

Correlation studies between electronic nose response and headspace volatiles of *Tongkat Ali* extracts

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Introduction

Herbal medicines are special due to their nutraceutical and medicinal values, making quality control of raw herbs and their products essential to ensure consistency in quality, safety and efficacy. Traditionally odour analysis and food regulation involve gas chromatograph-mass spectrometry (GC-MS) and human taste panels which can give detailed information about the contents of the odour. GC-MS can separate, identify and quantify individual volatile chemicals, but difficult to correlate the data with sensory evaluation. Since odours are complex mixtures of many volatiles, the technique is highly impractical. Human sensory evaluation is useful but expensive, time consuming, subjective, as well as biased from illness and other factors.

As an alternative to both, electronic nose is gaining popularity in odour analysis which is essentially an instrument to mimic the human sense of smell and consists of chemical imaging and multiparameter sensing systems. The sensing system can be a single sensor device or an array of sensors providing "fingerprints" that are characteristics of a particular odour. The advantages of which are rapid, real-time detection of volatiles, lower cost and easy automation.

Medicinal plant formulations may consist of hundreds of phytochemical and is very difficult to identify most of these components by usual methods. Generally, only a few pharmacologically active components are employed for evaluating their quality and authenticity. The volatile components contained in extracts are normally lower than the detection limits of the detector. Thus some form of pre-concentration becomes mandatory. Among these, simultaneous distillation-extraction

(SDE), dynamic headspace analysis or purge and trap methods using porous polymers and solid-phase micro-extractions (SPME) are the most popular methods in aroma analysis. *Eurycoma longifolia* Jack, commonly known as Tongkat Ali, belong to the Simaroubaceae family and grows wild in Southeast Asia. The plant contains a series of quassinoids, e.g., eurycomanone, 14,15-dihydroxyklaineaneone, eurycomanol and eurycomalactone; alkanoids, e.g., 9-methoxycanthin-6-one and together with others such as 13,21-dihydroeurycomanone, 13,21-epoxyeurycomanone. We have developed a smell sensor using gas chromatographic stationary phases and lipid materials to mimic the olfactory system. In the present study, Tongkat Ali extracts are analysed with GC-MS using solid-phase micro-extraction sampling technique and correlated 12 identified compounds with a QCM array sensor.

Experiment

Sample

Twelve Tongkat Ali Jack samples were extracted using water and methanol and either freeze or spray dried. Some samples were commercial samples obtained from various manufacturers.

Electronic nose - Instrumentation

The frequency changes of the sensors were monitored by a Universal Sensor Array System (QTS-3) connected to a personal computer for data acquisition and processing.

Electronic nose - QCM array sensors

The array sensor consisted of eight AT-cut quartz crystals with gold electrodes on both sides. Eight different types of gas chromatographic stationary phases and lipid materials were used to fabricate the array sensor. The sensing materials

consisted of polar, non-polar and amphiphilic materials. The sensing materials were coated on both sides of the quartz crystal either using simple ultrasonic spray coating device utilizing an ultrasonic atomizer. The coated crystals produced a relative standard deviation of less than 2 in terms of frequency changes. 100mg samples were taken in 20 ml vials and heated to 60°C for 15 min. The headspace vapours were funneled into the sensor chamber through a dried silica gel column, as shown in Fig. 1. The sequence of the sample injection was: baseline 60s, sample injection time 10s and recovery time 200s.

The response of the sensor was considered as the difference between the baseline and their maximum frequency change when samples were injected. Initially, compressed air was passed over the sensor array via valve 1 (valve 2 inactive) until a stable baseline was achieved (~ 1 min). Next the headspace volatiles in the vials were flowed over the sensor array by activating valve 2 and circulated for 1 min before the signals were recorded. The sensors were cleaned by compressed air for 3 min. A silica column was used to remove traces of moisture. Each sample was measured six times and the average was analysed. The relative standard deviations of the responses were less than 2.

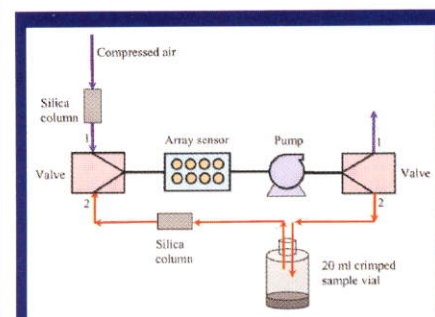


Fig.1 : Schematic diagram of the headspace sampling in quartz crystal array sensor.

GC-MS analysis

Samples were taken in 20 ml vials and heated at 60°C for 15 min. The headspace volatiles were adsorbed on Supelco SPME fiber No. 57318 for 15 min. The use of PDMS/wax with carbon sorbent led to more effective trapping of volatile compounds. The SPME fiber was inserted to the GC-MS injection port and held for 5 min to ensure that the adsorbed components were effectively desorbed. The trace GC and MS unit was used while the column was a fused silica capillary column. The injection temperature was 210°C. The initial oven temperature was 50°C and was held for 5 min, then raised to a final 210°C at 5°C/min and held for 5 min. He gas was used with a 1 ml/min flow rate. The GC-MS was operated with an ionization voltage of 70eV and an ion source temperature of 180°C. Compounds were identified by matching their mass spectra with those in the Wiley Library, NIST Library and also on retention indices. The relationship between QCM array sensor response and headspace volatile compounds from GC-MS was carried out by using multiple factorial analyses.

Results and discussion

QCM

GC materials used in the sensor fabrication were of different polarities. Lipids were of amphiphilic character due to the hydrophilic head groups and hydrophobic tail. The electronic nose gives an overall response to the complex mixture and not just a single component. The sensors are not compound specific but rather to chemical classes.

The volatile vapours are reversibly adsorbed when in contact with the sensing layer causing a temporary but reversible "detuning" of the quartz resonator, resulting in vibrational frequency variation. Although the aromatic compounds were of very low concentration, the array sensor responds to overall content and gives the characteristic chemical fingerprint of the headspace volatile vapor. Principal component analysis was carried out on the sensor array data in order to find out the classification ability of the array sensor. The data were usually preprocessed before analysis.

The principal component analysis showed that 99.24% of the total variance was obtained by five principal components (81.365 + 10.670 + 3.709 + 1.880 + 1.613). The plot of first and second principal component was able to classify freeze and spray dried extracts, as seen in fig.2.

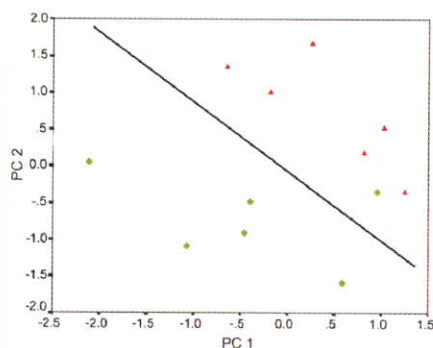


Fig. 2 : PC1 and PC2 plot of QCM analysis of spray and freeze dried extracts: (◆) spray dried and (▲) freeze dried.

GC-MS

More than 65 different volatiles have been reported to be present in the Tongkat Ali extracts. A GC-MS profile of the headspace vapor of *E. longifolia* extracts using solid-phase micro-extraction method is shown in Fig. 3. A total of 133 volatiles were found. Freeze dried extracts contained a maximum of 83 volatiles whereas spray dried contained maximum of 28 volatile compounds. Correlation analysis was based on the quantification of peak area of the volatiles. A total of eight major volatiles that are common in all the samples were used as the predicted variables. Principal component analysis was applied to the peak area of the eight major components. The eigenvalue of the PC1, PC2, PC3, PC4 and PC5 were 52.0, 23.3, 12.59, 9.47 and 1.5, respectively. This classification indicates that in spray and freeze dried extracts the compounds are of different composition. The correlation between QCM array sensors and eight identified compounds using GC-MS was done using varimax rotated principal component analysis. The identified compounds, 3-phenoxy-1-propanol, 2-phenoxy-ethanol and benzoic acid, have high loading values on PC1, while curcumene and 4-ethynyl-4-hydroxy-3,5,5-trimethyl-2-cyclohex-1-enone have high loading on PC2. Acetic acid and octanoic acid have high loading on PC3 and (R)-(-)-massoilactone has high loading on PC4. Sensors APZ-L,

PEG-4000, TOMA and OAm have high loading on PC1, while DOP has high loading on PC2, and EC was found to have high loading on PC3, respectively.

This result indicates that APZ-L, PEG-4000, TOMA and OAm sensors are more sensitive to acetic acid and octanoic acid while the DOP sensor is more sensitive to (R)-(-)-massoilactone and the ethyl cellulose sensor responds to (R)-(-)-massoilactone.

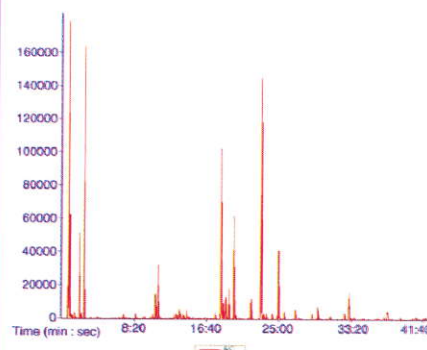


Fig. 3 : SPME-GC-MS profiles of *Eurycoma longifolia* extracts. TA Sabah acetone freeze dried.

Conclusion

The Tongkat Ali extracts were classified based on the eight common volatiles identified by SPME coupled with GC-MS. The headspace volatiles have been successfully used to classify either freeze or spray dried extracts. Correlations found between the headspace volatiles and the quartz crystal sensors for the classification of plant extracts, indicates sensitivity of the technique. Although individual compounds could be used to indicate the sensitivity of the sensors, whole headspace volatiles that are usually present in very low concentrations (ppm or ppb level) are used. Thus, the ability of the electronic nose to detect small changes of the volatiles (smell) offers an interesting approach to evaluate herbal extracts. The method could be useful for the rapid evaluation of herbal volatiles.

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