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Original Research Article

A novel strategy for rapid identification of the fruits of *Illicium verum* and *Illicium anisatum* using electronic nose and tongue technology

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Abstract

Purpose: *To develop an effective and rapid strategy for the identification of fruits of I. verum and I. anisatum based on their odor and taste.*
Methods: *Electronic nose (E-nose) and electronic tongue (E-tongue) technology was used to identify the fruits of I. verum (FIV) and I. anisatum (FIA). Samples of FIA, FIV, and FIA : FIV mixtures in different proportions (1 : 3, 1 : 1, and 3 : 1) were prepared to evaluate the identification abilities of E-nose and E-tongue methods. Samples were powdered and sifted through a standard sieve (aperture size 355 ± 13 μm) for E-nose analysis. Each sample was refluxed with water for 1 h before E-tongue analysis. The acquired data were analyzed by principal component analysis (PCA) and discriminant factor analysis (DFA).*
Results: *Based on the signals acquired by E-nose and E-tongue analyses, a total of 90 data points each were used for PCA. The three principal component values for E-nose analysis were PC1 = 93.89 %, PC2 = 6.08 %, and PC3 = 0.03 %, and those for E-tongue analysis were PC1 = 98.72 %, PC2 = 0.68 %, and PC3 = 0.57 %. The sample data were significantly divided into two groups representing FIV and FIA. Furthermore, E-nose and E-tongue assessments combined with PCA and DFA analyses effectively identified FIV, FIA and their mixtures.*
Conclusion: *The use of E-nose and E-tongue technology is an effective and rapid strategy to identify the fruits of I. verum and I. anisatum and their mixtures. This strategy may also offer an effective method for detection of adulterants.*

Keywords: *Illicium verum, Illicium anisatum, Discrimination, Electronic nose, Electronic tongue, Safety, Principal component analysis, Discriminant factor analysis*

INTRODUCTION

Illicium verum Hook (*I. verum*) is an evergreen tree that belongs to the Illiciaceae family and is mainly distributed in southern China and Vietnam [1,2]. The fruit of *I. verum*, commonly known as star anise, is an important aromatic according to the theory of traditional Chinese Medicine (TCM)

[3,4]. Star anise is recorded in TCM as an agent to alleviate pain and is commonly used to treat abdominal pain, hernia pain, back pain and vomiting, [4,5]. Moreover, star anise is widely used as one of the most popular spices and main component of famous condiments in China [4,6,7]. However, in recent years, increasing reports have indicated that the fruit of *Illicium anisatum* (*I. anisatum*), which is highly toxic, is used as an adulterant in star anise [8,9].

Star anise fruit is characterized by an outline with eight follicles and has a special odor and taste. In contrast, the fruit of *I. anisatum* commonly possesses more than eight follicles and has a much weaker odor and taste [1-4,10]. These differences have afforded experienced pharmacists the ability to identify and discriminate between the two fruits. However, star anise is often prepared as a powder, resulting in substantial difficulty in discriminating between the two substances on the basis of aroma. Analytical methods used to address this problem (e.g., gas chromatography–mass spectroscopy, high-performance liquid chromatography–mass spectroscopy, and thin-layer chromatography) are costly with regard to time and require large amounts of material standards [4,9,11]. These drawbacks prompted us to investigate an effective and rapid strategy for discriminating between the fruits of *I. verum* and *I. anisatum* based on their odor and taste.

EXPERIMENTAL

Plant materials

The fruits of *I. verum* (star anise, FIV) and *I. anisatum* (FIA) used in this study were purchased from Chengdu Lotus Pond market (Chengdu, China) and identified by Professor Chun-Jie Wu (College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China). The voucher specimens of fruits of *I. verum* (no. S-20151013-1) and *I. anisatum* (no. S-20151013-2) were deposited in the herbarium of College of Pharmacy, Chengdu University of Traditional Chinese Medicine (Chengdu, China).

Electronic nose

The FOX- 4000 E-nose system (Alpha M.O.S., France) with pattern recognition software (Alpha M.O.S., Version 2012.45) was used to investigate the odor of the fruits of *I. verum* and *I. anisatum* according to a previously reported method [12]. Eighteen metal oxide semiconductors (MOS) were used in the sensor array in this study (Table **1**). Test samples were powdered and sifted through standard 50 mesh (internal diameter 355 ± 13 μm). Subsequently, 0.1 g samples were accurately weighed and placed in a 20 mL sealed headspace vial that was then loaded into the auto sampler tray of the E-nose. Dry air was pumped into the sensor chambers at a constant rate of 150 mL/min, and 2 mL of headspace air was automatically injected into the E-nose system. The injection rate was 2 mL/s, the incubation temperature was 50°C, the incubation time was 1080 s, and the time interval between different injections was 600 s.

Electronic tongue

Following the method introduced by Yang *et al* [13], an αAstree E-tongue system (Alpha M.O.S., France) with Astree II software (Alpha M.O.S, Version 2012.45) was used to investigate the taste of the fruits of *I. verum* and *I. anisatum*. In this investigation, the sensor array consisted of seven cross-selective potentiometric sensors (ZZ, AB, GA, BB, CA, DA, and JE). The powdered test samples (2.0 g) and 80 mL distilled water were placed into a stoppered conical flask and refluxed for 1 h. Filtrates were diluted to 200 mL. The prepared solutions (80 mL) were placed into the auto-sampler tray of the E-tongue apparatus for analysis. Each sample was analyzed for 120 s, and all samples were analyzed three times, Data were recorded using the Astree II software.

Statistical analysis

All data were analyzed using Alpha M.O.S. statistical software. Principal component analysis (PCA) and discriminant factor analysis (DFA) were used for discrimination analysis of the samples.

RESULTS

Typical E-nose and E-tongue sensor responses and repeatability based on E-nose and E-tongue methods

The typical recorded sensor responses of the samples and the maximum responses collected as the output values are shown in Figure 1. Data for the triplicate sample analyses that were recorded by the pattern recognition software showed good repeatability and are shown in Table 1.

Typical sensor responses recorded with the E-tongue apparatus are shown in Figure 2. An average value was acquired based on the stable sensor responses of E-tongue analyses from 100 to 120 s and was used as the output data. The repeatability of the E-tongue results using this method of study is shown in Table 2.

Table 1: Repeatability based on E-nose method (n = 6)

MOS	RSD (%)	MOS	RSD (%)
LY2/LG	1.023	P40/1	0.356
LY2/G	1.255	T70/2	0.166
LY2/AA	0.052	PA/2	2.092
LY2/GH	0.451	P30/1	3.145
LY2/gCTL	0.662	P40/2	0.978
LY2/gCT	0.458	P30/2	0.838
T30/1	0.144	T40/2	1.7477
P10/1	1.125	T40/1	2.052
P10/2	1.522	TA/2	0.822

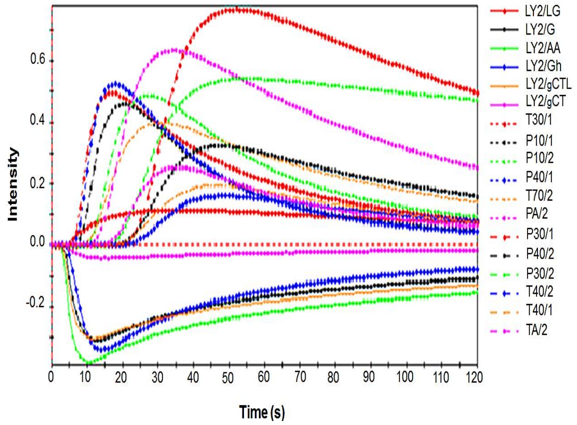

Figure 1: Typical sensor responses of E-nose during the measurement

Table 2: Repeatability based on E-tongue detection method (n = 6)

Sensor	ZZ	AB	GA	BB	CA	DA	JE
RSD (%)	0.25	0.32	1.55	0.65	0.88	0.29	0.52

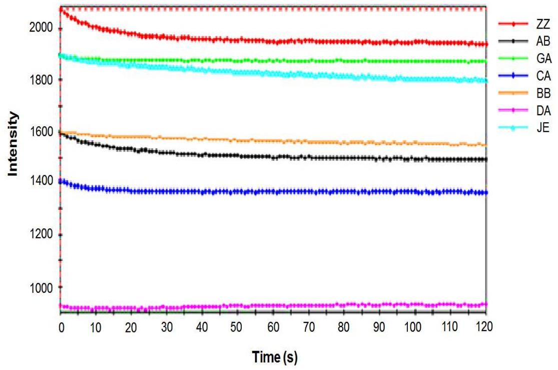

Figure 2: Typical sensor responses of E-tongue during the measurement.

E-nose and E-tongue for FIA and FIV

According to the signals acquired by E-nose measurements, a total of 90 data points were used for PCA. Three-dimensional scores plots from the E-nose PCA results are shown in Figure 3A. The three principal components are PC1 = 93.89 %, PC2 = 6.08 %, and PC3 = 0.03 %. The sample data were also divided into two groups that represent FIV and FIA, indicating E-nose assessment combined with PCA effectively discriminated between FIV and FIA. Similar to the E-nose measurements, a total of 90 data points obtained by E-tongue measurements were also used for PCA. Three-dimensional scores plots from the E-tongue PCA results are presented in Figure 3B. The three principal components were PC1 = 98.72%, PC2 = 0.68%, and PC3 = 0.57%. These results suggest that E-tongue measurement combined with PCA effectively discriminated between FIV and FIA.

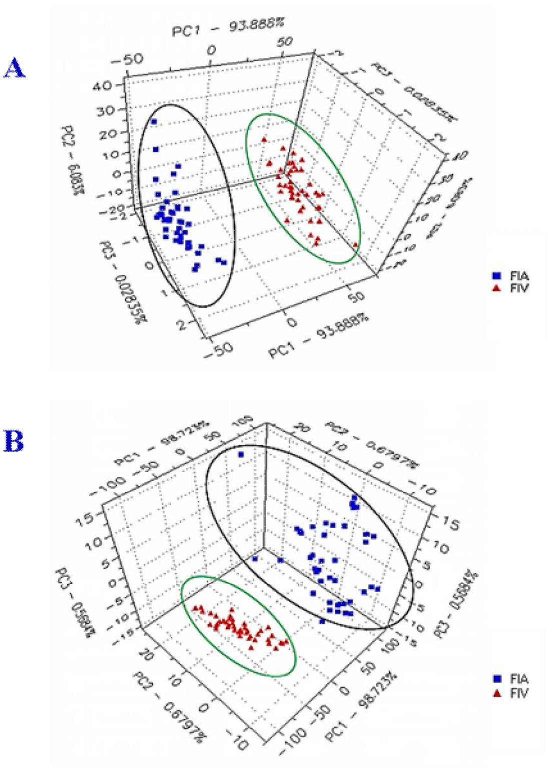

Figure 3: PCA score plots of E-nose and E-tongue measurements of FIA (n =15) and FIV (n = 15). Each sample was measured and analyzed three times.

E-nose and E-tongue data for FIA and FIV

Five mixture samples, including FIA, FIA:FIV (1 : 3), FIA : FIV (1 : 1), FIA : FIV (3 : 1), and FIV, were prepared to evaluate the ability of E-nose and E-tongue technology to identify FIA, FIV, and their mixtures. Next, five samples were analyzed by E-nose and E-tongue (each sample was repeated three times), and a total of 75 data points were acquired. As shown in Figure **4A**, the three principal components detected by E-nose are PC1 = 88.29%, PC2 = 11.65%, and PC3 =

0.053%. Results showed that the E-nose method effectively identified FIV, FIA, and their mixtures. The PCA scores plots of E-tongue are shown in Figure 4B. Like the E-nose method, the E-tongue method also effectively identified the FIV, FIA and their mixtures (PC1 = 97.17 %, PC2 = 2.53 % and PC3 = 0.29 %).

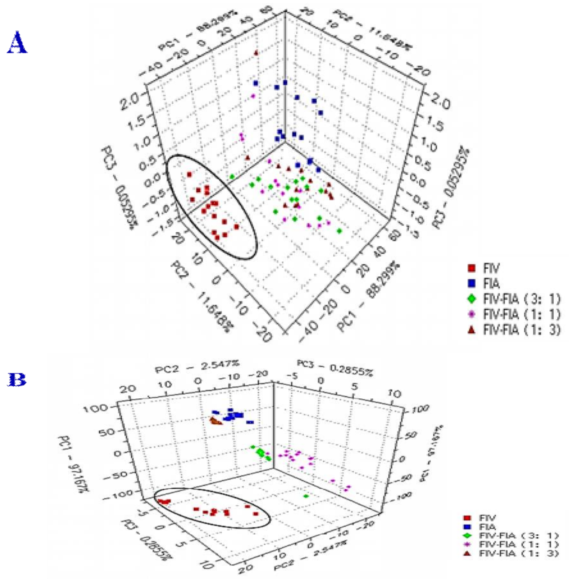

Figure 4: PCA scores plots of E-nose and E-tongue for discriminating mixtures of FIA and FIV. There were 5 samples for five mixtures [FIA, FIA : FIV (1 : 3), FIA : FIV (1 : 1), FIA : FIV (3 : 1), FIV], and each sample was repeated for three times

The DFA model, another classic method, was used in addition to the PCA model to analyze the data acquired by E-nose and E-tongue measurements in this study. There were five groups in the DFA analyses; namely, FIA, FIA : FIV (1 : 3), FIA : FIV (1 : 1), FIA : FIV (3 : 1) and FIV. Each group consisted of five samples. All the samples were analyzed three times, and a total of 75 data points were acquired. Of the total data points, 60 were selected randomly to establish the DFA model (12 data points per group), and the other 15 (three data points per group) were used for a confirmatory experiment. As can be seen in Figure 5A, the two principal components for the E-nose analysis are DF1 = 87.195 % and DF2 = 11.447 %, and the discriminating rate for the unknown sample is 70.8 %. The two principal components for the E-tongue method were calculated as DF1 = 65.41 % and DF2 = 29.56 % (Figure 5B), and the discriminating rate for the unknown sample was calculated as 98.7 %.

The present results indicate that E-nose and E-tongue measurements, combined with PCA and DFA analysis, can be used to identify FIV, FIA and their mixtures.

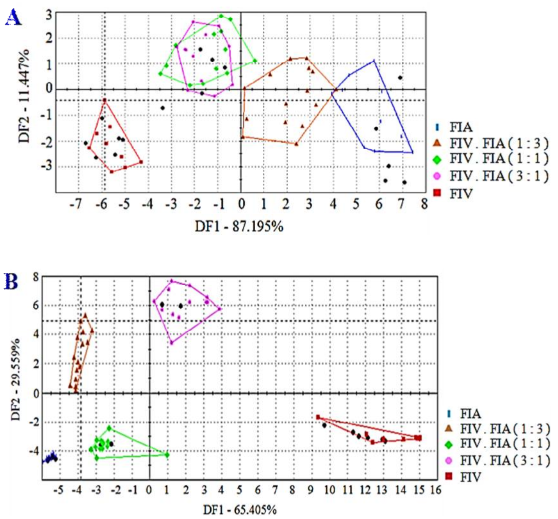

Figure 5: DFA score plots of E-nose and E-tongue for discriminating mixtures of FIA and FIV. There were five samples for five mixtures [FIA, FIA: FIV (1 : 3), FIA : FIV (1 : 1), FIA : FIV (3 : 1), FIV], and each sample was repeated three times

DISCUSSION

Food and medicine safety has always been a primary concern, so finding effective, rapid, and simple methods for ensuring the safety of food and medicine is always necessary [14,15]. To the best of our knowledge, star anise (*I. verum*) has no toxicity, but *I. anisatum* possesses high toxicity. These two fruits also differ distinctively with regard to odor and taste. These distinguishing parameters might prove to be two key points with which to rapidly and effectively differentiate the fruits of *I. verum* and *I. anisatum*.

New intelligent sensory technologies (IST), like E-nose and E-tongue systems that are able to imitate human smell and taste senses effectively and objectively, may present a feasible way to objectively discriminate between *I. verum* and *I. anisatum* fruits [12]. The E-nose is an intelligent apparatus and can be used to detect complex odors by an array of metal oxide sensors made by various odor-sensitive materials [16]. Recently, increasing numbers of research studies have demonstrated that E-nose is an effective and rapid way to discriminate foods, TCM materials, and agricultural products [16-18]. Similar to E-nose, E-tongue is another intelligent machine system designed to detect complex taste sensations [19]. Previous reports have revealed that the E-tongue possesses sensitivity capability for analyzing and discriminating between various liquid agents, such as wine, honey and tea, and plant or herbal extracts [12,13,18]. PCA is a popular multivariate statistical method used to reduce data dimensionality with minimal information loss. DFA is another effective tool for analyzing complex data based on supervised classification [13,20]. Previous research has demonstrated that these two statistical techniques could be effectively used to analyze data obtained by E-nose and E-tongue techniques.

CONCLUSION

The results of this study indicate the successful development of a novel strategy for rapid and effective differentiation of FIV and FIA based E-nose and E-tongue measurements. These ISTs may also offer an effective method for detection of adulterants.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Liang Li and Chun-Jie Wu conceived and designed the study. Mei-bian Hu and Zhi-Qiang Wei collected and analyzed the data. Jiao-Long Wang and Yu-Jie Liu wrote the manuscript. Jiao-Long Wang and Wen-Xiang Fan did the detail experiments. All authors have read and approved the manuscript for publication.

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