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Scent analysis of *Rosa laevigata* through metal oxide sensor array electronic nose

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Abstract: The scent fingerprint of *Rosa laevigata* Michx., Rosaceae, samples harvested at different periods was investigated. Principal component analysis (PCA) and discriminant factor analysis (DFA) were done on the scent response values measured by an electronic nose (EN) sensor. Statistical quality control (SQC) analysis was also conducted. The *R. laevigata* samples were clustered into two categories after being analyzed by PCA and DFA. One cluster consisted of samples No. 1 to No. 6, and the other consisted of samples No. 7 to No. 10. The combination of EN, PCA, and DFA for scent analysis can be used for the quality control of traditional Chinese medicines.

Introduction

Rosa laevigata Michx. is a member of the Rosaceae family. Its fruits are commonly used as traditional Chinese medicine (TCM). It is used as a kidney tonic to reduce urination; it is also used for cleansing the intestines and for arresting leucorrhea. According to the *Chinese Pharmacopoeia*, *R. laevigata* fruit can be harvested when it is ripe and red (The Pharmacopoeia Committee of People's Republic of China, 2010). Some references ascribe its anti-uretic and astringent properties to its acerbity; when it is ripe and red, it tastes sweet and loses its medicinal value. It should therefore be harvested when it is partly yellow but not yet ripe (Shen, 2003). There are two harvest periods for *R. laevigata*: the first is when it is ripe, and the second is when it is partly yellow but not yet ripe.

Secondary metabolites, which are highly dynamic in different periods of growth of a medicinal plant, directly influence its medicinal effect (Stitt & Fernie, 2003). Hence, the seasonal changes in secondary metabolites of *R. laevigata* should be fully studied to reasonably harvest. In spite of some well documents concern about chemical compounds of *R. laevigata*, such as Laevigatins E, F, G, 2 α -methoxyursolic acid, 11 α -hydroxytormentonic acid, loliolide, 6,7-diethoxycoumarin, diethylmalate et al has been reported

(Yoshida et al., 1989; Fang et al., 1991a; 1991b; Yuan et al., 2008), and some studies on the dynamic changes in its non-volatile components (such as flavones and polysaccharides) have been done (Zhou et al., 2005; Han et al., 2008; He et al., 2009; Liu et al., 2011). However, there is no paper about its volatile components. The present work used an electronic nose (EN) to detect the dynamic changes in its volatile components as it was harvested at different periods. Principal component analysis (PCA) and discriminant factor analysis (DFA) were adopted to cluster the *R. laevigata* samples and provide a reference for the systematic harvesting of *R. laevigata* and the promise of drug effect.

Materials and Methods

Rosa laevigata samples

Samples of *Rosa laevigata* Michx., Rosaceae, were collected from the hill behind Shipo Dian Town, Liu An City, Anhui Province (31°64'75"N, 116°06'36"E), and were identified by Prof. Z. Ouyang from Jiangsu University. From August 25, 2009 to December 27, 2009, ten batches of samples were regularly harvested and sequentially numbered from 1 to 10. The samples were air dried and ground.

Determination of the EN response resistance

An α FOX 4000 sensor array system and eighteen metal oxide sensors (Alpha M.O.S.) were used to determine the dynamic scent changes in the *R. laevigata* samples. The amount of sample used was 1 g. Detection conditions were: dry air carrier gas at 150 mL \cdot min $^{-1}$; 55 °C headspace temperature; 300 s residence

time; 120 rpm stirring speed; 1000 μ L injection volume; 120 s acquisition time; 600 s lag time. The detection of each sample was repeated five times.

Statistical analysis

The data were analyzed by PCA, DFA and SQC analysis using the α SOFTV9.1 software.

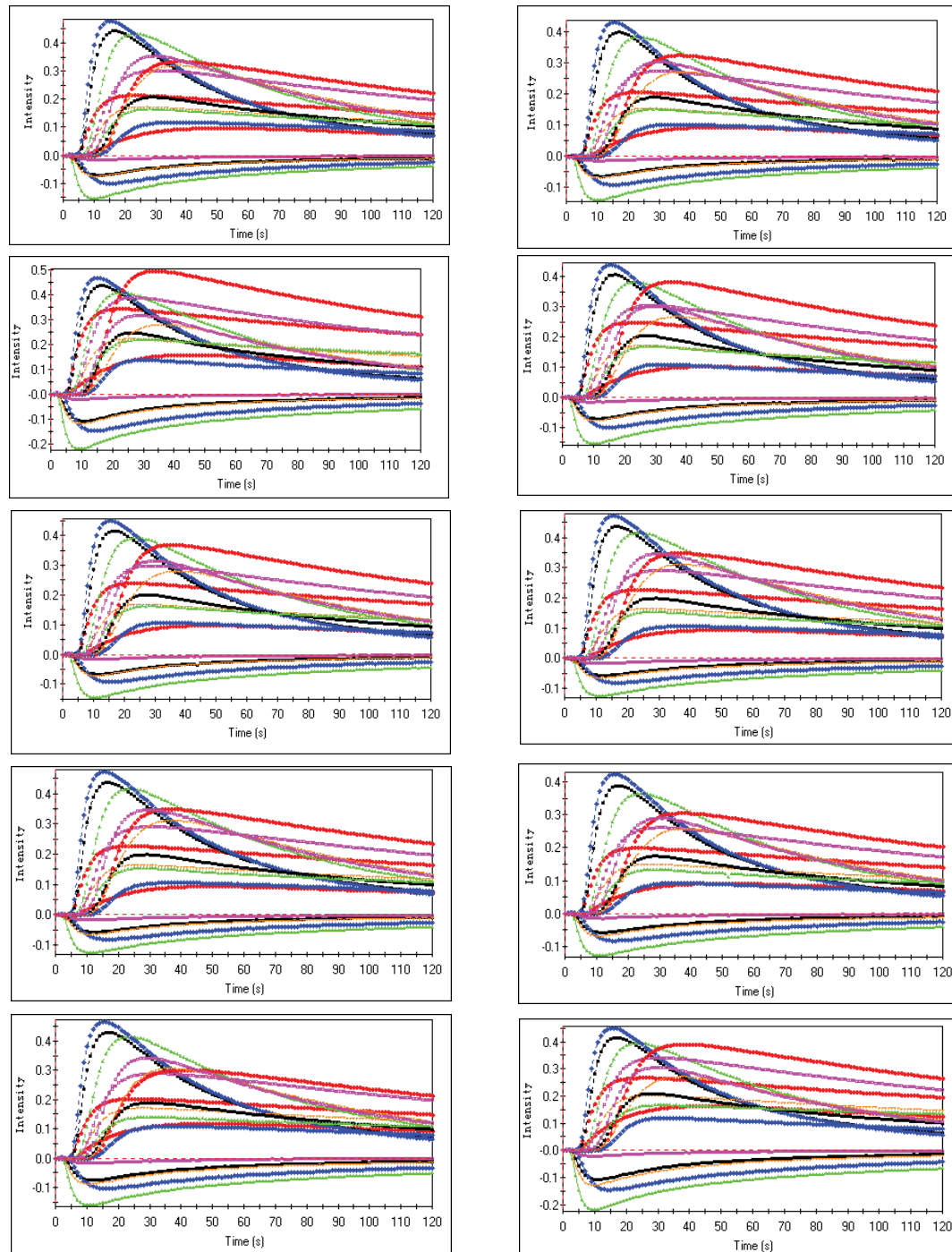


Figure 1. The sensing signals of *Rosa laevigata* samples No. 1 to No. 10.

Results

Results of the sensor signals of *R. laevigata* samples

Figure 1 shows the sensor signals produced by *R. laevigata* samples. The response intensity on the ordinate axis corresponds to the rate of change of the relative resistance of the different sensors $[(R_0 - R) / R_0]$, where R_0 is the resistance value at $t=0$, and R is the resistance value with the changes in time. Figure 1 shows that the sensors detected a small difference in the scent *R. laevigata* samples; only a few sensor signals differ.

The fingerprint map of the EN sensor signals were analyzed to understand better the response of the 18 metal-oxide sensors of the EN toward the scent of *R. laevigata* at different harvest periods. The fingerprint map was prepared by evenly placing the 18 sensors at 20-degree intervals along the circumference of a circle. The maximum response value for each sensor was recorded and marked. There are small differences in the samples harvested at different periods, and some small differences were detected by the T30, P30 and PA-type sensors (Figure 2).

PCA

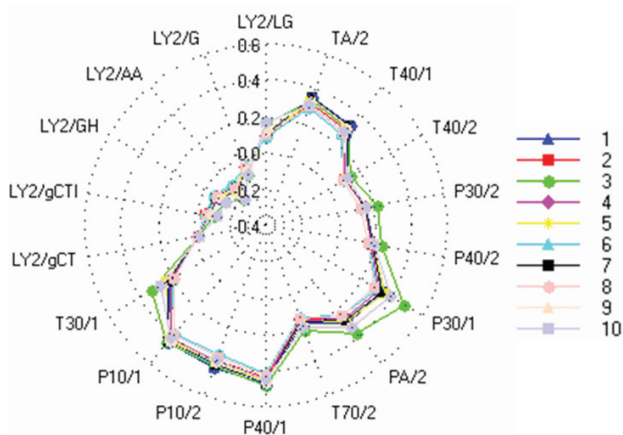


Figure 2. Scent fingerprint map of *Rosa laevigata* samples.

A credible conclusion could not be drawn due to the a large amount of information in the original signal map and the small difference between the signals of the samples. Therefore, stoichiometry data processing was adopted to analyze intuitively and scientifically the results. PCA is a type of multivariate statistical method, which data-converses and dimensionality-reduces the extracted multi-index information of sensors. It linearly classifies the characteristic vector after dimensionality reduction and displays the major two-dimensional map on a PCA analysis map. In the case of the absence or lack of sample information, PCA can quickly scan all data to determine the

associated features of the samples and make a conclusion based on the available information (Hansen et al., 2005; Pereira et al., 2006; Park et al., 2010).

PC1 and PC3 are taken as the coordinate axes to conduct a PCA analysis on the samples. Figure 3 shows that the response data points are close to the coordinate axis, and are clustered about 0.1. The response values of the sample data points collected before October 27, 2009 (sample No. 6) are negative in PC3, while the response values after the harvest period are positive. This implies that samples No. 1 to No. 6 may be clustered as one category, while samples No. 7 to No. 10 may be clustered as another category (Figure 3).

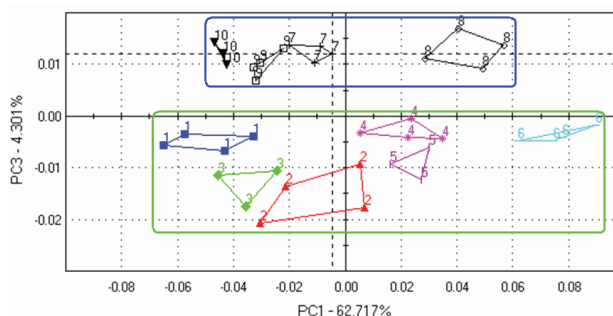


Figure 3. The PCA map of 10 samples of *Rosa laevigata*.

DFA

DFA is a classification technique that optimizes the distinguishability by recombining sensor data (Rosselli et al., 2005). With mathematical manipulation, it minimizes the differences between data of the same type and broadens the disparities between data from different categories to establish a data recognition model. Figure 4 shows that EN was able to distinguish between the *R. laevigata* samples harvested at different periods. Samples No. 1 to No. 6 evidently cluster together as one category, and the differences between their signals are small; samples No. 7 to No. 10 belong to one category, but the differences between them are larger.

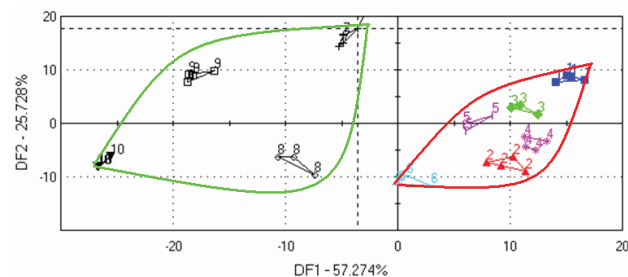


Figure 4. DFA map of ten samples of *Rosa laevigata*.

SQC analysis

The SQC analysis is based on the sample diversity, and is performed by acquiring an acceptance region and a rejection region by calculating data from a reference sample. Unknown samples are mapped in the figure, from which a conclusion (acceptance or rejection) may be made (Bleibaum et al., 2002; Munoz, 2002). A single olfactory value can be achieved by computing the sample mean and standard deviation. For every data point, the distance in scent units demonstrates the difference in scent.

SQC analysis was carried out by taking sample No. 1 as the reference. Small differences between the samples were detected by the EN; this illustrates the high sensitivity of the EN (Figure 5).

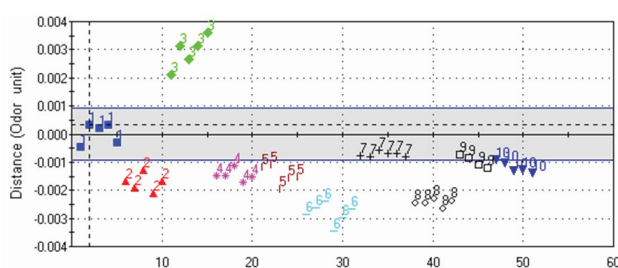


Figure 5. SQC analysis of ten samples of *Rosa laevigata*.

Discussion

The scent of a TCM is the major basis of judging its quality. Scent analysis a TCM with modern technologies such as gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC-MS in itself yields information on the concentration of volatiles present in a sample, but little is known about the relationship between these volatiles in a mixture and how they contribute to perceived sensory attributes (Hansen et al., 2005). Moreover, GC and GC-MS are still expensive and require trained personnel, EN instruments impress by virtue of simple or no sample work-up, automated measurement capability and easy, but mostly black box, pattern recognition and interpretation software (Alexandros et al., 2002; Hans et al., 2008; Han et al., 2009).

The EN is designed with electrochemical sensor arrays and proper identification equipment. It differs from GC, GC-MS, and other analytical methods in that it obtains not the qualitative and quantitative results of the individual components of the samples, but rather the overall information of the volatile components in the samples, that is, the fingerprint data. The EN can compare and analyze the scent information of different samples as well as establish an information database using standard samples, by adopting stoichiometry to qualify and quantitatively analyze unknown samples (Gardner & Bartlett, 1994). Currently, the EN has been

extensively applied in food analysis (Dutta et al., 2003; Thi et al., 2004; Gan et al., 2005; Wolfrum et al., 2006), environmental quality monitoring (Zhang et al., 2003), scent pattern analysis (Been, 2010), disease diagnosis (Di et al., 2003), and in other fields. This study used the EN to detect the changes in the volatile components of *R. laevigata* samples harvested at different periods to provide a reference for quantifiable scent indicators for *R. laevigata*.

Research results by Han show that the flavone content of *R. laevigata* harvested at different periods tends to increase initially and then decrease, and generally reaches its highest content in late September and early October (Han et al., 2008). The polysaccharide content also initially increases and then decreases, and is highest in November and December (Zhou et al., 2005). Flavone and polysaccharide content are used as standards. There are apparently two reasonable periods for harvesting *R. laevigata*, which is consistent with documentary records. The results of the present study show that the *R. laevigata* samples harvested from different periods are clustered according to their amount of volatile components. As they were harvested during the last third of October, the *R. laevigata* samples are divided into two categories. It is common knowledge that the same plant can have different medicinal materials if harvested at different times, as in the case of the ripe and unripe *Forsythia suspense* (Li & Li., 2006), and in *Pericarpium citri reticulatae viriole* and *Pericarpium citri reticulatae* (Zhou et al., 2009) Further pharmacological studies are required to confirm the practicality of having two different harvesting periods for *R. laevigata*.

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