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ORIGINAL ARTICLE

Rapid Qualitative and Quantitative Analysis of Volatile Components and Quality Identification of Amomi Fructus Based on Bionic Olfactory System

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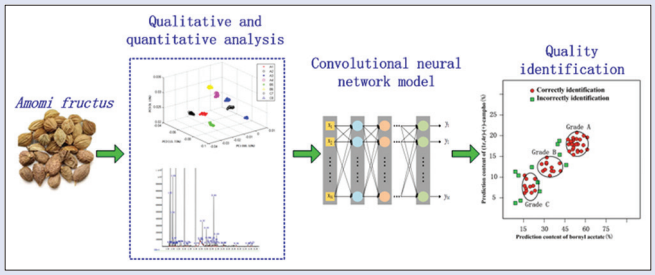
ABSTRACT

Background: *Amomi fructus* (AF) (a dried fruit of *Amomum villosum* Lour.) has been used in the treatment of digestive diseases such as abdominal pain and dysentery and in the prevention of abortion. The active ingredient of AF is its volatile oil. The volatile oil contains bornyl acetate and (1R,4R)-(+)-camphor, which are the primary active ingredients of AF that are analyzed for the quality assessment. Therefore, it is important to find an accurate and easy method to analyze the aforementioned volatile components of AF. **Materials and Methods:** In this study, 8 samples (A1–A4, B5 and B6, and C7 and C8) were collected and divided into Grades A, B, and C, respectively. The characteristics of volatile oils (the aroma) in these samples were analyzed using an electronic nose (E-nose) and a gas chromatography–mass spectrometry. In this study, we proposed a bionic olfactory system based on E-nose technology combined with a convolutional neural network algorithm for component identification. This system can qualitatively evaluate AF from different quality grades and quantitatively predict the contents of the two aforementioned primary chemical components. **Results:** The accuracy of qualitative identification was over 95% for Grade A samples and over 90% for Grade B and Grade C samples. **Discussion:** Based on our identification of the quality, Grade A samples were detected with an accuracy of 86.7%. However, Grade B and C samples were identified with lower accuracies (80% and 73.3%, respectively). **Conclusion:** The identification of quality of AF was successfully evaluated by two primary volatile components: bornyl acetate and (1R,4R)-(+)-camphor. The bionic olfactory system combined with an appropriate prediction model might be used as a potential quality control tool for Chinese herbal medicines.

Key words: Amomi fructus, bionic olfactory system, quality identification, quantitative analysis, volatile component

SUMMARY

- In this article, a model based on E-nose technology combined with a convolutional neural network (CNN) algorithm was proposed for the qualitative evaluation of different Amomi fructus (AF) grades and prediction of content of the two primary chemical components. From the experimental results, the following conclusions were drawn:
- For qualitative identification, the identification accuracy of three different grades of AF was over 90%.
- For quantitative analysis and quality identification, according to the analysis results of two main volatile components, bornyl acetate and (1r,4r)-(+)-campho, the identifcation accuracy was 86.7%, 80%, and 73.3% for Grade A, B, and C samples, respectively.
- It was revealed that the quality identification of AF could be evaluated by two main volatile components, bornyl acetate and (1r,4r)-(+)-campho



Abbreviations used: CHMs: Chinese herbal medicines; AF: Amomi fructus; GC-MS: Gas chromatography–mass spectrometry; E-nose: Electronic nose; PHWE: Pressurized hot water extraction; LPME: Liquid-phase microextraction; HPLC: High-performance liquid chromatography; CNN: Convolutional neural network; RSD: Relative standard deviation; RC: Relative content; PCA: Principal component analysis; NIST: National Institute of Standards and Technology; CAS: Chemical Abstracts Service; SD: Standard deviation; (Rc) ^{2}: Determination coefficient of calibration; (Rp) ^{2}: Determination coefficient of prediction; RMSEC: Root mean square of calibration; RMSEP: Root mean square error of prediction.

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INTRODUCTION

Amomi fructus (AF, called Sharen in Chinese) is a dry and mature fruit of the genus *Amomum* of the ginger family. The main species of AF include *Amomum villosum* Lour., AF Lour. var. xanthioides T. L. Wu et Senjen, or *Amomum longiligulare* T. L. Wu. As one of the most famous Chinese herbal medicines (CHMs), AF has been used in the treatment and prevention of digestive ailments such as abdominal pain and dysentery and in the prevention of abortion.[1-3] The high medicinal value

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of AF is mainly due to the presence of components in volatile oil such as bornyl acetate, (1R,4R)-(+)-camphor, borneol, D-limonene, myrcene, and camphene. Among these, bornyl acetate and (1R,4R)-(+)-camphor account for more than 65% of the total content of the volatile oil in AF. These two ingredients have been approved as the quality standard of AF due to their significant effect on anti-inflammatory, analgesic, and anticancer activity.[4-6] Deng presented a sampling technique using pressurized hot water extraction combined with liquid-phase microextraction to analyze the essential oil in AF samples from five different growing areas. He found that (1R,4R)-(+)-camphor and borneol acetate were the primary active ingredients of AF.[7] Dong identified different quality grades of AF by ten assessors, gas chromatography (GC), and an electronic nose (E-nose). He found that the content of bornyl acetate was the most crucial index to distinguish the quality of AF.[8]

Aroma is an essential characteristic used in the determination of AF quality.[2] Therefore, volatile components are detected using conventional methods, such as high-performance liquid chromatography and GC–mass spectrometry (GC-MS).[9-11] Using headspace GC-MS, seventy volatile components have been analyzed in AF samples. Among the components, bornyl acetate, (1R,4R)-(+)-camphor, and borneol accounted for more than 70% of the total content of the volatile oil, and therefore, they play a significant role in identifying the AF from different growing areas.[12] Zou *et al.* presented a method for quality evaluation of AF based on bornyl acetate and (1R,4R)-(+)-camphor content in different samples using GC-MS and E-nose.[13] Their results confirmed that bornyl acetate was one of the primary active components that can be used in quality assessment of AF. However, these widely used methods have disadvantages as being complex in operation and are time-consuming.[14-16] In recent years, bionic olfactory sensing systems (E-nose) that are rapid, secure, and reliable have been proven to be promising tools for the quality assessment of CHMs.

E-nose has been used in the food industry,[17,18] pharmaceutical industry,[19,20] environmental monitoring,[21] and so on. Mohammad-Razdari *et al.* investigated a method to assess the quality of tomato paste using an olfactory machine system coupled with chemometric tools and found that an E-nose system with TGS2610, MQ3, TGS2620, and TGS2600 sensors was effective in monitoring the adulteration of the paste.[22] Dong presented a new headspace integrated E-nose combined with pattern recognition analysis to distinguish between 13 species of Chinese medicinal herbs. According to his results, there was a 100% classification rate. The bionic olfactory sensing system is suitable for the analysis of volatile compounds, especially for the volatiles of complex samples, such as medicine, food, and cosmetic industry.[23]

In this study, a grade identification system of AF was established based on E-nose technology combined with a convolutional neural network (CNN) algorithm. In this study, we aimed to study the quantitative profiles and build a content prediction model for index components by comparing E-nose and GC-MS data. With this system, AF samples of unknown grade can be identified by quantitative analysis of composition and identification of grade based on aroma characteristics detected by E-nose technology without the complicated and time-consuming chemical compositional analysis. This study might propose a fast, reliable, and straightforward method for the quality control analysis of CHMs.

MATERIALS AND METHODS

Collection of study materials

AF (dried and mature fruit of *A. fructus* Lour.) samples were collected from four main growing locations: Guangdong, Yunnan, Guangxi, and Hainan provinces in China. The samples A1–A4, B5 and B6, and C7 and C8 were respectively labeled as Grades A, B, and C. This information was verified by B. H. and is listed in Table 1. All samples were harvested in October 2018 and dried at 30°C for 4 h in the same oven. They were collected by Z. L. (College of Traditional Chinese Medicine, Guangdong Pharmaceutical University) and were verified by B. H. (College of Traditional Chinese Medicine, Guangdong Pharmaceutical University). Then, all samples were crushed into powder, filtered through No. 1 sieve, divided into two groups, and sealed. In order to ensure the accuracy of detection, we optimized the experimental conditions for E-nose testing and GC-MS analysis.

Table 1: Labels and grades of Amomi fructus samples from different origins that harvested during October 2018

Sample label	Origin	Place	Grades
A1	Xingfu village	Guangdong province	A
A2	Xin village	Guangdong province	A
A3	Mengla county	Yunnan province	A
A4	Mengla county	Yunnan province	A
B5	Ningming county	Guangxi province	B
B6	Xingfu village	Guangdong province	B
C7	Chengmai county	Hainan province	C
C8	Ningming county	Guangxi province	C

Electronic nose

In this study, we employed PEN3 (Airsense Analytics, Schwerin, Germany) to detect the odor characteristics of AF samples. PEN3 is made up of an automatic sampling device, an array of sensors, and a data processing system. It works as follows: (1) samples are heated and concentrated in the automatic sampling device; (2) during the detection, the volatile compounds react with the surface of the sensors, causing them to respond and output the signals; and (3) finally, signal preprocessing and data analysis are completed by the data processing system. Table 2 shows the list of sensors and their characteristics of PEN3.

Before detection, PEN3 was first running for more than 60 min to ensure that all the sensors were heated up to the working temperature (above 200°C) and the gas path was cleaned by clean air. Each sample was heated at 40°C and concentrated in the automatic sampling device, keeping for 30 min. During detection, we set the chamber flow rate and injection flow rate at 150 mL/min. Each sample was continuously tested 10 times and each time we included the monitoring process (120 s, the measurements were recorded from 1 s to 120 s) and cleaning process (180 s).

Gas chromatography–mass spectrometry

Preparation of volatile oil

According to the General Principle 2204 given in Chinese Pharmacopoeia,[2] all samples were sliced, powdered, and sieved using 24 meshes. Each sample was weighed (50 g) and extracted. The samples were extracted with ethyl ether as the solvent in a Soxhlet Extractor (SER148/6; VELP, Usmate Velate, Italy) for 5 h at 110°C–120°C. The solvent was extracted with 500 mL of water in a steam distillation for 10 h, and the yield rate was calculated using the following equation:

$$W(\%) = \frac{m_1}{m_2} \times 100 \quad (1)$$

where W is the yield rate, m_1 is weight of the volatile oil, and m_2 is the sample weight. The recovered volatile oil was diluted and further analyzed using GC-MS.

Table 2: The name and characteristics of each sensor in performance of electronic nose-3

Sensor number	Sensor name	Object substances for sensing	Threshold value (mL/m^3)
S1	W1C	Aromatics	10
S2	W5S	Nitrogen oxides	1
S3	W3C	Ammonia and aromatic molecules	10
S4	W6S	Hydrogen	100
S5	W5C	Methane, propane, and aliphatic non-polar molecules	1
S6	W1S	Broad methane	100
S7	W1W	Sulfur-containing organics	1
S8	W2S	Broad alcohols	100
S9	W2W	Aromatics and sulfur- and chlorine-containing organics	1
S10	W3S	Methane and aliphatics	10

Analysis of volatile components

Before conducting GC-MS analysis, each volatile oil was diluted 100 times with ethyl ether separately to obtain an effective GC-MS spectrogram. We used a GC system (Agilent 7890A, USA) equipped with HP-5 capillary column (30 m × 250 μm × 0.25 μm film thickness) and MS system (Agilent 5975C, USA) for the analysis of volatile oil. The oven temperature was initiated at 60°C, held for 3 min, increased to 130°C at 20°C/min, and again increased to 160°C at 5°C/min, held for 10 min, and finally increased to 230°C at 20°C/min and held for 2 min. The temperature of the injector was maintained at 250°C, and helium was used as the carrier gas with a flow rate of 1.00 mL/min. The mass spectrometer was operated in electron bombardment ionization source mode, with the ionization temperature, the interface temperature, and the quadrupole temperature held at 230°C, 280°C, and 150°C, respectively. The quantity scanning range was set to 40–500 amu and the quantity scanning speed was held at 2.94 times/s.

Finally, each volatile oil was analyzed three times by GC-MS within the same condition to calculate the average values of the relative standard deviation (RSD). The relative content of each volatile oil was calculated.

Data analysis

The E-nose data analysis was conducted by bionic olfactory odor analysis software (BOOAS version 2.0, Guangdong University of Technology, Guangzhou, China). The GC-MS data analyzed each volatile oil by comparing the recorded mass spectra with the National Institute of Standards and Technology (14.L) mass-spectral library. Principal component analysis (PCA) and CNN were used to analyze the odor differences of AF of different grades. CNN is a multi-layer feedforward network trained by error backpropagation with deep structure. Its fundamental theory is to use translation, distortion, and scaling procedure to minimize the mean square error of the actual output value and the expected output value of the network using gradient search technology.[24,25] CNN has been one of the classical and widely used deep learning algorithms in many fields.[26]

The GenStat (Version 18th, VSNC) and MATLAB (version R2013a, MathWorks Inc., Natick, USA) software packages were employed to conduct the data analysis, including PCA and CNN.

RESULTS

E-nose sensor response

In this section, sensors' response and qualitative analysis of AF samples based on PCA are discussed. Figure 1 shows all sensor responses for three phases of initialization (from 1 s to 5 s), changing (from 6 s to 40 s), and stabilization (from 41 s to 120 s). In this figure, A1–A4 represent the responses of sensors to Grade A samples; B5 and B6 represent the responses of sensors to Grade B samples; C7 and C8 represent the responses of sensors to Grade C samples. The trend observed for A1–A4 samples is very similar which confirms that they belong to the same grade. However, the trend of Grade A response is different from that of Grades B and C. It can be seen that sensors S2 and S6–S9 show different values for Grade A, B, and C samples. It was found that despite the similarity of volatile components among different grades of AF samples, the concentration of bornyl acetate and (1R,4R)-(+)-camphor was varied according to the different grades of AF samples. This is demonstrated by the following GC-MS component analysis.

Gas chromatography–mass spectrometry component analysis

Figure 2 and Table 3 show the presence of bornyl acetate and (1R,4R)-(+)-camphor as identified by GC-MS analysis. As listed in Table 3, the content of bornyl acetate in A1–A4 samples was, respectively, 51.24%, 50.17%, 49.52%, and 50.48%; the content of (1R,4R)-(+)-camphor in A1–A4 samples was, respectively, 17.73%, 18.48%, 17.85%, and 17.28%. Meanwhile, the content of bornyl acetate was more than 3.3 mg/g in A1, A2, A3, and A4 samples, which was in agreement with the evaluation indexes that the content of bornyl acetate shall not be < 3.0 mg/g. The ratio of bornyl acetate to (1R,4R)-(+)-camphor was more than 2.0 in Grade A of AF.[2,3] In addition, the content of bornyl acetate in B5, B6, C7, and C8 was, respectively, 32.04%, 30.89%, 22.64%, and 18.85%; the content of (1R,4R)-(+)-camphor in B5, B6, C7, and C8 was, respectively, 10.76%, 11.38%, 7.14%, and 8.12% in C8. It is shown that both contents of bornyl acetate and (1R,4R)-(+)-camphor in Grade B and C samples were less than that of in Grade A samples. Thus, in all the tested volatile components, we found that bornyl acetate and (1R,4R)-(+)-camphor were the primary active ingredients. Therefore, Chinese Pharmacopoeia 2015 Edition[2] and literature[7,8] recommend checking the quality control of AF based on these two primary ingredients.

Principal component analysis

The qualitative analysis of AF from different grades was conducted by applying PCA to the E-nose data. A total of 240 samples (30 samples of each group) were divided into two parts: 160 samples (20 samples of each group) for the training set and 80 samples (10 samples of each group) for the testing set. The 10-fold cross-validation was applied to evaluate the performance of the model. The dataset was randomly divided into 10 subsets, where 9 out of the 10 subsets were used for training and the remaining one for testing. The cross-validation process was repeated 10 times. Thus, 10 identification accuracy rates were acquired, and the mean accuracy was calculated as the evaluation index. The results of the qualitative analysis of AF from different grades based on PCA are presented in Figure 3.

Table 3: Relative content of two main volatile compounds (bornyl acetate and (1R,4R)-(+)-camphor of Amomi fructus from different labels identified by gas chromatography-mass spectrometry

CAS	Compounds*	Formula	Relative content (%), mean±SD							
			A1	A2	A3	A4	B5	B6	C7	C8
5655-61-8	bornyl acetate	$C_{12}H_{20}O_2$	51.24±5.18	50.17±5.51	49.52±4.63	50.48±6.36	32.04±4.22	30.89±3.71	22.64±2.38	18.85±3.14
464-49-3	(1R,4R)-(+)-camphor	$C_{10}H_{16}O$	17.73±2.08	18.48±2.45	17.85±3.04	17.28±2.47	10.76±2.29	11.38±3.62	7.14±1.73	8.12±2.81

*Compounds identified via GC-MS analysis based on comparison with the retention indices and the mass spectra of standard compounds (similarity≥90% was listed). CAS: Chemical Abstracts Service; SD: Standard deviation; GC-MS: Gas chromatography-mass spectrometry

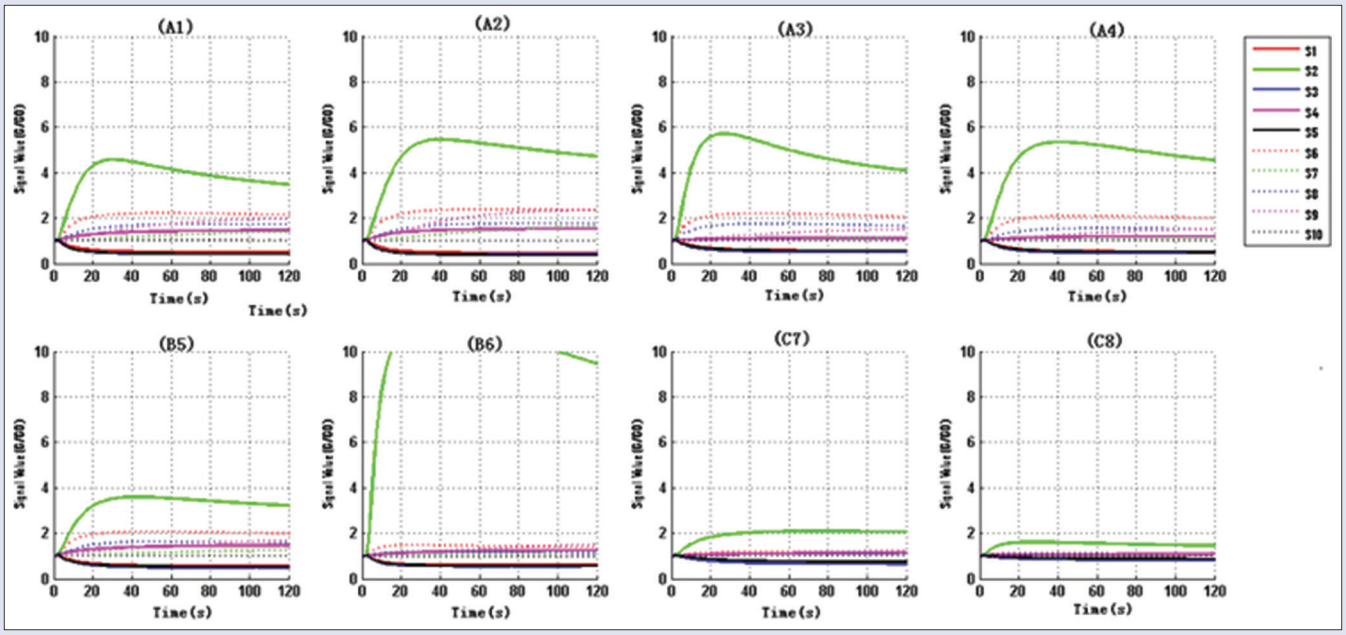

Figure 1: Sensors' response to the selected Amomi fructus samples for different grades. G0 and G represent the electronic conductivity of the sensors when detecting clean air and the Amomi fructus samples, respectively. (A1), (A2), (A3), and (A4) represent responses of Grade A samples; (B5) and (B6) represent responses of Grade B samples; (C7) and (C8) represent responses of Grade C samples

DISCUSSION

Qualitative analysis

The qualitative analysis of AF as shown in the three-dimensional spatial distribution plot of AF samples based on PCA [Figure 3] confirms that the first, second, and the third principal components demonstrated more than 91.39% of the total variance. Therefore, the samples of three selected grades of AF could be separated efficiently.

Table 4 shows the accuracy of identification of different AF grades. The identification accuracy of the training set was more than 95%, and the accuracy of the testing set was more than 90%. The results show that the characteristics of volatile components of Grade A samples were significantly different from those of Grade B and C samples. However, the difference between Grades B and C was negligible, which led to the low accuracy of identification [Table 3]. Figure 3 and Table 4 show the results of the analysis. According to our results, the performance of identification of different grades of AF samples based on E-nose technology was accurate and reliable.

Quantitative prediction

Evaluation parameters

For further quantitative analysis of bornyl acetate and (1R,4R)-(+)-camphor, we proposed a prediction model based on CNN regression models. Four most important parameters were used to evaluate the performance of the model: the square correlation coefficient of the training set (determination coefficient of calibration [R_c^2]), the square correlation coefficient of the testing set (determination coefficient of prediction [R_p^2]), the root mean square error of calibration (the root mean square error of the training set, RMSEC), and the root mean square error of prediction (the root mean square error of testing set, RMSEP). They were calculated according to the following equations.

$$(R_c)^2 = 1 - \frac{\sum_{i=1}^{n_1}(y_c^i - f_c^i)^2}{\sum_{i=1}^{n_1}(y_c^i - \overline{y_c})^2} \quad (2)$$

$$(R_p)^2 = 1 - \frac{\sum_{i=1}^{n_2}(y_p^i - f_p^i)^2}{\sum_{i=1}^{n_2}(y_p^i - \overline{y_p})^2} \quad (3)$$

$$RMSEC = \sqrt{\frac{1}{n_1}\sum_{i=1}^{n_1}(y_c^i - f_c^i)^2} \quad (4)$$

$$RMSEP = \sqrt{\frac{1}{n_2}\sum_{i=1}^{n_2}(y_p^i - f_p^i)^2} \quad (5)$$

where y_c^i and f_c^i are the values obtained from the models during the training process and the values obtained from GC-MS analysis, respectively; y_p^i and f_p^i are the values obtained from the models

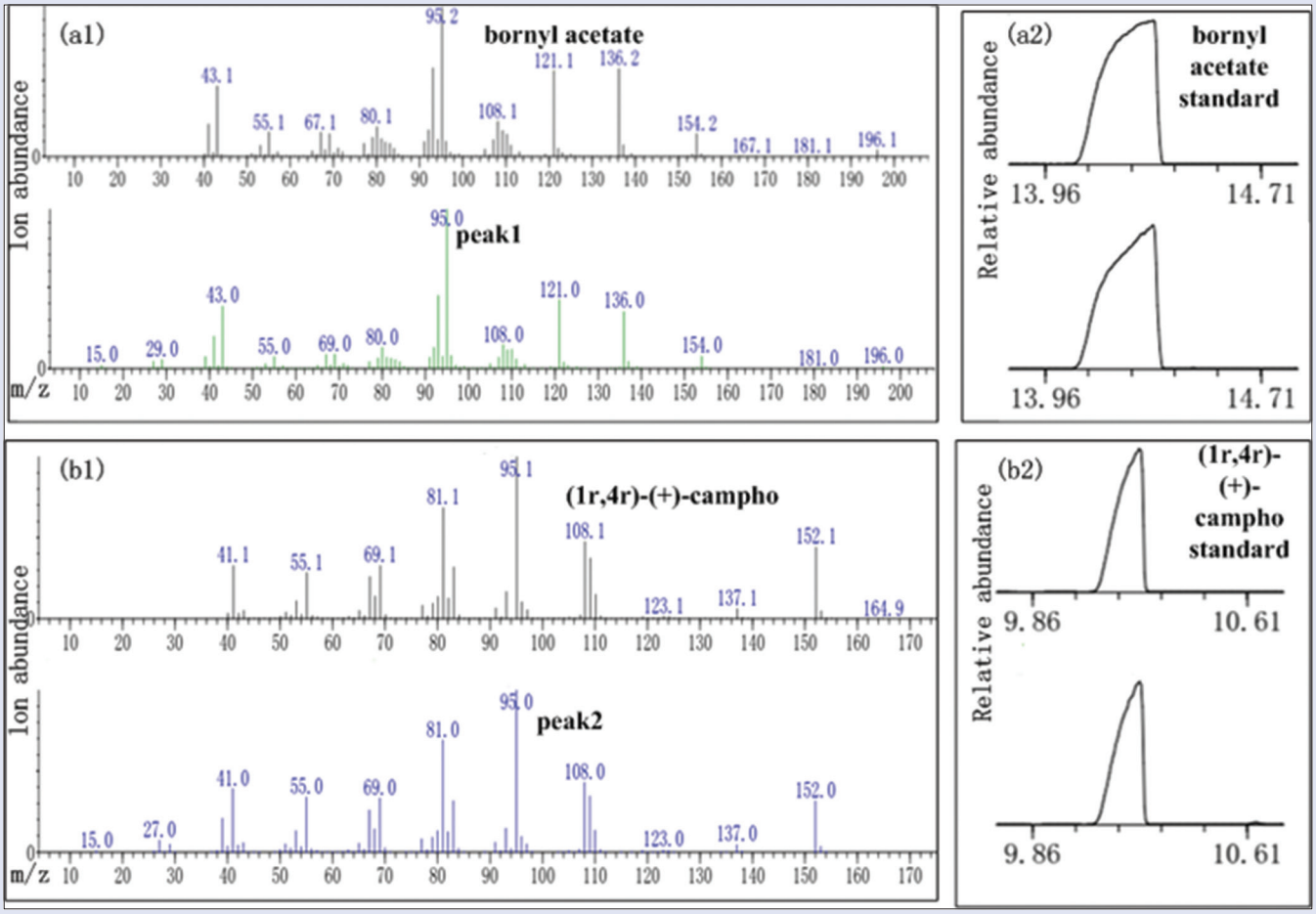

Figure 2: Analysis of odor components: bornyl acetate and (1R,4R)-(+)-camphor generated by gas chromatography–mass spectrometry from A2 sample: (a1) and (b1) were mass spectrometry of peak 1 and peak 2 in the National Institute of Standards and Technology 14.L library compared with the mass spectrometry of the bornyl acetate and (1R,4R)-(+)-camphor standard, separately; (a2) and (b2) were the bornyl acetate and (1R,4R)-(+)-camphor standards, the gas chromatography–mass spectrometry chromatogram of compounds of bornyl acetate and (1R,4R)-(+)-camphor, separately

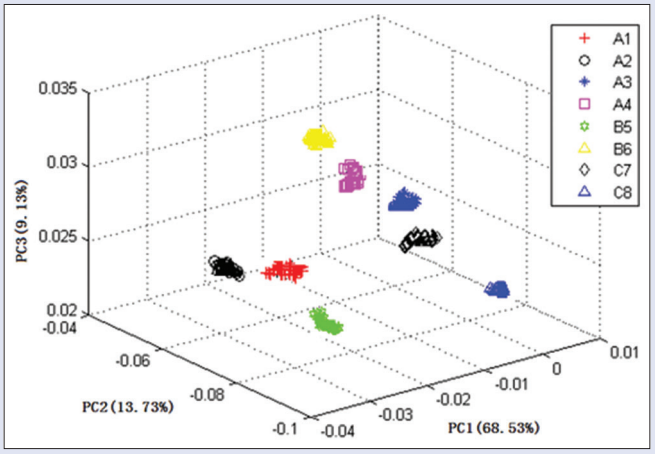

Figure 3: Qualitative analysis of Amomi fructus from different grades based on principal component analysis. (A1), (A2), (A3), and (A4) were Grade A samples; (B5) and (B6) were Grade B samples; (C7) and (C8) were Grade C samples

during the testing process and the value obtained from GC-MS analysis, respectively; $\overline{y_c}$ and $\overline{y_p}$ are the mean values of y_c^i and y_p^i; n_1 is equal to 20 (20 samples in the training set of each group); and n_2 is equal to 10 (10 samples in the testing set of each group). Thus, if the values obtained from the CNN model are close to those values analyzed by GC-MS, the higher values for the R_c^2 and R_p^2 as well as the lower values for RMSEC and RMSEP would be obtained. Therefore, for a superior regression model, it is aimed to achieve a high correlation coefficient (R_p) and a low RMSEP for testing.

Convolutional neural network prediction model training

Compared with some traditional algorithms, CNN is an artificial neural network with a deep structure, which is one of the first classical and widely used deep learning algorithms.[26] CNN has a strong non-linear mapping ability and good adaptability. It is a feedforward neural network, which consists of an input layer, hidden layer, and output layer. The hidden layer includes convolution layer, pooling layer, and full connection layer. CNN can solve non-linear problems and present superior performance in comparison with other neural networks.[27]

In this study, CNN regression model was set up to predict the contents of bornyl acetate and (1R,4R)-(+)-camphor in AF samples. It was designed as seven layers: one input layer, two convolution layers, two subsampling layers (called pool layer), one full connection layer, and one output layer.

Table 4: Identification of accuracy of different Amomi fructus grades and sample labels

Sample label	Training set			Testing set		
	Number of samples	Correctly identified samples	Accuracy (%)	Number of samples	Correctly identified samples	Accuracy (%)
A (A1-A4)	80	79	98.8	40	38	95
B (B5, B6)	40	38	95	20	18	90
C (C7, C8)	40	40	100	20	18	90

Table 5: Quantitative analysis and volatile component identification based on convolutional neural network

Volatile constituents	Training set		Testing set	
	$(R_c)^2$	RMSEC	$(R_p)^2$	RMSEP
bornyl acetate	0.914	0.962	0.893	1.046
(1R,4R)-(+)-camphor	0.907	1.027	0.884	1.109

$(R_c)^2$: Determination coefficient of calibration; $(R_p)^2$: Determination coefficient of prediction; RMSEC: Root mean square of calibration; RMSEP: Root mean square error of prediction

In the convolution layer and pool layer, which were interleaved, the sigmoidal function was used for convolution layer activation function. The sigmoid function is given as follows:

$$g(x) = \frac{1}{1+e^{-x}} \quad (6)$$

where x is the input value.

Before training the model, all data were divided into two parts: 160 samples (20 samples of each group) for the training set, which was applied to determine the optimal prediction model, and 40 samples (5 samples of each group) for the testing set, which was used to evaluate the predictive performance of the model. The E-nose data were used as independent variables, and the contents determined by GC-MS were used as dependent variables. These variables were introduced into the model as input and output variables. In order to ensure that the CNN model can be fully trained and limit the training time, we introduced criteria to stop training when the number of training iterations is more than 500, and the total error of the last 100 training iterations is <0.01. Finally, at the end of the training, the parameters of the best CNN prediction model were as follows: α = 0.023 (learning rate), β = 25 (batch size, the number of training samples for input each time), and n = 2300 (training times), which showed that all parameters in CNN network were automatically adjusted to a perfect condition, and then, the CNN prediction model was constructed. Finally, the evaluation parameters, R_c^2, R_p^2, RMSEC, and RMSEP, were calculated [Table 5].

According to Table 5, the predicted results (for bornyl acetate – R_c^2 = 0.914 and RMSEC = 0.962 in training set and R_p^2 = 0.893 and RMSEP = 1.046 in testing set and for (1R,4R)-(+)-camphor – R_c^2 = 0.907 and RMSEC = 1.027 in training set and R_p^2 = 0.884 and RMSEP = 1.109 in testing set) were found to be satisfied. The results demonstrated that the CNN model could perfectly predict the contents of bornyl acetate and (1R,4R)-(+)-camphor in AF samples.

Identification of different grades

Based on the prediction values, the following three evaluation parameters were proposed in the identification of qualitative assessment and grading of AF samples: prediction content of bornyl acetate (a), prediction content of (1R,4R)-(+)-camphor (b), and the ratio of prediction content of bornyl acetate to prediction content of (1R,4R)-(+)-camphor (λ). Table 6 shows the specific settings of each parameter.

After the CNN prediction model finished training, sixty unknown samples were selected to evaluate the predictive performance of the model according to the evaluation parameters listed in Table 6. Figure 4 and Table 7 show the results.

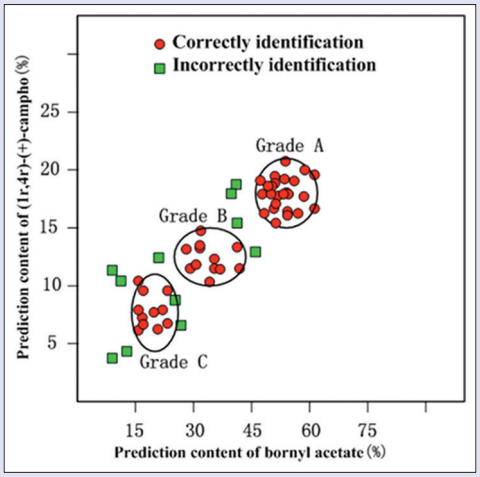

Figure 4: Identification results for unknown Amomi fructus samples of different grades based on convolutional neural network prediction model

According to Figure 4, we clearly differentiated the three different grades of AF samples. Based on our analysis, 26 samples were identified as Grade A and 4 samples were identified as Grade B. The contents of bornyl acetate for Grade B were below 45% (a <45) and the content of (1R,4R)-(+)-camphor in one sample was below 15% (b <15). In 15 Grade B samples, 12 were identified as Grade B and 3 samples were identified as Grade C. The contents of bornyl acetate in two samples of Grade C were below 25% (a <25) and the content of (1R,4R)-(+)-camphor in one sample was below 10% (b < 10); in 15 Grade C samples, 11 were identified as Grade C and 4 samples were identified as Other because of the contents of bornyl acetate were below 15% (a <15) and the contents of (1R,4R)-(+)-camphor were below 5% (b <5). Figure 4 shows that it is reasonable and reliable to identify the quality grade of AF according to the contents of bornyl acetate and (1R,4R)-(+)-camphor predicted by the CNN model. It is further clarified that bornyl acetate and (1R,4R)-(+)-camphor are the common factors which contribute to the aroma of AF, indicating that these two compositions might weigh more in differentiating.

As listed in Table 7, the Grade A samples were easy to identify than that of the other two grades. The accuracy of identification for Grades A, B, and C was 86.7%, 80%, and 73.3%, respectively, which means that the Grade A samples possess a significantly different concentration of bornyl acetate and (1R,4R)-(+)-camphor. Figure 4 and Table 7 confirm that good quality AF samples contained higher concentrations of bornyl acetate and (1R,4R)-(+)-camphor, whereas low-quality AF samples emit lower concentrations of volatile compounds.

Given the complexity of the sample pretreatment, the time-consuming procedures during the measurement, and the amount of data analysis and the cost, we propose that E-nose technology might be a good alternative in determining the quality and grading of AF samples. The

Table 6: Evaluation parameters for different quality grades of Amomi fructus

Content of bornyl acetate (%) (a)	Content of (1R,4R)-(+)-camphor (%) (b)	Ratio of bornyl acetate to (1R,4R)-(+)-camphor (λ)	Result
a≥45	b≥15	$\lambda \geq 2.5$	A
45>a≥25	15>b≥10	$2.5 > \lambda \geq 2.0$	B
25>a≥15	10>b≥5	$2.0 > \lambda \geq 1.5$	C
a<15	b<5	$\lambda < 1.5$	Other

Table 7: Identification results of different Amomi fructus grades based on convolutional neural network prediction model

Grades	A	B	C
Number of unknown samples	30	15	15
Correctly identified	26	12	11
Accuracy (%)	86.7	80	73.3

CNN prediction model might show the concentrations of separated volatile compounds, which can help explain the inner causes from a chemical viewpoint. Therefore, the prediction system of volatile components based on E-nose technology might be a simple and reliable method for determining AF grade. In addition to improving the accuracy of grading identification of AF, it is necessary to integrate other information-processing technologies, such as the electronic tongue, in future studies.

Repeatability of electronic-nose and gas chromatography–mass spectrometry

In this study, the repeatability of PEN3 was evaluated by applying five parallel measurements of A2 samples and the GC-MS method was executed using B5 samples. In the E-nose case, the RSD of the relative peak area and the relative retention time were both <1.70%, whereas the RSD of volatile components was 1.90% via GC-MS analysis. These results show that both methods had good repeatability.

CONCLUSION

In this study, a model based on E-nose technology combined with a CNN algorithm was proposed for the qualitative evaluation of different AF grades and prediction of content of the two primary chemical components (i.e., bornyl acetate and (1R,4R)-(+)-camphor). The detailed profiles of the two components were used to distinguish different quality grades of AF. The performance of the model was verified using four statistical parameters: R_c^2, R_p^2, RMSEC, and RMSEP.

1. For the qualitative identification, the accuracy of identification of the training set and the testing set was, respectively, 98.8% and 95% for Grade A samples, 95% and 90% for Grade C samples, and 100% and 90% for Grade C samples. It was found that the results of accuracy test of qualitative analysis based on E-nose technology were higher
2. For the quantitative prediction, the contents of bornyl acetate and (1R,4R)-(+)-camphor were predicted based on E-nose data combined with CNN algorithms. The prediction results of R_c^2 were over 0.90 and of R_p^2 were over 0.884, which was reliable and better than previous work[13]
3. For the quality grade identification, Grade A samples were identified better than the other two grades. The identification accuracy was 86.7%, 80%, and 73.3% for Grade A, B, and C samples, respectively
4. It was confirmed that good quality AF samples contained higher concentrations of bornyl acetate and (1R,4R)-(+)-camphor, whereas low-quality samples had lower concentrations.

Finally, we can conclude that the CNN-based prediction model using E-nose technology is a robust, reliable, and nondestructive approach for determining AF grades and predict the contents of their critical aroma components, offering an alternative method for future studies on intelligent monitoring and quality control for CHMs.

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Conflicts of interest

There are no conflicts of interest.

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