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IOP Conf. Series: Materials Science and Engineering **546** (2019) 032012 doi:10.1088/1757-899X/546/3/032012

Identification of Bioactive Compounds in Ginger Based on Molecularly Imprinted Polymer Quartz Crystal Microbalance Gas Sensor

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Abstract. Borneol, citral, and geraniol have been investigated as the major bioactive compound commonly found in ginger. In this work, a molecularly imprinted polymer (MIP) coated quartz crystal microbalance (QCM) sensor array has been employed for selective recognition of bioactive compounds in the ginger essential oil. In the experiment, the concentration of these bioactive compounds previously was measured using solid phase micro extraction gas chromatography-mass spectroscopy (SPME-GC/MS). Design of MIPs as the template of target molecules was created using polyacrylic acid (PAA) polymer matrix and three molecular targets (borneol, citral, and geraniol). An array of QCM sensor was prepared using four 9-Mhz AT-cut quartz crystal embedded between vacuum-deposited Au electrodes. For data recording, the headspace system flew the odorant of three varieties of ginger essential oil as positive control odorant and wild ginger essential oil as negative control odorant into the QCM sensor chamber. Then, mass loading in the MIP films caused frequency change of QCM sensor array due to odorant adsorption in a thin layer of MIP. Principal component analysis (PCA) and linear discriminant analysis (LDA) were applied to analyse the QCM response sensor. PCA score plot showed segregation of feature response of ginger essential oil with and without the molecular target in the coordinate of principal components. Meanwhile, LDA was able to discriminate training datasets of 80 ginger samples containing borneol, citral, and borneol with accuracy more than 92.50%.

Keywords: quartz crystal microbalance, molecularly imprinted polymer, borneol, citral, geraniol, principal component analysis, linear discriminant analysis

1. Introduction

Ginger (*Zingiber officinale Roscoe*) has been well-known as one of phytomedicines since years ago. In Indonesia and other countries, ginger has been utilized as beverage, food product, and herbal medicine. The people utilizes ginger as beverage and food product due to its specific warmness, flavour, and taste. In several countries, ginger was used as raw material for some popular ginger drink: *Wedang jahe* in Indonesia, *Syabaji* in Nepal, *shogayu* in Japan, and *sujeonggwa* in Korea. Meanwhile, efficaciousness

of ginger as phytomedicine for human health caused several the people in the world utilized ginger for raw material such as in Java [1], India [2], and China [3].

In the modern era, previous research reported that efficaciousness of ginger was contributed by the presence of bioactive compounds in the ginger. Several bioactive compounds in ginger which had beneficial properties for human health such as antiradical activity, smoothing muscle relaxation, and anticancer. Based on experiments, geraniol, gingerol, β-myrcene, citral, α-zingibene, and 6-shogaol were the major bioactive compounds that was effective for human health. Geraniol grafted chitosan oligosaccharide was investigated as a potential antibacterial agent [4]. Other experiments showed that geraniol was effective as anti candidis agent [5], improves the impaired vascular reactivity in diabetes and metabolic syndrome [6]. Gingerol induced cell cycle arrest and apoptosis in triple-negative breast cancer cells [7]. Borneol was efficacious as anti-influenza virus and anti-depressant [8, 9]. Moreover, citral was bioactive compound which is useful for anti-inflammatory [10], cytotoxic effects on tumour cell cultures [11], anti-microbial agent [12], anti faciola larva [13], and etc.

Indonesia has been well-known as one of ginger producer countries in the world. Based on observation of the size and color of rhizome, three varieties of ginger well-grown in Indonesian archipelago including: small-white ginger or *jahe emprit* (*Zingiber officinale var amarum*), big-white ginger or *jahe gajah* (*Zingiber officinale var officinale*), and red ginger or *jahe merah* (*Zingiber officinale var rubrum*) [14]. Identification of bioactive compounds such as geraniol, borneol, and citral in these ginger varieties with a simpler method is necessarily conducted to investigate the superiority of ginger as a high quality of agro-industrial commodity from Indonesia. In addition, recognition of ginger that is rich in bio-active compounds will increase economic price due to quality assurance of this agro commodities for human's health.

Based on previous references, standard analytical techniques carried out for identifying chemical constituents as well as bioactive compounds in phytomedicine used chromatographic techniques and non-chromatographic techniques. The most common chromatographic techniques for identification of major compounds in herbals used gas chromatography/ flame ionized detector (GC/FID) [15], gas chromatography/mass spectroscopy (GC/MS) [16] and high-performance liquid chromatography (HPLC) [17]. In addition, Fourier transforms infra (FTIR) spectroscopy [18] was also used as non-chromatographic to identify the chemical constituents of the organic sample. These conventional methods are efficient and accurate for identifying volatile and bioactive compounds in phytomedicine. However, limitations such as large size, high cost, and complicated technical processing and time-consuming in the analysis are still a big challenge for researchers. In another side, the identification of volatile and bioactive compounds in phytomedicine with small size apparatus, low cost, simple technical process, and time-consuming is necessarily conducted. Employment of sensory analysis has tried as another breakthrough to simplify the identification of bioactive compounds in phytomedicine with simpler, lower cost, and time-saving procedures.

In several studies, the researchers have employed sensory analysis based on metal oxide semiconductor (MOS) gas sensor to analyze sensory response of volatile constituents of aromatic samples including phytomedicine e.g.: ginger [19], pepper [20], vanilla, and coffee. However, e-nose based on MOS sensor was unable to identify the volatile constituents of the sample. The employment of MOS gas sensor was only used to measure the response of gas sensor array towards the volatile compounds of an aromatic sample, then two or more sample can be discriminated based on the response. Hence, GC/MS analysis was still used to complete the identification of volatile constituents of the sample previously analysed using MOS sensor.

Another type of sensory analysis used quartz crystal microbalance (QCM) sensor. QCM sensor is now being employed as a gas sensor beside the MOS sensor due to highly sensitivity and selectivity to target molecules. Compared to MOS gas sensor, QCM is able to identify the volatile constituents of several aromatic samples including phytomedicine, e.g. linalool in black tea [5] β-caryophyllene in mango[21], and 3-carene in mango [22]. A thin plate of quartz crystal blanks with metal electrodes on each side is prepared to make a QCM sensor. For sensing applications, QCM sensors must be coated with appropriate polymer. The target compound is adsorbed on the coating surface increasing the mass

of QCM sensors and results in a change in its resonance frequency. An alternating electrical excitation is flown to the electrodes, then quartz crystal faces deformation and relaxation and results in a change in its resonance frequency. According to Sauerbrey's equation, the QCM resonance frequency decreases linearly with the adsorbed mass. Identification of the target compound can be conducted by observing different frequency deviations. In other words, identification of specific compound in the tested sample can be carried out by investigating the magnitude of frequency change in the QCM sensor array due to loading mass in the MIP film. The magnitude of frequency change is also used to indicate the sensitivity of the QCM sensor. In this case, MIP-QCM is low cost, simpler, and reliable apparatus used to identify bioactive compounds in phytomedicines.

The aim of this study is to identify bioactive compounds in ginger essential oil using MIP coated QCM sensor array. MIP films were prepared using polyacrylic acid (PAA) as host polymer, added with target bioactive compounds: borneol, citral, and geraniol for polymer coating of QCM sensors. The odour of ginger essential oil then was flown in the sensor chamber of the QCM sensor. GC/MS analysis of ginger essential oil was conducted for validating the quantity bioactive compound in the ginger.

2. Materials and Methods

2.1. Chemicals and Instruments

Borneol, citral, geraniol, and PAA were purchased from Sigma-Aldrich, Japan, while ginger essential oil were obtained from steam distillation. Three varieties of ginger including small-white ginger (*Zingiber officinale var. amarum*), big-white ginger (*Zingiber officinale var. officinale*), and red ginger (*Zingiber officinale var. rubrum*) was used as positive control odorant (PCO), while wild ginger (*Curcuma xanthorhiza*) was used as negative control odorant (NCO). These materials were purchased from a local traditional market in Purwokerto, Central Java, Indonesia. All materials were washed using water to remove the soil which sticks to the skin of the rhizomes. Once the washing process, the materials were sliced. The dimension of the sliced sample is approximately 1cm×1cm×0.2 mm. The sliced samples then put in drying cabinet at 60 ˚C from 24 hours. A boiling tank contained 10 L of water was prepared, then all dried small white ginger (SWG) was put in the boiling tank for running steam distillation for about 8 hours. The essential oil of SWG was taken out into glassware tube by turning down the faucet adapter. These procedures were replied for others samples, i.e. big white ginger (BWG), red ginger (RG) and wild ginger (WG) investigated in this study. In average, 10 ml of ginger essential oil was obtained from 5 kilograms of wet ginger rhizome.

2.2. Instruments

We employed four 9-Mhz AT-cut quartz crystal embedded between vacuum-deposited Au electrodes (Seiko EG&G, Japan) as QCM sensor array for sensing bioactive compounds from samples. Frequency changes of QCM were calculated using Sauerbrey equation as [23]:

$$\Delta f = \frac{-2f_0\Delta m}{A\left(\mu_g d_g\right)^{\frac{1}{2}}} \quad (1)$$

Where f_0, μ_g, d_g, A, and Δm stated original frequency, shear modulus, density, surface area and mass loading of QCM respectively. Each QCM electrode was put in a cylindrical crystal holder and housed in the sensing chamber. The QCM sensor response was measured by connecting QCM electrode to the QCM analyser (QCA 922, Seiko EG & G, Japan and recorded using a portable computer (Panasonic, Japan) equipped with WinQCM software. The apparatus of MIP coated QCM sensor used in this study presented in Fig. 1.

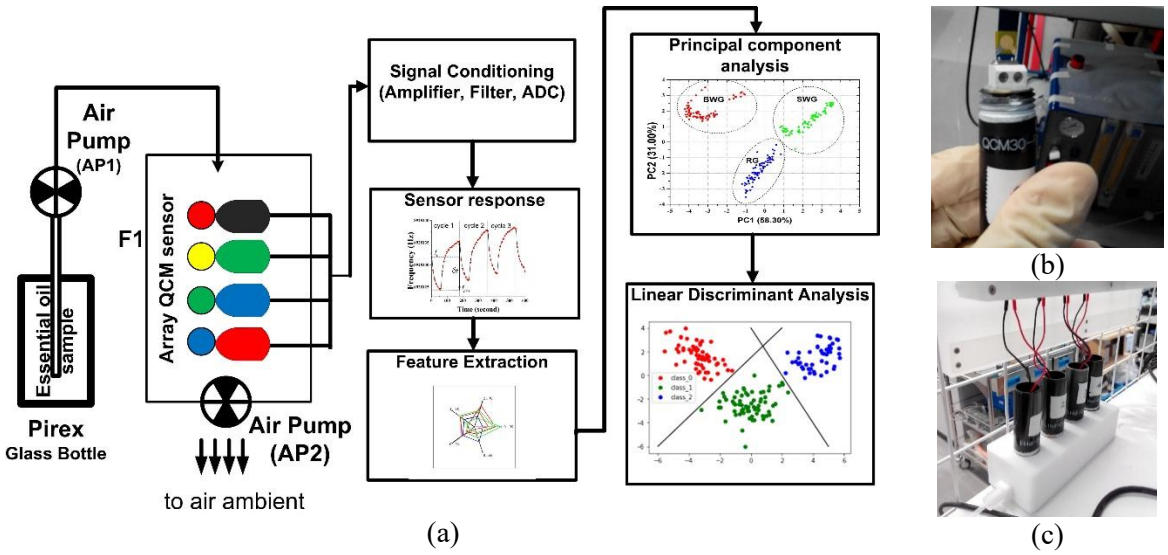

Figure 1. Apparatus of MIP coated QCM sensor array used in this study: (a) the block diagram of MIP coated QCM sensor array ; (b) 9-Mhz AT-cut quartz crystal microbalance ; (c) the chamber of QCM sensor

The procedure of MIP films preparation used a simple approach in the following steps: (a) each 250 mg of PAA was dissolved in 5 ml ethanol for preparing three solutions of polymer; (b) template molecules were created by adding each 10 µL of pure borneol, citral, and geraniol separately into three polymer solutions; (c) 25 µL of ginger essential oil solutions (SWG, BWG, and RG) was added into each polymer solutions; (d) mixture solutions were put in magnetic stirrer, stirred for 2 hours continuously; (e) 5 µL of each solution was dropped on the surface of each different QCM electrode using micro-pippet (Nichipet, Japan); (f) each QCM electrode was run in spin coating separately at 500 rpm for 30 s to obtain a typical thickness MIP films coating in few hundred nm; (g) Three QCM electrodes were put in a vacuum oven simultaneously at 40 ° C for 12 hours to remove the PAA matrix in template molecules; (h) The QCM electrodes were ready for odour sensing measurement.

2.3. SPME-GC/MS Analysis

SPME-GC/MS was employed for identifying target bioactive compounds both in the PCO and NCO group sample. A PDMS/DVB fibre extracted four types of odorant: SWG, BWG, RG was identified as PCO; and WG as NCO. The fibres were conditioned for 10 min at 250 °C in the GC/MS injector before SPME-GC/MS analysis. For each sample, 1 µL of essential oil was dropped in a 10-mL of the vial using micro syringe. The fibre coating was embedded into the headspace to determine temperature and time value set in the experiment. The temperature was set at 50 ºC while incubation and extraction time were set 5 min and 10 min, respectively. The fibre containing the extracted volatile compounds of ginger was injected into GC/MS injector. Direct injection of helium was used as carrier gas in the split mode. Injector and detector temperature were maintained at temperature 200-280 ºC. The measurement of each ginger sample using GC/MS equipped with auto-sampler was set for about 60 minutes. The temperature of the column was programmed initially at 70 ºC and then increased at 250 ºC for 10 minutes (at a rate of 18 °C min-1). Mass conditions were followed: ionization voltage, 70 eV; ion source temperature, 200°C; full scan mode in the 30–450 amu mass ranges with 0.2 s scan-1 velocities. Identification of compounds was identified by using NIST 08 database (NIST mass spectral database, PC version 2008). The total ion current from GC/MS spectra was used to calculate the relative percentages of separated compounds by a computerized integrator. From SPME/GC-MS analysis, the concentration of target molecules (borneol, citral, and geraniol) was identified.

2.4. Data recording of MIP coated QCM sensor

We prepared 100 ml Pyrex glass bottle for placing ginger essential oil samples. The glass bottle had 2 holes, a hole connected to air pump, and the other was connected to sensor chamber. A cotton paper (20 cm×10 cm) was placed inside the glass bottle. 100 µL of ginger essential oil contained target molecules was injected over the cotton paper separately using micro syringe. A cycle of data recording consisted of sensing and flushing carried out alternately. The control system of QCM sensor was set to be 1 minute for sensing process and 2 minute for flushing. In the sensing process, the control system set the air pump machine to flow dry air (flow rate 1L/min) into glass bottle for 1 min (AP_1 was in ON condition), the odour of ginger essential oil flowed into sensor chamber containing four electrodes of QCM sensor. During the sensing process, gas molecules interacted with MIP films, added mass loading in the surface of QCM electrodes. Increasing of mass loading due to interaction between volatile compound target and MIP films change the QCM sensor frequency referred to Sauerbrey equation shown in Eq. 1. The QCM frequency change was converted into a frequency signal and presented in personal computer.

Oppositely, during the flushing process, AP_1 was set in OFF, while AP_2 was set in ON. The second air pump machine (AP_2) pumped the odorant outside from the sensors chamber, the accumulation of target molecules in the sensor chamber decreased gradually. Hence, the frequency of QCM sensor returned to the baseline value. In data recording, the sensing and flushing of target molecules were carried out alternately in four cycles. We recorded three types of odorant: PCO, NCO, and unknown odorant (UKO). PCO positively contained target molecules (borneol, citral, and geraniol) obtained from SWG, BWG and RG, while NCO contained no bioactive target molecules obtained from WG. Meanwhile, group of UKO was obtained from SWG, BWG, and RG in which the presence of target molecules has not been identified yet. Each odorant was measured alternately for 20 times, hence, totally 80 dataset of sensor response were obtained for data analysis.

2.5. Feature Extraction

The response of all sensors was converted into 4×80-dimensional feature vector upon the following relative frequency change parameter [24].

$$RFC = \frac{\Delta f}{f_b} = \frac{f_b - f_{min}}{f_b} \quad (2)$$

Where f_b and f_{min} are sensor responses calculated at "Odorant in" and "Odorant out", respectively.

2.6. Data analysis

Multivariate techniques based on principal component analysis (PCA) and linear discriminant analysis (LDA) were used to analyse the dataset of MIP coated QCM sensor response. We also used Minitab ver. 17.0 as statistical software to simplify computational data analysis.

3. Results and Discussion

3.1. Composition of target bioactive compound

The composition of target bioactive compounds in three varieties of ginger (SWG, BWG, and RG) as PCO and WG as NCO previously investigated using SPME-GC/MS is presented in Table 1. Two target bioactive compounds identified in SWG. Borneol and neral were identified at 5.79% and 5.10%, respectively. In BWG, borneol and neral appeared in different quantities. Borneol appeared only 1.22%, while neral appeared at 11.90%. In RG, three target bioactive compounds were measured higher concentration. Geraniol was measured 9.99%, citral and borneol were identified 29.45% and 5.22% respectively. The quantity of neral (17.32%) was contributed in the calculation for citral due to isomeric properties of these compounds.

Table 1. List of major chemical compounds identified in three gingers using GC/MS analysis

Compound name	Concentration (%)			
	SWG	BWG	RG	WG
borneol	5.79	1.22	5.22	n/a
citral	n/a	n/a	12.13	n/a
geraniol	n/a	n/a	9.99	n/a
neral	5.10	11.90	17.32	n/a

The efficaciousness of these target bioactive compounds for human's health has been investigated in several research activities. Geraniol has been investigated as a bio-active compound that was efficacious for anti-inflammatory, cancer chemotherapy, cytotoxicity against fibrosarcoma, anti-bacterial agent, and anti-Parkinson disease [25-27]. Neral, a monoterpenoid aldehyde, has been widely used as a powerful lemon-fragrance chemical, fruit odour with a woody, balsamic undernote and a sweet, warm, powerful, and spicy taste [28]. In previous research, this compound has anti-inflammatory activity [10]. Citral, the isomer of neral has similar odour and taste with neral. In addition, citral has a spicy, fruit odour with a woody, balsamic undernote and a sweet, warm, powerful, spicy taste [28]. Previous research investigated that citral was also efficacious as antifungal, antimicrobial, antioxidant, and anti-tumour activity [29, 30]. Furthermore, borneol, the third of the target bioactive compound is a bicyclic organic compound and a terpene derivative had a camphor-like odour and burning taste somewhat reminiscent of mint, [29]. Borneol was efficacious as anti-influenza virus and anti-depressant. [8, 9].

3.2. Array sensor response of MIP coated QCM

The typical array sensor response of MIP coated QCM obtained by measuring the odorant response of a SWG sample without any feature extraction is presented in Fig. 2. The sensor response describes three parameters: baseline frequency, the minimum frequency, and the relative frequency change (RFC). Each sensor has different value for these parameters due to different sensitivity and selectivity toward target molecules captured by MIP templates. The difference of sensor response between three varieties of ginger (SWG, BWG, and RG) as PCO and WG as NCO can be distinguished by calculating the RFC magnitude, the frequency change of the QCM electrodes divided by the frequency baseline (see Fig 4.a.). The RFC magnitude corresponded to the mass loading change on the surface of MIP films while interacting with odorant of gingers. The variation of RFC magnitude at QCM sensor array occurred while sensing the odorant grouped both in PCO and NCO.

Data in Table 2 shows that the variation of RFC magnitudes occurs in four sensors and different kinds of ginger sample. This indicates that all sensor is selective and sensitive to each target molecules through the proper design of MIP film templates. The pattern of data also shows that the RFC magnitudes obtained from the group of PCO samples are relatively higher than the NCO sample. This indicates that the QCM sensor array is selectable to the target molecules identified in this study. Oppositely, in the measurement of NCO (the sample without target molecules), the pattern of RFC magnitudes are lowest than others due to the absence of borneol, citral, and geraniol in MIP films.

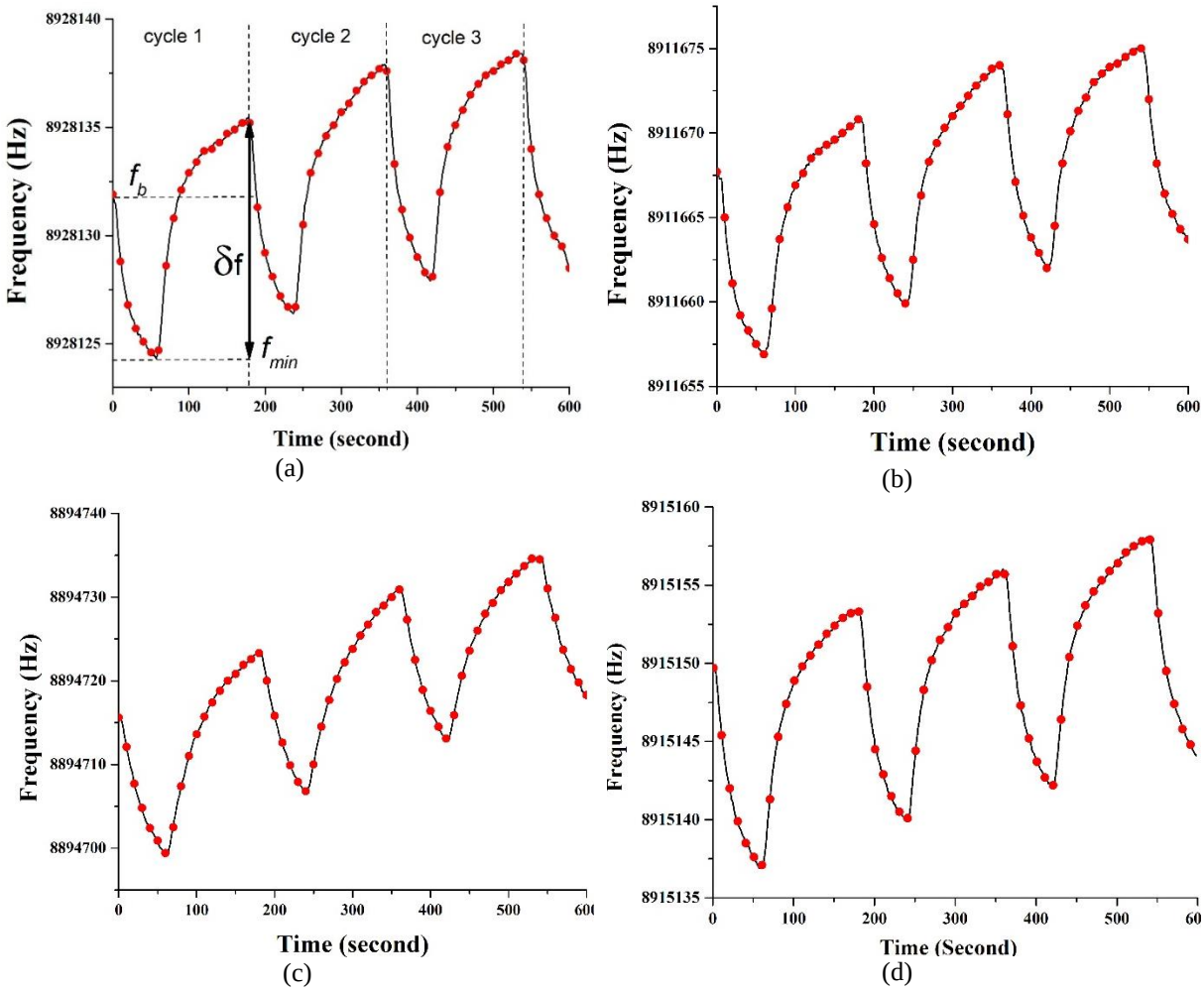

Figure 2. Typical array of MIP coated QCM sensor response obtained by measuring SWG odorant: (a) response obtained from sensor 1 for borneol identification; (b) response obtained from sensor 2 for citral identification; (c) response obtained from sensor 3 for geraniol identification; (d) response obtained from sensor 4 for arbitrary compound identification.

Table 2. Magnitude of the relative frequency change (RFC) obtained from PCO, NCO, and UKO

Class of sample	Sample name	Sample code	RFC ($\times 10^{-6}$)			
			Sensor 1	Sensor 2	Sensor 3	Sensor 4
PCO	Small white ginger sample 1	SWG_1	1.01	1.21	1.84	1.48
PCO	Big white ginger sample 1	BWG_1	1.06	1.30	1.86	1.47
PCO	Red ginger sample 1	RG_1	1.05	1.28	2.00	2.10
NCO	Wild ginger sample 1	WG_1	0.74	1.09	0.81	0.90
UKO	Small white ginger sample 11	SGW_{11}	1.03	1.22	1.75	1.47
UKO	Big white ginger sample 11	BWG_{11}	1.08	1.35	1.96	1.48
UKO	Red ginger sample 11	RG_{11}	0.65	0.69	1.00	1.01

3.3. Principal component analysis

The RFC magnitudes obtained from the sensor array then were analysed using principal component analysis (PCA) and linear discriminant analysis (LDA). The PCA is a statistical technique used to simplify the dimensionality of numerical data sets and convert a set of observations of correlated variables into a set of values of uncorrelated variables called principal components. In this case, PCA is applied to reduce the data dimension of RFC values obtained from array QCM sensor. With PCA, the feature vector of RFC was converted into 2 principal components with highest eigen values and also reduce feature vector dimension from 4×80 to 2×80.

Fig. 3 presents the score plot in the PC1-PC2 coordinate of four different clusters of 80 samples. In total, 96.40% of variance has been retained from two principal components. PC1 contributed 89.60% of the variance, while PC2 contributed 6.90%. Clearly separation of samples can be observed in the visualization of PC coordinates. This indicated that group of PCO samples (SWG, BWG, and RG) were clearly different from group of NCO sample (WG). In group of PCO samples, RG was separated from SWG and BWG. However, SWG and BWG were not clearly separated. Two samples of SWG and five samples of BWG is located in similar principal component coordinates. The visual pattern of group samples separation in Fig. 3 indicated that the MIP coated QCM array sample was able to discriminate the odorant containing target molecules (PCO) and the odorant without target molecules (NCO).

Furthermore, the array sensor was also able to discriminate groups of RG containing borneol, citral, and geraniol with groups of SWG and BWG containing only borneol and citral. The array sensor was also able to predict the existence of target molecules in the group of UKO sample. The point location of UKO samples is nearby PCO samples. Hence the QCM sensor array considered that borneol, citral, and geraniol were identified in UKO samples.

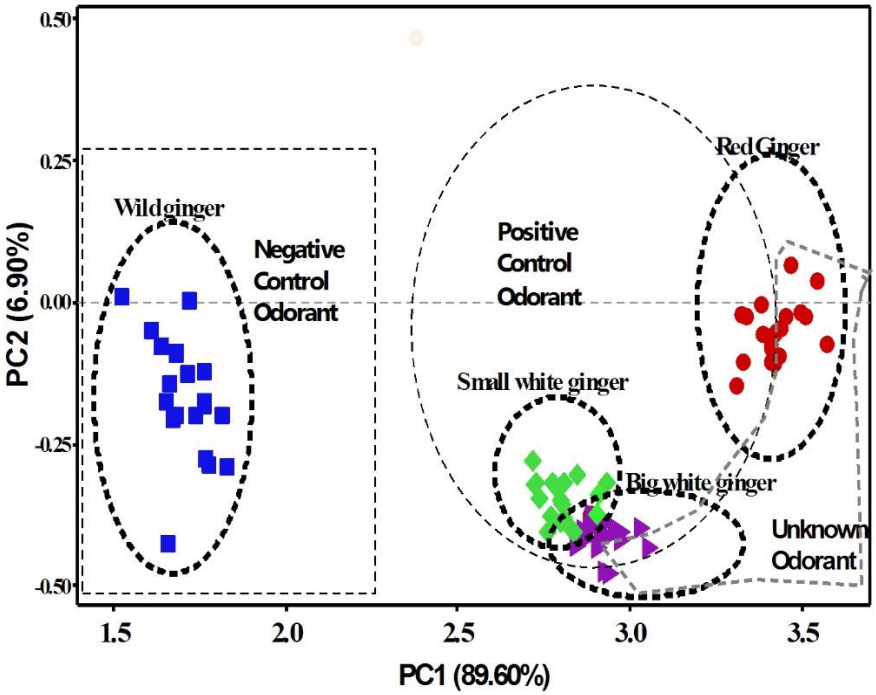

Figure 3. PCA score plot of 4×80 feature vectors of MIP coated QCM array sensor using relative frequency change in two dimensional of principal components obtained from 80 samples grouped in PCO, NCO, and UKO

3.4. Linear discriminant analysis

Furthermore, linear discriminant analysis (LDA) was applied for classification and avoiding the process of variable reduction. LDA was implemented in two categories. In the first category, LDA was applied to discriminate the samples between groups of PCO and NCO, while the second category was used to discriminate three varieties of gingers: SWG, BWG, and RG. In the first category of discrimination, implementation of LDA in the training dataset with 80 samples provided a perfect classification of the samples. With the cross-validation, LDA was able to obtain 98.80% accuracy rate, only a sample in the group of PCO was incorrectly assigned to group of NCO. Implementation of LDA in the second category of training dataset classified 80 samples into four different classes: BWG, LWG, RG, and WG. The percentage of training data sets correctly classified was 93.80%, since of 80 predictions made only two samples from SWG were incorrectly assigned to BWG and only three samples from BWG were incorrectly assigned to SWG. With the cross-validation, the percentage accuracy of classification obtained 92.50%. Three samples from SWG was incorrectly assigned to BWG category, and oppositely three samples from BWG were incorrectly assigned to SWG.

Table 3. The results obtained on applying LDA using 80 samples in the training dataset

Discriminant category	Method	Number of sample	True assign	False assign	Accuracy (%)
PCO-NCO	Without cross validation	80	80	20	100.00
	With cross validation	80	79	1	98.30
SWG-BWG-RG-WG	Without cross validation	80	75	5	93.80
	With cross validation	80	74	6	92.50

4. Conclusion

MIP coated QCM sensor array has been employed to identify borneol, citral, and geraniol as target bioactive compound from three varieties of gingers. QCM sensors are highly sensitive and selective for identifying target bioactive compound, shown by PCA and LDA analyses. The visualization of PCA score plot in two principal component coordinates shows that groups of the PCO samples and the NCO samples is clearly separated with 96.40% of the variance. PC_1 contributed 89.60% of the variance, while PC_2 contributed 6.90% of the variance. Implementation of LDA in training datasets used for classification of 80 samples into four different classes: BWG, LWG, RG, and WG obtained accuracy level at 93.80% without cross-validation and 92.50% with cross-validation, respectively. Identification of borneol, citral, and geraniol using MIP coated QCM sensor array shows that this apparatus can be developed as a rapid and low-cost instrument for identification of specific major compound in other herbal medicine samples.

Acknowledgements

This work was supported by the project of "*Penelitian Pembinaan/Peningkatan Kualitas* – fully funded by DIPA IAIN Purwokerto 2019, provided by Research and Community Services Office, LPPM IAIN Purwokerto". The authors also thank for GC-MS technician from the Laboratory of Organic Chemistry, Faculty of Mathematics and Natural Science, Universitas Gadjah Mada, Yogyakarta, Indonesia who have conducted GC/MS analysis for three varieties of Indonesian ginger.

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