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Short communication

Rapid assessment of quality of deer antler slices by using an electronic nose coupled with chemometric analysis

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ABSTRACT

Deer antler is a precious animal-sourced traditional Chinese medicine. We aimed to rapidly assess the quality of deer antler slices by electronic nose so that we can ensure medical safety. In this study, response intensity of the electronic nose was favorably optimized, and samples were well assessed by using an electronic nose based on LDA model. The results obtained herein suggested that electronic nose could be an effective method to rapidly assess the quality of deer antler slices, and could also be an important tool for categorization of complex aroma mixtures for the control of quality of drugs or food.

Introduction

Deer antler (Lu-rong in China) is a precious animal-sourced traditional Chinese medicine. Several studies have proven its functions of anti-bone resorption, anti-arthritis and promoting chondrocyte proliferation (Kim et al., 2005; Lin et al., 2011; Wu et al., 2013). Generally, deer antlers are always cut into slices as end products, which have four presentations according to the difference of quality, including wax slices, powder slices, sand slices and bone slices. It is known that deer antler slices of different quality have diverse active ingredients and efficacies. Wax slices, from top of deer antlers, are of higher quality than powder slices, sand slices and bone slices. Bone slices, from partially ossific part of deer antlers, are of low quality (Tseng et al., 2014). However, they are always easily mixed together because of their similar appearance, which may lead to medical and safety problems. Thus, it is necessary to provide an effective method for assessment of the quality of deer antler slices so that we can ensure safety.

Traditionally, the quality assessment of deer antlers was mainly based on the experts' senses (Chen et al., 1999; Ye, 1986). Traditional methods are prone to lack objectivity and accuracy, especially those in powder forms. Molecular identification methods had successfully discriminated deer antlers of different sources (Fajardo et al., 2006; Haynes and Latch, 2012; Jeong et al., 2007; Kim et al., 2012; Zhang et al., 2011; Zhao et al., 2010), but they are not able to identify deer antlers of different quality. In contrast, chemical methods, such as chromatography and spectrometry, can evaluate the chemical constituents of antlers (Yan et al., 2009), which are able to assess deer antlers of different quality. However, they are time-consuming and pose a high cost.

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The electronic nose, which can detect odorous volatile components, is an artificial olfactory system. It has been applied in the field of agriculture, food and environmental protection (Pacioni et al., 2014; Wilson, 2013). In comparison to other methods, the electronic nose is more simple and rapid. It can rapidly detect smells of samples, transfer them into digital signals and directly analyze them by attached software's so that we can intuitively and rapidly assess the results. It has also been investigated the great richness of odorous proteins and lipids in deer antlers (Wu et al., 2013). Therefore, using an electronic nose is suitable for discriminating different deer antler slices. Here, we first tried to use an electronic nose to assess the quality of deer antler slices. Moreover, multiple data analysis models were tested to get optimized analytical method. This study may expand the application of the electronic nose into the field of Chinese medicine and allow experts to rapidly assess the quality of odorous animal-sourced traditional Chinese medicines and other drugs or foods.

Table 1
The components and main applications of the sensors of the FOX-3000 electronic nose.

Sensor markers	Name	Detection of Chemical components
S1	LY2/LG	Oxidizing gas
S2	LY2/G	Ammonia, carbon monoxide
S3	LY2/AA	Ethanol
S4	LY2/GH	Ammonia/organic amine
S5	LY2/gCTL	Hydrogen sulfide
S6	LY2/gCT	Propane/butane
S7	T30/1	Organic solvents
S8	P10/1	Hydrocarbons
S9	P10/2	Methane
S10	P10/2	Fluorine
S11	T70/2	Aromatic compounds
S12	PA/2	Ethanol, ammonia/organic amine

Material and methods

Samples

Deer antlers in four presentations, including wax slice, powder slice, sand slice and bone slice were obtained from Anguo, Hebei, China. They were species of *Cornu Cervi Pantotrichum* described in the Chinese Pharmacopoeia 2010. All the samples were deposited into the specimen room of Beijing University of Chinese Medicine.

Electronic nose

The Alpha M.O.S FOX 3000 with 12 metal oxide sensors consisted of a sampling apparatus, array of sensors, a HS-100 auto sampler, an air generator equipment and software (Alpha Soft V11) for data recording. The sensor array was composed of 12 metal oxide sensors divided into three chambers: T, P and LY (Table 1).

Experimental procedures

The samples were accurately weighed to 0.6 g and were then placed in 10 ml headspace vials. The headspace time and temperature were 500 s and 70°C, respectively. The carrier gas was air with a flow rate of 150 ml/min. The injection volume was 2500 µl, the injection rate 1500 µl/s and the stirring rate 250 rpm. The acquisition time and the time between injections were 200 s and 400 s, respectively. The response value of each of the 12 sensors for every sample was recorded, and response curves were generated (Lin et al., 2013).

Statistical processing

Two response-feature values of the 12 sensors were recorded, including the first-feature values and the second-feature values. The first-feature values represented the 12 maximum response values of 12 sensors. 120 response values at 10 time points (5s, 10s, 15s, 20s, 25s, 30s, 35s, 40s, 45s and 50s) of 12 sensors were taken as the second-feature values. The data sets of the samples were analyzed using SPSS 19.0 based on cluster analysis (CA), principal component analysis (PCA), linear discriminate analysis (LDA) and artificial neural network (ANN) of radial basis function (Lin et al., 2013).

Results and discussion

Optimization of experimental conditions

Previous studies had not been reported regarding the application of an electronic nose in animal-sourced materials in traditional Chinese medicine. Therefore, we optimized the experimental conditions of electronic nose to get better response values. In this study, four factors, including sample quantity (A), injection volume (B), headspace time (C) and headspace temperature (D), were used to optimize the response intensity in an orthogonal assay (Table 2). The results showed that the most effective factor was Factor D. The next important factor was Factor B, followed by Factor A and C. The optimized conditions were D3B3A3C2, described in Section 2.

Fig. 1 shows the typical sensor responses for samples of deer antler slices in the four presentations. The curves represent the intensity of each sensor against time, when the volatiles reach the measurement chamber. It indicated that the sensors exported richness of useful information. The maximum response values for each sensor were between 0.3 and 0.9 and the best periods of time were from 10 to 20 s. The result suggested that the detection conditions of the electronic nose were favorably optimized.

Discrimination of deer antler slices of different quality by electronic nose

Chemometric analysis is an important part in terms of the application of electronic noses. In this study, four models, including cluster analysis (CA), principal component analysis

Table 2
Orthogonal design table L_9 (3^4).

Level	Factors			
	Sample quantity (A)	Injection volume (B)	Headspace time (C)	Headspace temperature (D)
1	0. 2 g	1500 µl	300 s	50°C
2	0. 4 g	2000 µl	500 s	60°C
3	0. 6 g	2500 µl	700 s	70°C

(PCA), linear discriminated analysis (LDA) and artificial neural network (ANN) models were used to test the applicability of quality assessment of antler slices. CA and PCA models based on first-feature values or second-feature values were successfully built using SPSS software in this study. However, both of them failed to discriminate antler slices of different quality (data not shown). This indicated that CA and PCA models were not suitable for discrimination of different antler slices.

ANN model was also performed in this study (Fig. 1); 80% of the samples were used as the training data and 20% of samples were used as the testing data. The results showed that correct classification rate of ANN for the testing samples varied from 20% to 100%, which suggested that quality of samples could also be generally evaluated by ANN model. We deduced that similar smells between powder sand slices might contribute to low classification rate, while specific smells of wax and bone slices might result in high classification rate, which could be visually reflected in LDA model (Fig. 1).

Fig. 1 also shows the discrimination of deer antlers of four specifications based on LDA model of second feature values. As shown, contribution rates of LD1 and LD2 were 62.6% and 33.0% respectively, and importantly, deer antler slices of four presentations were well distinguished. It implied that the LDA model based on second-feature values was an effective way to discriminate antler slices of different quality and an electronic nose should be able to rapidly assess the quality of deer antler slices.

Notably, we also found that some fake deer antler slices were mixed with authentical ones and they were identified as *Rangifer tarandus*. These fakes may also affect the quality of deer antlers, which can be regarded as the followed theme of study in the future.

Association analysis between smells and chemical components

To further understand the reasons inducing the quality differences between deer antler slices, we analyzed their chemical constituents. As shown, several volatile components in high content, including propane, butane, organic solvents, hydrocarbons, methane, fluorine, aromatic compounds, ethanol, ammonia and organic amines, were detected by electronic nose (Table 1). But notably, the contents of volatile components in wax slices, reflected by response intensity of sensors, were the highest, followed by powder slices, sand slices and bone slices, which indicated that volatile components content might be associated with quality differences between deer antler slices and could be effective markers for rapid quality identification of deer antler slices (Fig. 2).

Currently, amino acids are considered as important internal index components for quality control of deer antler slices (Wu et al., 2013). It had been investigated that wax slices of deer antlers have high content of amino acids, followed by powder slices, sand slices and bone slices (Song and Feng, 2013; Wang, 2009). Interestingly, it coincided with the change trend of response intensity of sensors (Fig. 2). We could infer that the intensity response of sensors should also be closely related to amino acids content. In conclusion, the results amazedly suggested that the electronic nose could mirror levels of both volatile components and amino acids, and should be effective for quality control assessment of deer antler slices.

However, the standard "deer antler slices of high quality" is still unclear, thus it is a hard question for many experts since deer antler slices have so many active chemical components we are not able to determine the effective markers for controlling quality (Tseng et al., 2014). This study can be a good supplement for traditional methods.

Perspective and application of the electronic nose

As a matter of fact, lots of TCM are aromatic, such as herbal medicines from Umbelliferae and Labiatae species. Also, most food or drink and even air have specific smells. The electronic nose is suitable for rapid identification of these materials, thus it has broad application prospects. Furthermore, there are not any effective tools for on-spot identification, and an electronic nose, as a mini-sensor, is expected to be developed as a wearable detector for on-spot identification, which will support and facilitate drug control. The electronic nose, we believe, will play an important role in drugs, agriculture, food and environmental protection.

Conclusion

In this study, we first described the use of the electronic nose for the assessment of quality of animal-sourced medicinal materials. As a result, the electronic nose combined with LDA model successfully discriminated deer antler slices of different quality. The result further proves that the electronic nose, like the human olfactory system, is able to discriminate different smells and even quality, and is effective for rapid quality identification. It is also believed that electronic nose can be an important tool for categorization of complex aroma mixtures and control of quality of drugs and food.

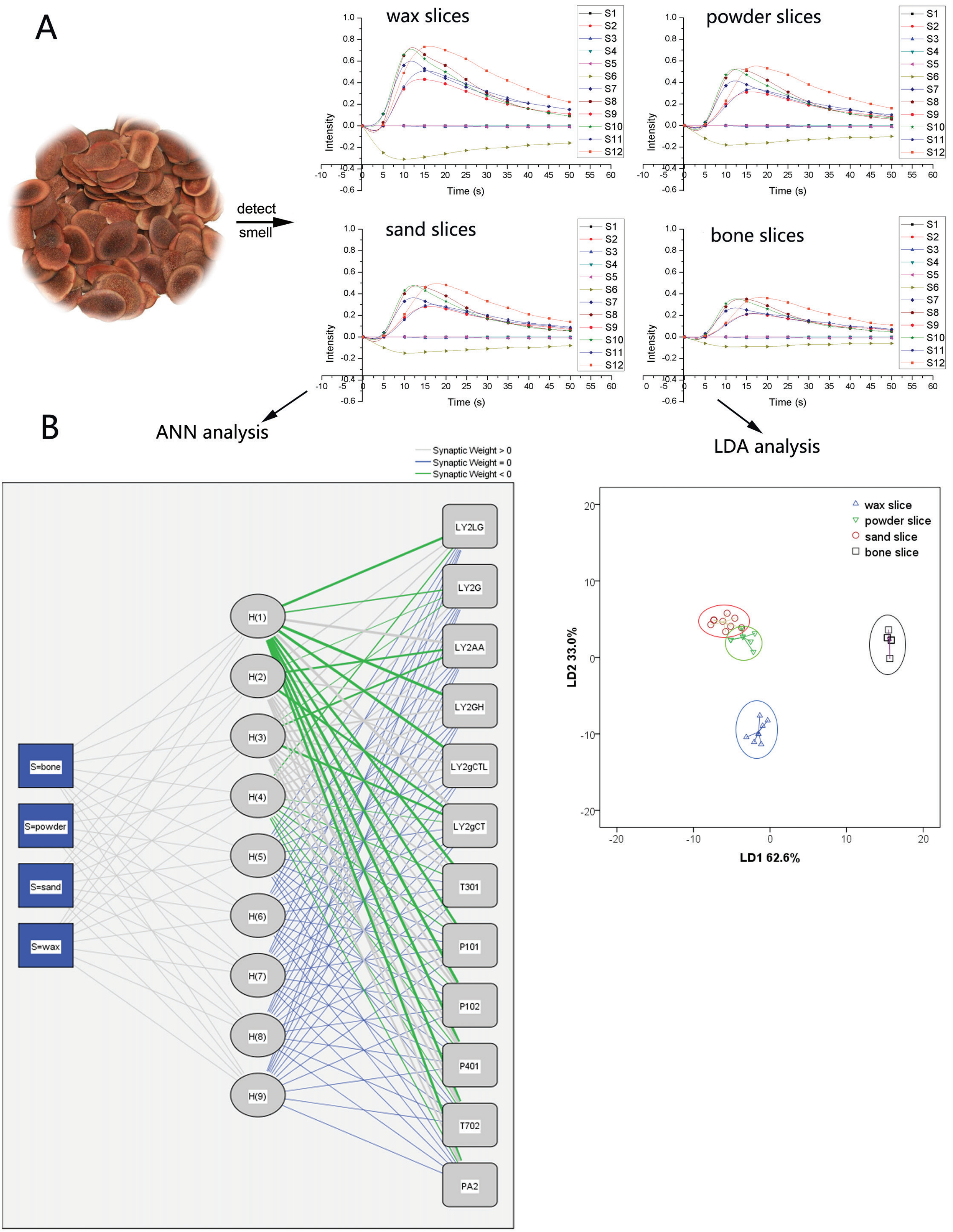

Figure 1 – Instruction of an electronic nose. **A**, Electronic nose detects the smells of samples ("S1-S12", the same as description in Table 1, represent markers of sensors.). **B**, Electronic nose transfers smells into digital signals and then directly analyzes them using attached software's based on linear discriminated analysis (LDA) and artificial neural network (ANN) models.

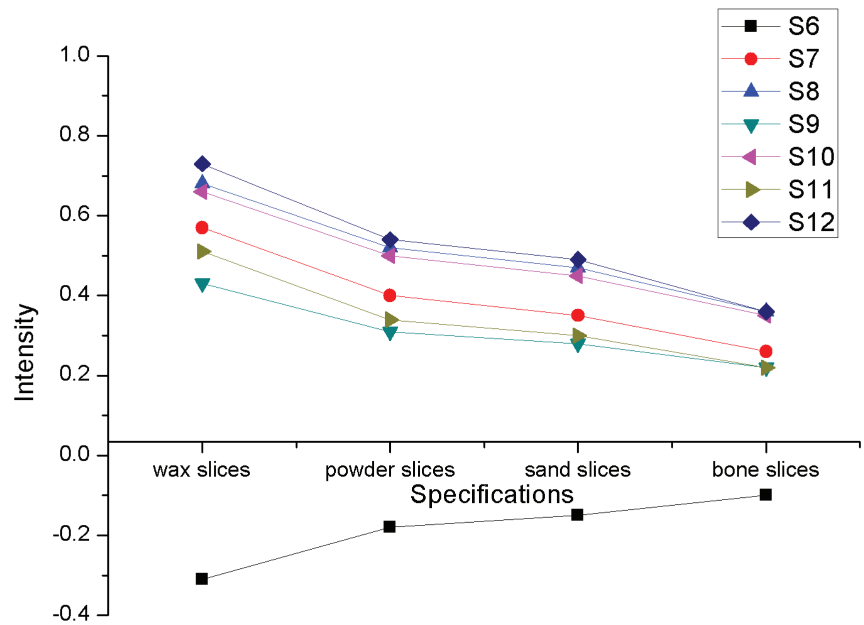

Figure 2 – Change trend of smell intensity of different antler slices. "S6-S12", the same as description in Table 1, represents sensors markers. The horizontal axis represents specifications of four slices. The vertical axis represents signal intensity of sensors.

Author's contribution

GX and CL accomplished the whole experiment. XW and CL guided the experiment and data analysis. HL, LH, HW, SL, XF, XR, XZ and XZ accounted for data analysis, collecting samples and processing samples.

Conflicts of interest

The authors declare no conflicts of interest.

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