Identification of sulfur fumed *Pinelliae Rhizoma* using an electronic nose

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ABSTRACT

Background: *Pinelliae Rhizoma* is a commonly used Chinese herb which will change brown during the natural drying process. However, sulfur fumed *Pinelliae Rhizoma* will get a better appearance than naturally dried one. Sulfur fumed *Pinelliae Rhizoma* is potentially toxical due to sulfur dioxide and sulfites formed during the fuming procedures. The odor components in sulfur fumed *Pinelliae Rhizoma* is complex. At present, there is no analytical method available to determine sulfur fumed *Pinelliae Rhizoma* simply and rapidly. To ensure medication safety, it is highly desirable to have an effective and simple method to identify sulfur fumed *Pinelliae Rhizoma*. **Materials and Methods:** This paper presents a novel approach using an electronic nose based on metal oxide sensors to identify whether *Pinelliae Rhizoma* was fumed with sulfur, and to predict the fuming degree of *Pinelliae Rhizoma*. Multivariate statistical methods such as principal components analysis (PCA), discriminant factorial analysis (DFA) and partial least squares (PLS) were used for data analyzing and identification. The use of the electronic nose to discriminate between different fuming degrees *Pinelliae Rhizoma* and naturally dried *Pinelliae Rhizoma* was demonstrated. **Results:** The electronic nose was also successfully applied to identify unknown samples including sulfur fumed samples and naturally dried samples, high recognition value was obtained. Quantitative analysis of fuming degree of *Pinelliae Rhizoma* was also demonstrated. The method developed is simple and fast, which provides a new quality control method of Chinese herbs from the aspect of odor. **Conclusion:** It has shown that this electronic nose based metal oxide sensor is sensitive to sulfur and sulfides. We suggest that it can serve as a supportive method to detect residual sulfur and sulfides.

Key words: Electronic nose, identification, *Pinelliae Rhizoma*, sulfur

INTRODUCTION

Pinelliae Rhizoma is one of the commonly-used Chinese herbs in traditional Chinese medicines against cough, inhibitting the emesis.[1,2] Its trade name is *Pinelliae Rhizoma*. The color of fresh *Pinelliae Rhizoma* is white. During the natural drying process, *Pinelliae Rhizoma* change brown. However, *Pinelliae Rhizoma* is fumed with sulfur during the natural drying process could be whiter and gets a more attractive appearance. The longer it fumes, whiter it looks. Sulfur dioxide and sulfites which are produced during the fuming procedures contribute to the conditioning by preventing oxidation, browning and microbial reactions.[3] Fuming with sulfur in drying process is a very common procedure for processing of *Pinelliae Rhizoma* in herbal medicine markets, so do other Chinese herbs such as Radix Astragali, Radix Angelicae Sinensis, Rhizoma Dioscorea, etc., Merchants will get a higher price and more profits to sale sulfur fumed Chinese herbs. Nevertheless, sulfur fumed *Pinelliae Rhizoma* is harmful to human body due to residual sulfur and sulfides in decoction. Excessive amounts of sulfur cause a sore throat and stomach discomfort to users[4] and the concentration of sulfur dioxide in drugs over $500\mu g \cdot g^{-1}$ will lead to uncomfortable tasting.[5] Due to the sulfur dioxide being sulfites produced by the reaction with *Pinelliae Rhizoma* chemical compositions (alkaloids, organic acids, Pinellia protein, starch, etc.),[2,6,7] the drug properties of *Pinelliae Rhizoma* may be changed. Oral or parenteral exposure to sulfites has been reported to induce a range of adverse clinical effects in sensitive individuals, ranging from dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhoea to life-threatening anaphylactic and asthmatic reactions.[8]

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In recent years, sulfur and sulfides detection have been a study of wide interest. Pauls.RE applied gas chromatography with on-column injection to determine elemental sulfur in gasoline.[9] The conventional determination of total sulfur dioxide (including sulfites) is Monier-Williams method.[3] Several researchers have studied the instrumental analysis of sulfites. Ina Kristiana *et al*., developed a solid-phase micro extraction and gas chromatography–mass spectrometry method for analysis of trace levels of polysulfides in drinking water distribution systems.[10] A pervaporation-flow injection method with a copper hexacyanoferrate-carbon nanotube (CuHCF-CNT)-modified carbon paste-electrode for determination of sulfite in food samples have been reported by Lori Shayne T. Alamo *et al*.[11] These conventional analytical tools such as gas chromatography-mass spectrometry (GC-MS), can provide accurate analysis for samples and give quantitative and qualitative information. Otherwise, these analytical instruments become less effective as sample complexity increases and time-consuming.[12] The odor components in sulfur fumed *Pinelliae Rhizoma* is complex and difficult to determine. However, no analytical method is able to identify whether *Pinelliae Rhizoma* was fumed with sulfur simply and rapidly. Therefore, it is highly desirable to have an effective and simple method for assessing sulfur fumed *Pinelliae Rhizoma*.

Dodd and Persaud first introduced the electronic nose designed to mimic the mammalian olfactory system.[13] The electronic nose can be an effective tool for odor analysis which consists of an array of sensors and an appropriate pattern-recognition system. Sensor array which reacts with volatile chemicals in the sample headspace result in a resistance change of sensor to form signals. The signal are then processed and analyzed using advanced pattern-recognition techniques such as principal components analysis (PCA), discriminant function analysis (DFA) and partial least squares (PLS).[14] The signals generated by samples are analyzed to extract features which can be evaluated as a whole to eliminate redundancy and to arrive at a description of the overall mix of volatiles and their intensity.[15] Electronic noses have been widely used in odor analysis and differentiate samples in recent years.[16] Rafi Haddad *et al*., applied an electronic nose system to predict the pleasantness of novel odorants and found it's similar to average human ratings.[17] A rapid method for identification of foodborne pathogens contamination in packaged fresh vegetable using electronic sensor array was developed by Ubonrat Siripatrawan.[18] Distinguished a diesel with sulfur content with chemical sensors based electronic nose have been reported.[19] Clinical evaluation of oral malodor caused by volatile sulfur compounds (VSC) using electronic nose have also been reported.[20] The metal oxide sensor to be considered as one of the standard sensors in the field of electronic noses,[15] since metal oxide sensor has a wide choice for different selectivity and a high sensitivity for sulfur and sulfides, we propose a method to identify whether *Pinelliae Rhizoma* was fumed with sulfur and predict the fuming degree of *Pinelliae Rhizoma* by using an electronic nose based on metal oxide sensors in this paper.

MATERIALS AND METHODS

Sample preparation

Fresh *Pinelliae Rhizoma* was purchased from a Good Agricultural Practices (GAP) planting base located in genuine production place of *Pinelliae Rhizoma*. Sulfur was obtained from a chemical raw material market. Sulfur fumed *Pinelliae Rhizoma* was prepared from fresh *Pinelliae Rhizoma*. The skin of fresh *Pinelliae Rhizoma* was peeled off at first. After cleaned with water the skinless herbs were exposed to air until almost dry. Then these herbs were transferred in an airtight metal container with a small opening on one side at the top. Some sulfur was put in the bottom of the container, whose amount is five thousands of herbs.[21] The sulfur was lighted. *Pinelliae Rhizoma* underwent fuming for hs till dryness to prepare the sulfur fumed samples. These samples were labeled as S1, S2 and S3 after being fumed for 1 h, 3 hs and 5 hs, respectively. A quantity of fresh *Pinelliae Rhizoma* was naturally dried, without being fumed with sulfur, to prepare a reference indicator. These samples were labeled as S0. We used diverse fuming time to indicate different fuming degrees. Samples S0-S3 was used as qualitative and quantitative analysis training standard samples.

Unknown samples were prepared to test the identification ability of the electronic nose system. *Pinelliae Rhizoma* fumed for 1h, 3h and 5h respectively were labeled as unknown 1, unknown 2, unknown 3, while unknown 4 was a naturally dried sample without being fumed with sulfur. Unknown 5-10 were purchased from different herbal medicine markets and already known as sulfur fumed *Pinelliae Rhizoma*. All these unknown samples were prepared by one person and analyzed by another person.

Electronic nose analysis

Operating mode and data acquisition

Analysis was preformed by a FOX 4000 electronic nose (Alpha MOS, Toulouse, France), which was equipped with 18 metal oxide sensors and a HS100 auto-sampler. All samples were analyzed on the FOX 4000 immediately after preparation. The data were processed by the Alpha Soft V12.3 software. Figure 1 shows a schematic diagram of the electronic nose system. This electronic nose includes 18 different metal oxide sensors (LY/LG, LY/G, LY/AA, LY/GH, LY/gCTL, LY/gCT, T30/1, P10/1, P10/2, P40/1, T70/2, PA2, P30/1, P40/2, P30/2, T40/2, T40/1 and TA/2) in three chambers, as a detector of electronic

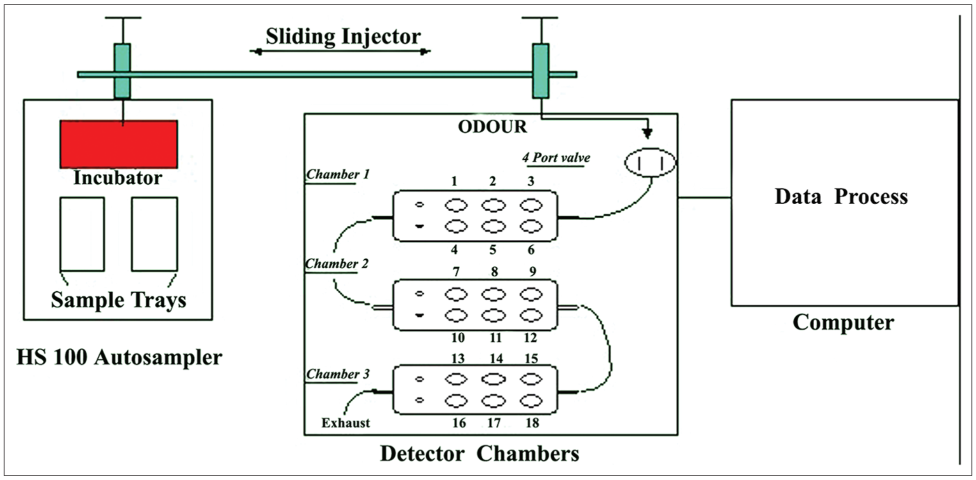

Figure 1: Electronic nose system includes auto sampler, injector, detector, and computer

nose. Samples were first put in headspace vials which were incubated at 40°C for 10 min to generate volatilization of odor components in headspace. Then 1ml was collected from the headspace of samples and injected into the electronic nose system by the auto-sampler. Odor components of samples will react with metal oxide sensors and change the resistances of sensors, only the maximum resistance change of each sensor is used for analysis.[12] The delay between two injections was 2 min, the carrier gas flushed over the sensors to their initial phase before the next injection.

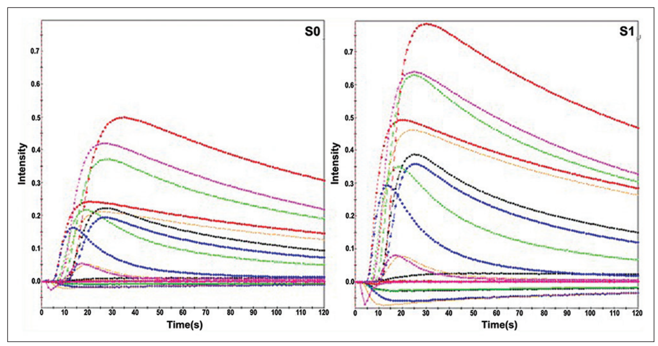

Figure 2: Original signals of naturally dried *Pinelliae Rhizoma* (S0) and the least fuming degree *Pinelliae Rhizoma* (S1) generated by sensors

Data analysis

Raw data acquired from the electronic nose system was analyzed using multivariate statistical methods such as PCA, DFA and PLS. All these data analysis procedures were completed by applying Alpha Soft software. PCA transforms the numerous original variables to fewer new variables, principal components are linear combinations of the original variables.[22] PCA shows inherent relationships and discriminations between samples as non-supervised analyses. DFA was applied to identify an unknown sample as supervised analyses. To predict fuming degree of samples, a calibration curve is developed by PLS mode, the correlation between electronic nose measurement and actual fuming time was evaluated.

Analytical conditions

Analytical conditions for electronic nose were: Carrier gas, synthetic dry air, flow rate (ml/min): 150, quantity of sample in the vial (g): 1.5, total volume of the vial (ml): 10, headspace generation time (min): 10, headspace generation temperature (°C): 40, agitation speed (rpm): 500, injected volume (ml): 1, injection speed (ml/s): 1, syringe temperature (°C): 50, acquisition time (min): 2, time between injections (min): 10.

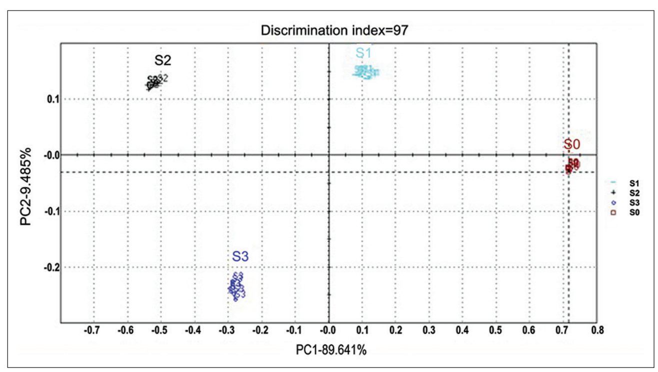

Figure 3: principal components analysis analysis of different fuming degrees *Pinelliae Rhizoma* (S1, S2, S3) and naturally dried *Pinelliae Rhizoma (S0)*

RESULTS AND DISCUSSION

Discrimination between sulfur fumed *Pinelliae Rhizoma* and naturally dried *Pinelliae Rhizoma*

Sulfur fumed *Pinelliae Rhizoma* (S1-S3) and naturally dried *Pinelliae Rhizoma* (S0) was analyzed. Figure 2 shows the original signals of S0 and S1 samples generated by sensors. There are obvious differences between naturally dried samples and the least fuming degree samples. PCA performed on the maximum resistance change of each sensor indicates that each of the four samples (S1, S2, S3 and S0) is discriminated from the others [Figure 3]. The samples located in the PCA diagram according to a clockwise direction with the increase of fuming degree. Two principle components are 89.641 and 9.485%. The discrimination index is 97 implying that a very high degree of discrimination was achieved. A successful discrimination model should have an index between 80 and 100.[12]

A high degree of discrimination index was achieved probably because the metal oxide sensor has a high sensitivity for sulfur and sulfides. In a word, the electronic nose has the capability of discriminating between sulfur fumed *Pinelliae Rhizoma* and naturally dried *Pinelliae Rhizoma* and also has the ability of identifying different fuming degrees samples.

Identification of unknown samples

S1, S2, S3 and S0 were analyzed as qualitative analysis training standard samples to build a model employing DFA. These training samples were divided into 2groups which were sulfur fumed group (S1-S3) and naturally dried group (S0). This model was built to identify whether *Pinelliae Rhizoma* was fumed with sulfur. Unknown samples would be identified by this model [Figure 4] and the recognition values were calculated. The results are shown in Table 1. Unknown 1-3, which were fumed with sulfur for different time periods, were correctly identified as sulfur fumed group. Unknown 4 which was naturally dried sample were correctly identified as naturally dried group. Unknown 5-10, which were the sulfur fumed samples purchased from herbal medicine markets were correctly identified as sulfur fumed group.

All these unknown samples obtained an acceptable recognition value which should be higher than 90. Unknown 1-3 were analyzed immediately after prepared while unknown 5-10 which purchased from herbal medicine markets had been stored for some time after the fuming procedures. The odors of the stored samples might be reduced, suggesting that the electronic nose also has the ability to identify a stored fumed sample. *Pinelliae Rhizoma* has some different planting bases in various regions. These regions have different growing environment for *Pinelliae Rhizoma* so the fresh samples from different regions may have different odors, so do sulfur fumed samples. It would be interesting to learn the discrimination among sulfur fumed samples from different regions. A statistical model is built using samples from different regions should have a better ability to identify whether *Pinelliae Rhizoma* was fumed with sulfur. This electronic nose system may be used to identify other sulfur fumed Chinese herbs to ensure medication safety. All in all, the electronic nose has promising abilities of discrimination and identification. It is able to identify the sulfur fumed *Pinelliae Rhizoma* simply and rapidly. Further more, it is possible that monitoring sulfur and sulfides using electronic nose in other fields such as environment analysis,[23] brewery industry and food safety control.

Quantitative analysis of fuming degree

To predict the fuming degree of sulfur fumed *Pinelliae Rhizoma*, S0, S1, S2 and S3 which had been fumed with sulfur for different hours were analyzed as quantitative training standards. A calibration curve was generated from these standards by using a PLS model, as shown in Figure 5.

Table 1: The results of identification of unknown samples

Sample name	Recognition group	Recognized	Recognition value (%)
Unknown 1	Sulfur fumed	Yes	100.0
Unknown 2	Sulfur fumed	Yes	100.0
Unknown 3	Sulfur fumed	Yes	100.0
Unknown 4	Naturally dried	Yes	100.0
Unknown 5	Sulfur fumed	Yes	98.9
Unknown 6	Sulfur fumed	Yes	97.2
Unknown 7	Sulfur fumed	Yes	97.6
Unknown 8	Sulfur fumed	Yes	96.8
Unknown 9	Sulfur fumed	Yes	97.0
Unknown 10	Sulfur fumed	Yes	97.1

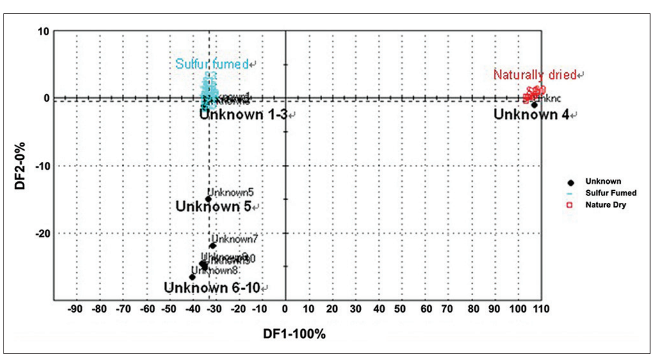

Figure 4: Unknown samples (Unknown 1-10) identification usingdiscriminant function analysis

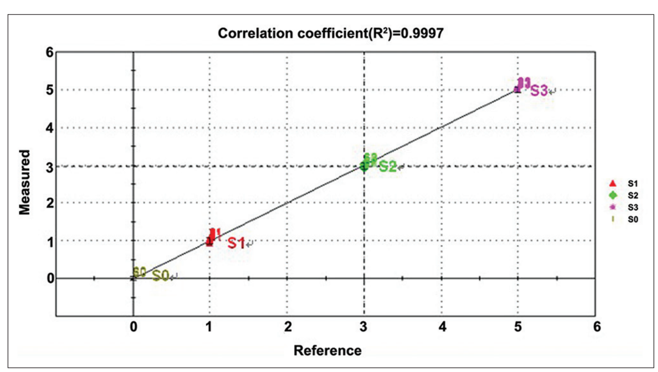

Figure 5: Calibration curve for quantitative analysis of fuming degree of sulfur fumed *Pinelliae Rhizoma*

Table 2: Fuming degree prediction of unknown 5-10

Sample name	Index	Measured (h)	Mean (h)
Unknown 5	1	2.17	1.89
Unknown 6	2	1.9	
Unknown 7	3	1.9	
Unknown 8	4	1.87	
Unknown 9	5	1.74	
Unknown 10	6	1.77	

The *X*-axis means the actual fuming time of samples, the *Y*-axis means the prediction fuming time of samples by PLS model. A good fit between the fuming time predicted by the PLS model and the actual fuming time of the samples was obtained. The correlation between actual values and prediction value is 0.9997, higher than 0.9. It implies that this calibration curve could be applied to predict fuming degree of sulfur fumed *Pinelliae Rhizoma*.

Unknown samples (Unknown 5-10) were analyzed by this model. The results are shown in Table 2. The mean fuming time of unknown samples which purchased from herbal medicine markets measured by electronic nose is 1.89h. It may not be the actual fuming time of these samples, because these samples have been stored for some time before analyzed. The measured values probably indicate the odors of unknown samples are similar to that of the *Pinelliae Rhizoma* fumed for 1.89h. To the stored fuming samples, the fuming time measured by the electronic nose should be a quantitative index of unpleasant odor produced by sulfur and sulfides. Overall, the electronic nose for quantitative analysis of fuming degree has been proved. It could be the support method for conventional odorant analytical methods.

CONCLUSION

We have demonstrated that the electronic nose has the qualitative capability of discriminating between sulfur fumed *Pinelliae Rhizoma* and naturally dried *Pinelliae Rhizoma* and also has the abilities of differentiating the samples with different fuming degrees. Using the electronic nose to identify whether *Pinelliae Rhizoma* was fumed with sulfur also has been proved in this paper. Quantitative analysis of sulfur fumed samples using electronic nose has been presented. The electronic nose can predict the fuming degree of *Pinelliae Rhizoma*. Sulfur fumed *Pinelliae Rhizoma* is very common in herbal medicine markets which is harmful to human body and difficult to be assessed using conventional analytical tools, while the electronic nose can identify the sulfur fumed *Pinelliae Rhizoma* rapidly and simply. It has shown that this electronic nose based metal oxide sensor has a high degree of sensitivity to sulfur and sulfides, this system may be used to identify others sulfur fumed Chinese herbs to ensure medication safety. It provides us a new quality control method of Chinese herbs from odor aspect. Using an electronic nose to monitor residual sulfur and sulfides rapidly is also promising.

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