information, a single gas sensor can be sensitive to various VOCs such as alcohol, ammonia, hydrogen, toluene, iso-butane, ethanol. This leads to low accuracy for odor classification of coffee flowers. Therefore, only single gas sensor is not enough to classify a difference in coffee flower odors before and after pollination. Classification techniques are essential for odor classification. In this work, PCA was used to classify coffee flower odors based on all eight sensor responses as input data.

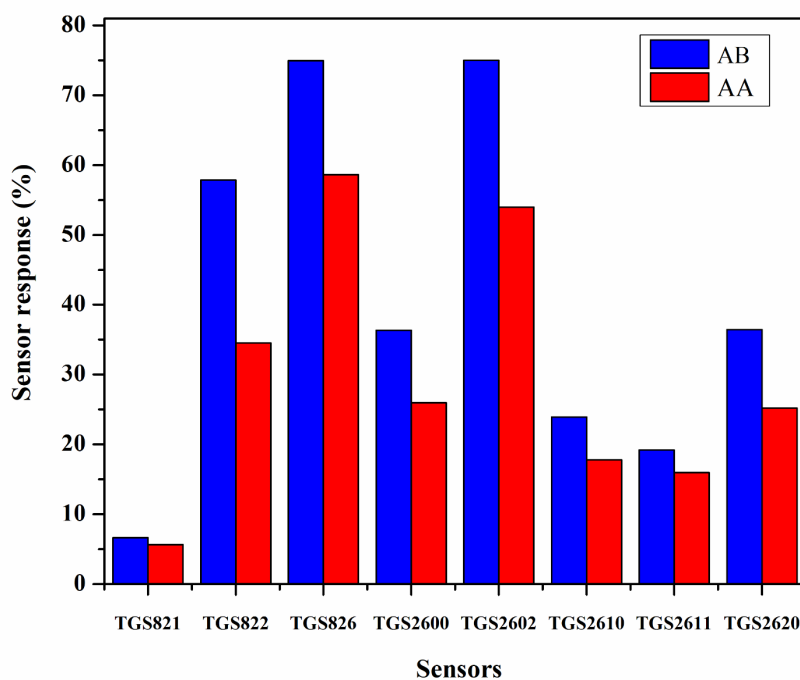


Figure 4. Sensor responses of all gas sensors to coffee flower odors.

Table 2. The identification of VOCs in odors of AB and AA samples using GC analysis.

Group (No.)	Volatile compound	RT (min)	Molecular formula	Before pollination (AB) (Peak Peak area x 10 ³ (Mean ± SD, n=3)	After pollination (AA) Peak area x 10 ³ (Mean ± SD, n=3)	Relevance index
Aldehyde						
1	Acetaldehyde	14.59	C ₂ H ₄ O	8.62 ± 0.40	12.10 ± 1.16	67.69
2	Propanal	15.76	C ₃ H ₆ O	63.18 ± 2.34	262.87 ± 12.09	93.14
3	2-methylpropanal	18.05	C ₄ H ₈ O	10.74 ± 0.89	17.20 ± 1.25	49.13
4	but-(E)-2-enal	27.61	C ₄ H ₆ O	22.01 ± 2.74	13.25 ± 1.49	85.88
5	(E,E)-2,4-hexadienal	54.07	C ₆ H ₈ O	3.29 ± 0.31	ND	98.98
6	2-Decenal, (E)-	77.58	C ₁₀ H ₁₈ O	2.79 ± 0.48	2.75 ± 0.24	89.81
Alcohols						
7	1-Propanol	19.69	C ₃ H ₈ O	8.79 ± 0.74	13.84 ± 1.21	81.05
8	1-Propanol, 2-methyl-	24.12	C ₄ H ₁₀ O	36.35 ± 2.06	21.81 ± 0.73	68.23
9	pentan-2-ol	30.93	C ₅ H ₁₂ O	ND	2.67 ± 0.02	93.13
10	2-Methyl-1-butanol	34.32	C ₅ H ₁₂ O	363.11 ± 27.84	16.58 ± 1.09	95.07
11	S(-)2-methyl-1- butanol	35.57	C ₅ H ₁₂ O	ND	32.83 ± 1.57	85.09
12	1-Hexanol	51.01	C ₆ H ₁₄ O	ND	1.84 ± 0.40	91.44
13	3-Octanol	61.45	C ₈ H ₁₈ O	1.07 ± 0.15	3.78 ± 0.38	92.24
14	2-nonanol	68.95	C ₉ H ₂₀ O	8.24 ± 0.80	2.09 ± 0.10	70.97
15	E-2-Nonen-1-ol	72.69	C ₉ H ₁₈ O	0.35 ± 0.24	ND	90.21
16	2-Undecanol	79.42	C ₁₁ H ₂₄ O	1.75 ± 0.11	ND	71.23
17	1-Tridecanol	92.28	C ₁₃ H ₂₈ O	3.44 ± 0.11	ND	70.39
Alkane						
18	Pentane	16.80	C ₅ H ₁₂	6.85 ± 0.50	6.82 ± 0.17	64.74
19	pentadecane	88.16	C ₁₅ H ₃₂	38.18 ± 1.16	5.92 ± 0.48	83.01
20	Tridecane	80.73	C ₁₃ H ₂₈	0.57 ± 0.03	ND	60.96
21	heptadecane	96.29	C ₁₇ H ₃₆	26.46 ± 1.14	4.09 ± 0.20	84.95
Ketones						
22	butane-2,3-dione	21.25	C ₄ H ₆ O ₂	ND	3.30 ± 0.07	88.62
23	butan-2-one	21.94	C ₄ H ₈ O	7.24 ± 0.05	2.69 ± 0.08	82.27
24	2-Heptanone	53.03	C ₇ H ₁₄ O	ND	3.87 ± 0.18	91.93
25	delta-Dodecalactone	96.96	C ₁₂ H ₂₂ O ₂	5.92 ± 0.26	1.10 ± 0.04	57.41
26	delta- Tetradecalactone	107.32	C ₁₄ H ₂₆ O ₂	2.40 ± 0.04	ND	3.54
27	2-decanone	73.91	C ₁₀ H ₂₀ O	1.33 ± 0.65	ND	99.38

*ND = not detected.

Table 2. (Continued) The identification of VOCs in odors of AB and AA samples using GC analysis.

Group (No.)	Volatile compound	RT (min)	Molecular formula	Before pollination (AB) (Peak area $\times 10^3$ (Mean $\pm$ SD, n=3))	After pollination (AA) Peak area $\times 10^3$ (Mean $\pm$ SD, n=3)	Relevance index
Ester						
28	Methyl isobutyrate	29.46	C ₅ H ₁₀ O ₂	1.11 $\pm$ 0.04	17.71 $\pm$ 0.14	83.43
29	Methyl 2-methylbutanoate	42.46	C ₆ H ₁₂ O ₂	0.40 $\pm$ 0.29	ND	96.14
30	Ethyl isovalerate	49.41	C ₇ H ₁₄ O ₂	2.66 $\pm$ 0.06	ND	83.61
Phenol						
31	Phenol, 2-methyl-	65.17	C ₇ H ₈ O	4.44 $\pm$ 1.17	6.77 $\pm$ 0.73	82.83
Acid						
32	acetic acid	23.12	C ₂ H ₄ O ₂	1.87 $\pm$ 0.08	1.09 $\pm$ 0.29	82.98
33	benzoic acid	71.97	C ₇ H ₆ O ₂	1.04 $\pm$ 0.90	ND	97.51
Others						
34	Trichloroethane	26.65	C ₂ H ₃ Cl ₃	5.05 $\pm$ 1.03	5.69 $\pm$ 0.68	79.55
35	pyrazine	36.04	C ₄ H ₄ N ₂	50.74 $\pm$ 3.76	ND	84.00
36	2-methylthiophene	41.34	C ₅ H ₆ S	48.92 $\pm$ 2.00	8.90 $\pm$ 1.29	92.62
37	3-methyl-3-sulfanylbutoanol-1-ol	59.92	C ₅ H ₁₂ OS	1.47 $\pm$ 0.47	2.55 $\pm$ 0.11	89.70
38	acetylpyrazine	64.07	C ₆ H ₆ N ₂ O	0.18 $\pm$ 0.01	ND	92.99
39	(2R,4R)-Tetrahydro-4-methyl-2-(2-methylprop-1-enyl)-2H-pyran	70.54	C ₁₀ H ₁₈ O	0.20 $\pm$ 0.28	1.46 $\pm$ 0.27	76.42
40	Limonene	67.73	C ₁₀ H ₁₆	1.04 $\pm$ 0.10	ND	72.08
41	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	76.44	C ₁₀ H ₁₄ O	3.79 $\pm$ 0.54	4.47 $\pm$ 0.16	96.83
42	alpha-Terpinen-7-al	78.62	C ₁₀ H ₁₄ O	1.07 $\pm$ 0.11	1.17 $\pm$ 0.12	94.31
43	Methyl eugenol	84.07	C ₁₁ H ₁₄ O ₂	1.39 $\pm$ 0.02	ND	91.16

*ND = not detected.

3.2 PCA Result

To evaluate performance of our developed portable E-nose based on eight metal oxide gas sensors for odor classification of coffee flowers, 192 sensor responses were input into PCA. Figure 5 illustrates two-dimensional plot of PCA. The first two PCs (PC1 and PC2) contributed 97.65% and 1.52% of the total variance, respectively. The accumulation of the first two PCs was 99.17% of

data variance which represents high information to explain an odor difference of coffee flower samples. Moving along PC1 axis, sensor responses to coffee flower odors were classified into two groups correctly corresponding to coffee flower samples before and after pollination. The two classified groups were quite close together but they did not overlap. It is revealed that sensor responses used as input data showed good quality

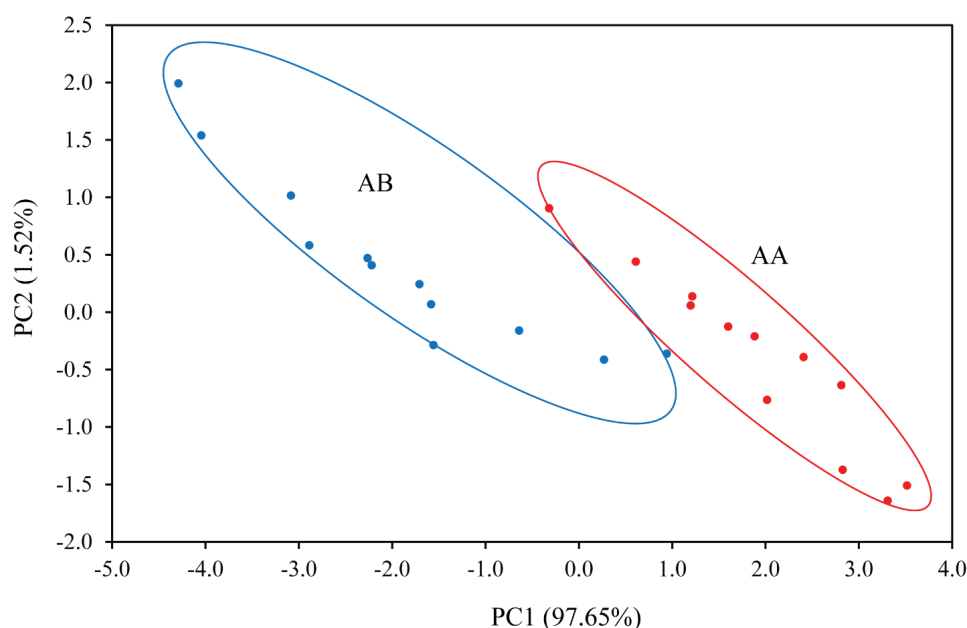


Figure 5. PCA score plot for odor classification of coffee flower samples.

of data for PCA analysis. From PCA result, our developed portable E-nose system based on eight metal oxide gas sensors with PCA analysis could classify the odor difference between coffee flowers before and after pollination.

3.3 GC Analysis

VOCs of coffee flower samples including AB and AA were analyzed using GC. Figure 6 depicts a comparison of peak area of VOCs obtained from columns MXT-5. It can be clearly seen that a significant difference in amounts of VOCs between AB and AA samples was found. Forty-three peaks were defined as highly specific to VOCs of coffee flowers samples. The VOCs were classified into eight groups based on their chemical properties including six aldehydes, eleven alcohols, four alkanes, six ketones, three esters, one phenol, two acids and ten others as presented in Table 2. A comparison of VOCs between AB and AA samples from the peak patterns of each compound appearing in the same retention time (see Figure 6), it was found that, after pollination, the amounts of some VOCs

decreased or increased or vanished. For instance, the amounts of all VOCs in the alkane group and some VOCs in the alcohol group (1-propanol, 2-methyl, 2-methyl-1-butanol, 2-nonanol etc.) decreased. In case of vanished amounts of VOCs, they were pyrazine, acetylpyrazine, limonene, delta-tetradecalactone, 2-decanone, ethyl isovalerate and (E,E)-2,4-hexadienal etc. For increased amounts of VOCs, they were propanal, acetaldehyde, 2-methylpropanal, 3-octanol, 2-heptanone and methyl isobutyrate etc. Meireles *et al.* [5] reported that pyrazine was regarded as one of impact compounds for coffee odors. In our study, the amount of pyrazine vanished after pollination. Disappearance of pyrazine might mainly lead to lower sensor responses. It is attributed that pyrazine may be one of major compounds of coffee flower odors before pollination. Moreover, we found a large decrease in amount of 2-methyl-1-butanol after pollination. The pyrazine and 2-methyl-1-butanol may be used as a key indicator for qualitative evaluation of coffee flower odors based on E-nose measurement.

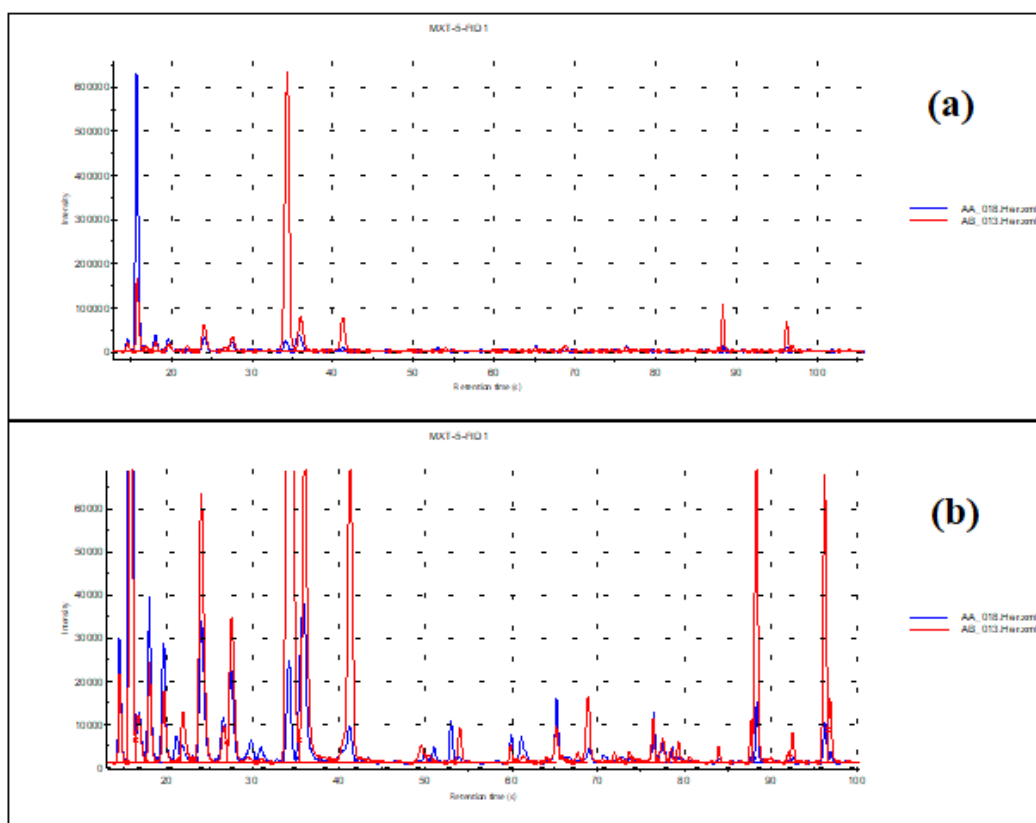


Figure 6. The chromatographic behaviors of VOCs of coffee flower samples in columns MXT-5 by GC; normal (a) and enlarged images (b).

4. CONCLUSIONS

In summary, we have successfully developed a portable electronic nose (lab made) based on eight metal oxide gas sensors for identification of a difference in coffee flower odors before and after pollination. All metal oxide gas sensors showed sensitivity to coffee flower odors. PCA result based on sensor responses as input data provided good classification of odors of coffee flower samples. Based on GC analysis, it was found that some VOCs with decreased or vanished amounts after pollination resulted in lower sensor responses. Pyrazine and 2-methyl-1-butanol may be used as a key compound for identifying a difference in odors of coffee flowers. It is concluded that our developed portable E-nose can be used as a

rapid tool for qualitative classification of coffee flower odors and it offers low cost, nondestructive measurement, real-time detection and easy to use.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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