Detection of Corn Quality Based on Surface-Enhanced Raman Spectroscopy and Electronic Nose Technology

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This study explored a corn quality detection method based on surface-enhanced Raman spectroscopy (SERS) and electronic nose technology. The content of aflatoxin (AFB1) and ochratoxin (OTA) in corn samples was detected by fluorescence immunoassay as the basic data for the experiment. Subsequently, the SERS curve of the corn samples was measured, and the electronic nose was used to analyze the odor of the samples. Combining the relationship between SERS curves, electronic nose data, and the toxin content in corn, a prediction model was established by using the random forest (RF) method. The results showed that the model's coefficient of determination of the test set for predicting AFB1 reached 0.70, and the model's coefficient of determination of the test set for predicting OTA reached 0.74. This experiment showed that SERS and electronic nose technology can effectively detect the mycotoxin content in corn samples, which provides a new method to predict the toxin content in corn.

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INTRODUCTION

Corn is one of China's four major food crops. In 2023, China's corn sown area reached 44.22 million hectares, and the corn output reached 289 million tons. Harvested corn inevitably mildews during storage, producing toxins such as aflatoxin, ochratoxin, zearalenone, and others (Xu *et al*. 2023). Aflatoxin damages animal livers, reduces animal production performance, and weakens animal immunity, fertility, and feed utilization. Feeding such degraded corn to dairy cows leads to a decrease in milk production (Zhang 2023). Ochratoxin is a mycotoxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum*. It leads to slow animal growth and reduced feed utilization. In severe cases, it causes renal tubular epithelial damage and necrosis of intestinal lymphoid glands (Zhang 2023). Consequently, the detection of corn quality is very important. Traditional chemical detection methods damage samples, are time-consuming and labor-intensive, and require professional technical personnel, making it difficult to meet the growing demand for corn detection. Therefore, it is necessary to develop a new method to solve the limitations of traditional methods.

Fluorescence immunoassay (FIA) is a detection technology based on the principle of immune reaction. It has been mainly used to detect the presence and concentration of specific molecules (such as proteins, antibodies, *etc*.) in liquids. It combines the advantages of immunology and fluorescence analysis technology and has the characteristics of high sensitivity, specificity, and degree of automation. In fluorescence immunoassay, fluorescently labeled antibodies or antigens are usually used for detection. The intensity of the fluorescent signal is proportional to the concentration of the target molecule in the sample. Therefore, the content of the target molecule in the sample can be quantitatively analyzed by measuring the fluorescence intensity. Fluorescence immunoassay has a wide range of applications in fields such as biomedical research (Ma *et al*. 2018), environmental monitoring (Wang *et al*. 2022) and clinical diagnosis (Hou 2023). In recent years, it has been applied in agricultural product detection. Miao *et al*. (2023) used fluorescence immunoassay to detect vomitoxin in cereal products. Compared with the methods described in the China national standard, the result error is ≤16.28% (Miao *et al*. 2023). Lu and associates used immunochromatographic fluorescence to detect lead content in wheat. The experimental result’s coefficient of variation is less than 15% (Lu *et al. 2*024). Mogos and associates used phycocyanin-based fluorescence immunoassay to detect vomitoxin, zearalenone, *etc*. in food and feed, and the results were found to be reliable (Girmatsion *et al*. 2024). The high sensitivity and automation characteristics make it an indispensable important technical means in modern biomedical and biochemical analysis.

Surface-enhanced Raman spectroscopy (SERS) is an analytical technique that uses the Raman scattering phenomenon to obtain structural and compositional information of samples. When a sample is irradiated by excitation light, some photons will undergo Raman scattering, resulting in a slight change to the energy and frequency of the incident photons. This change reflects the vibration and rotation states of the sample molecules, and it reveals the chemical composition, crystal structure and intermolecular interactions of the sample. Compared with infrared spectroscopy, Raman spectroscopy does not need to significantly resonate with the molecular vibrations of the sample, so it avoids the overlap of certain spectral peaks and provides clearer analysis results. Surface enhancement technology increases the signal intensity of Raman spectroscopy by several orders of magnitude, so the detection can be performed at very low concentrations. The core principle of SERS involves surface or chemical enhancements. For the surface enhancement, local electromagnetic field enhancement is generated on the surface of metal nanoparticles (usually silver or gold). These nanoparticles produce a local surface plasmon resonance effect under light irradiation, thereby significantly enhancing the intensity of the scattered light. This enhancement effect increases the intensity of the Raman signal, making Raman spectroscopy more sensitive. For the chemical enhancement effect, the interaction between molecules and the metal surface, such as electron transfer or resonance effect, increases the light scattering of molecules. The application of SERS has a wide range in multiple fields, including drug development (Liu *et al*. 2021), life science (Artem Tabarov *et al*. 2022), environmental science (Liu *et al*. 2023), and materials science (Laden *et al*. 2024). In agriculture, surface-enhanced Raman spectroscopy was used to detect AFB1 in corn and soybean samples, and the relative error of the detection results was -14% and 4.9% (Zhu *et al*. 2024). Raman spectroscopy has been used to detect *Fusarium* toxins in winter wheat, with an accuracy rate of 96% (Moskovskiy *et al*. 2021). Yang *et al*. 2023) used line-scanning Raman hyperspectral imaging technology to detect various aflatoxins in peanuts, and achieved reliable results. Raman spectroscopy has the characteristics of non-destructiveness, high sensitivity, and real-time analysis.

Electronic nose technology simulates the human olfactory system and is used to identify and analyze gases or odors. Multiple different types of gas sensors are installed inside the electronic nose. According to different principles, these sensors can be classified into metal oxide type, electrochemical type, conductive polymer type, mass type, photoionization type, and so on. Commonly used electronic noses include semiconductor type, electrochemical type, and infrared gas sensors. Each type of sensor has specific sensitivity and response characteristics to different odor molecules. When gas molecules come into contact with the sensors, chemical reactions or physical adsorption will occur, which will lead to changes in the electrical properties of the sensors and generate corresponding electrical signals. Electronic nose technology has a very wide range of applications in the agricultural field. Wang (2023) used an electronic nose to detect the storage quality of peanuts and rice. Compared with the results of physical and chemical property detection, the accuracy rate reaches more than 80%. Zhang (2024) performed qualitative analysis on moldy grains based on electronic nose technology, and the average accuracy rate of the model reaches 93.3%. Ali *et al*. (2023) used an electronic nose combined with machine learning to predict the carbohydrate content of potatoes during storage, and the accuracy rate was higher than 90%.

Although the detections based on surface-enhanced Raman spectroscopy (SERS) and electronic nose are currently widely used, there are few reports on combining the two to conduct research on the quality of corn. SERS can provide detailed information on molecular vibrations and accurately identify the structures and types of molecules. The electronic nose can detect and analyze the overall odor characteristics of gases. The combination of the two can obtain multiple pieces of information ranging from molecular structures to odor characteristics, thus enhancing the ability to distinguish complex samples. Meanwhile, the combination of the two can also improve the detection accuracy. SERS is very sensitive to the detection of certain components, but it may be interfered with by other substances. The electronic nose can make judgments on odors as a whole. After combination, they can verify and complement each other and reduce misjudgment cases. This study aims to explore a new method for detecting the quality of corn.

EXPERIMENTAL

Materials

Detection of toxin content by fluorescence immunoassay

The fluorescence immunoassay was used to detect aflatoxin (AFB1) and ochratoxin (OTA) in corn samples. The instrument used in the experiment was the fluorescence immunoassay quantitative POCT analyzer of Shanghai Xiongtu Technology Company (Shanghai, China). The aflatoxin and ochratoxin immunoassay kits used were purchased from Shanghai Xiongtu Technology Company (Shanghai, China). The corn samples were collected from the Chifeng area in Inner Mongolia Autonomous Region of China.

Surface-enhanced Raman spectroscopy measurement

For the Surface-Enhanced Raman Spectroscopy (SERS) technique, the Raman spectrometer used was the CORA 5001 (Anton Paar, Austria) Raman spectrometer. The reagents used included silver nitrate (Sinopharm, Beijing, China), deionized water, and sodium citrate (Sinopharm, Beijing, China).

Electronic nose detection

Electronic nose technology detects the odor characteristics of corn samples through a gas sensor array and generates corresponding data. The electronic nose device used was the PEN3 model electronic nose produced by AIRSENSE Company (Schwerin, Germany). This device is equipped with 10 different types of gas sensors, including metal oxide semiconductor sensors and electrochemical sensors.

Methods

Detection of toxin content by fluorescence immunoassay

For sample processing, the corn sample was ground into powder. Approximately 1.0 ± 0.02 g of powder was mixed with 5 mL of sample extraction solution. After shaking and extracting for 5 min with a vortex oscillator, the sample was centrifuged at 4000 rpm for 2 min, and 100 µL of the supernatant was transferred to a new container with 600 µL of sample dilution solution. The sample was filtered to remove suspended particles.

For the immune reaction, 100 µL of the treated sample liquid was added to the sample application hole of the test strip. The strip was placed horizontally in a constant temperature incubator and heated for 6 min to allow the toxin to bind to the antibody. After the reaction, the test strip was placed into the fluorescence immunoassay quantitative POCT analyzer, and the toxin concentration in the sample was obtained. All steps were carried out under specified temperature and time conditions to ensure the accuracy of the results. The equipment and reagents used in the experiment are shown in Fig. 1.

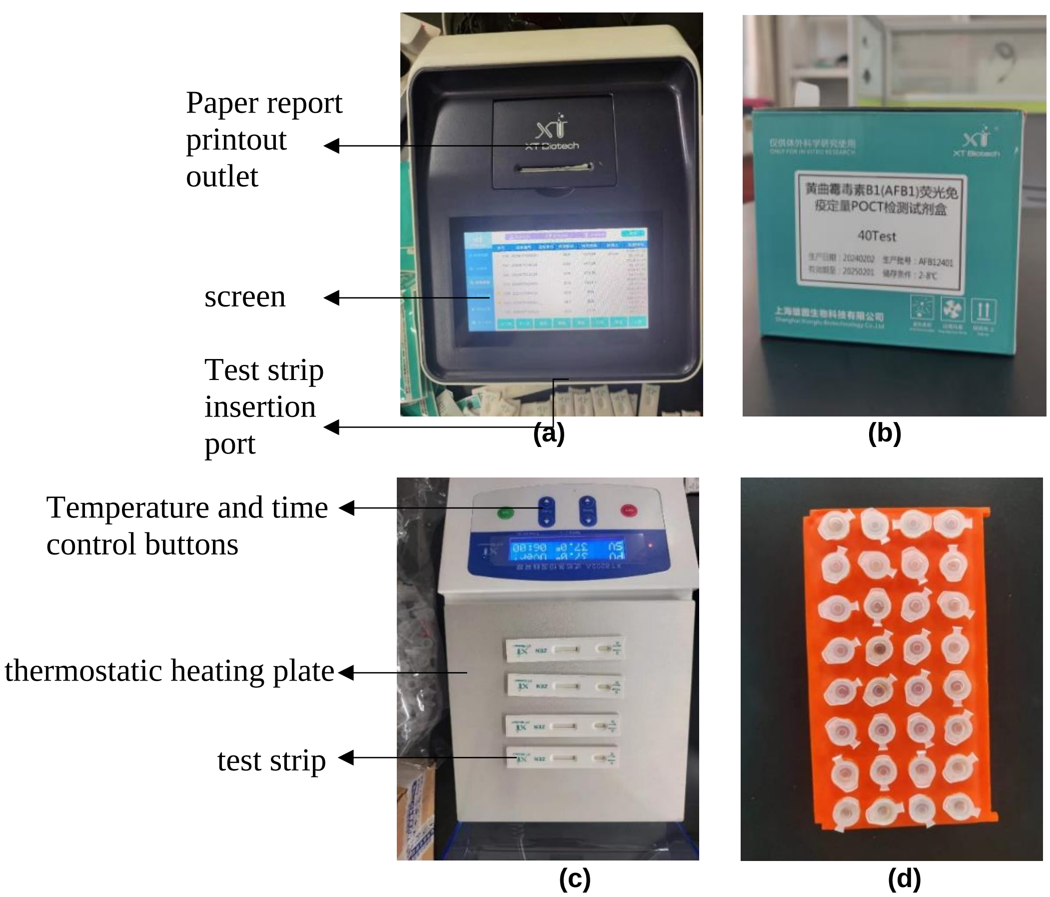


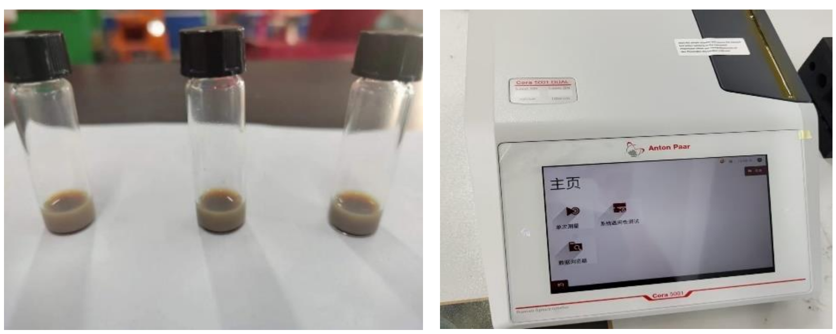
Fig. 1. (a) Fluorescence immunoassay quantitative POCT analyzer, (b) Detection kit, (c) Constant temperature incubator, (d) Sample

Surface-enhanced Raman spectroscopy measurement

To measure the Raman spectral data of corn samples, measurements were carried out under a 1064 nm laser to ensure high-quality spectra. First, silver nanoparticles were prepared as the SERS substrate by adding 200 mL of deionized water and 33.72 mg of silver nitrate (Sinopharm, Beijing, China) to a clean and sterile conical flask. They were gently mixed and heated on a magnetic stirrer. After boiling, 8 mL of a 1% by mass sodium citrate solution was added to the mixture, which was heated at a stirring rate of 650 r/min for 15 min. After heating, the solution was stirred until it cooled to room temperature. Then, deionized water was added to bring the final volume to 200 mL. Next, 1 mL of the final solution was placed in a sterile centrifuge tube and centrifuged at 7000 r/min for 7 min. After centrifugation, the supernatant was discarded, and the pellet was resuspended it with 100 μL of ddH_20 to obtain a uniform milky gray solution. This solution is a negatively charged silver nanoparticle substrate with a pronounced optical enhancement effect.

To prepare samples for SERS, the sample solution of corn fluorescence immunoassay was mixed with the SERS substrate to ensure uniform distribution. The mixed solution was placed in a Raman spectrometer for detection. The spectral data of each sample was recorded, and baseline correction was performed on the spectra to eliminate background interference.

The instruments and reagents used in the experiment are shown in Fig. 2, and the obtained spectral data are shown in Fig. 3.



(a) (b)

Fig. 2. (a) Prepared silver ion substrate; (b) Raman spectrometer

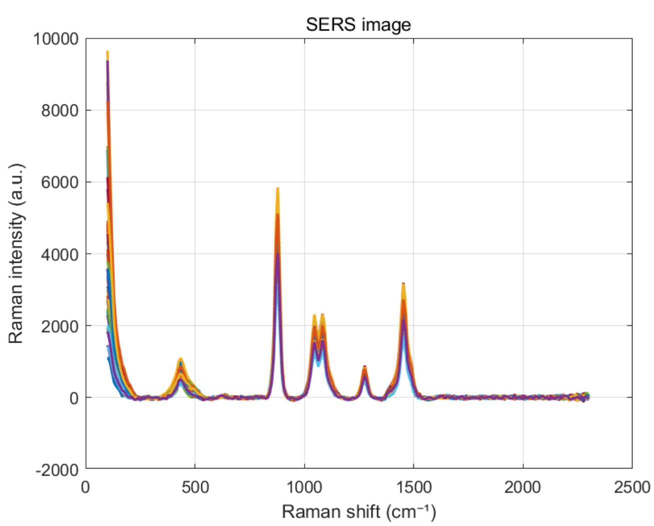


Fig. 3. Surface-enhanced Raman spectroscopy data

Electronic nose detection

First, the corn sample was placed in a sealed centrifuge tube. To ensure the stability and repeatability of the data, the sample was left at room temperature to reach a stable odor release state. Before measurement, the centrifuge tube was placed in a water bath at 50 °C for 30 min. After starting the electronic nose device, the cleaning time was set to 60 seconds, and the measurement time was set to 120 seconds for odor sampling. The device analyzes the collected gas through the sensor array, and each sensor generates a corresponding response signal. The odor sensor data was recorded for each sample, including the response value and change trend of the sensor. The instruments used in the experiment are shown in Fig. 4. below.

Fig. 4. Electronic nose instrument

Data Processing and Model Construction

Data preprocessing

Because the spectral data obtained by the spectrometer, in addition to the information of the component to be tested of the measured sample, is easily interfered with by irrelevant information such as stray light, baseline drift, noise, and sample background, it had an effect on the modeling. To improve the accuracy of spectral measurement and the signal-to-noise ratio of spectral data, denoising processing and baseline correction were performed on the SERS spectral data. Most of the data collected by the electronic nose presented a waveform that first rose, then fell, and finally stabilized. The odors or volatile compounds produced by moldy corn usually reach a stable state after molding, which continue to be released for a period of time. Therefore, analyzing the data in the final stable stage can better capture the odor characteristics related to mildew, while eliminating possible interferences at the beginning of collection, such as environmental odors or other noises. Additionally, the relatively stable data at the 60th second is collected for analysis. At the same time, the electronic nose data were standardized and detrended to eliminate the influence of environmental changes on sensor responses.

Feature Extraction

Feature extraction of spectral data

The main Raman peaks and their intensities were extracted from the SERS spectrum, and spectral features related to toxins were identified (Gao *et al*. 2012; Li *et al*. 2014; Zhu *et al*. 2024). The experimental environmental factors, sample factors, spectrometer laser power, and other factors were considered to determine that the Raman shifts of 798, 1050, 1082, and 1276 cm^{-1} were the characteristic peaks of AFB1. According to the literature (Hu *et al*. 2017; Martinez *et al*. 2020; Serebrennikova *et al*. 2024), the Raman shifts of 1064, 1266, and 1452 cm^{-1} are the characteristic peaks of OTA.

Feature extraction of electronic nose data

The characteristic parameters of sensor responses were extracted from electronic nose data, including response intensity and correlation between sensors. The odors released when corn molds are usually related to volatile organic compounds, including but not limited to the following categories: ketones, alcohols, aldehydes, acids, and other volatile organic compounds. Therefore, the sensor signals in the electronic nose that are sensitive to such substances were selected for analysis. The principal component analysis (PCA) method selected to reduce the dimension of electronic nose data. To test whether the data were suitable for principal component analysis, a KMO test and a Barlett spherical test on the data of principal component analysis were performed. The results showed that the KMO sampling adequacy measure was 0.638, greater than 0.6, and the value of p was less than 0.05, indicating that the data supports principal component analysis. According to the principle that the eigenvalue was greater than 1, two common factors were extracted, and the cumulative variance contribution rate was 88.274%. Accordingly, by extracting two common factors, the variance of 88.274% of the original data was reflected. Thus, the dimensionality reduction of electronic nose data was completed.

Fusion of Spectral Data and Electronic Nose Data

The fusion method for Surface-Enhanced Raman Spectroscopy (SERS) data and electronic nose data adopted the weighted fusion method in feature-level fusion. Different weights were assigned to the data of four Raman characteristic peaks extracted for AFB1 and the data of two principal components extracted by principal component analysis from the electronic nose. Through the cross-validation method, it was found that the model achieved the best performance when the weight of the Raman data was 0.77 and the weight of the electronic nose data was 0.23. Similarly, for the data of three Raman characteristic peaks extracted for OTA and the data of two principal components extracted from the electronic nose, the weighted fusion method was also adopted. After cross-validation, it was obtained that the model had the best performance when the weight of the Raman data was 0.8 and the weight of the electronic nose data was 0.2.

Construction of Mycotoxin Content Prediction Model

Random Forest (RF) is a common machine learning algorithm used for classification and regression problems. The working principle of random forest in classification problems is as follows: construct multiple decision trees; for each decision tree, randomly select a part of features and samples for training; each decision tree makes classification predictions on samples; and a new sample is determined by the joint voting of all decision trees. The working principle of random forest in regression problems is similar. Each decision tree makes regression predictions on samples, and the predicted value of a new sample is the average of the predicted values of all decision trees. The advantages of random forest lie in having high accuracy and stability, not being prone to overfitting, being able to handle high-dimensional data, being insensitive to feature selection, and being able to evaluate the importance of features.

The extracted spectral feature data and electronic nose feature data, together with the toxin content data detected by fluorescence immunoassay, were trained by the RF algorithm to construct prediction models for AFB1 and OTA. During the model training process, model parameters such as the number of decision trees and the minimum number of leaves were optimized through cross-validation methods, and the optimal feature variables were selected to improve prediction accuracy. It was found that the model had the best performance in predicting AFB1 and OTA when the number of decision trees and the minimum number of leaves were 100 and 5 respectively.

RESULTS AND DISCUSSION

The contents of aflatoxin (AFB1) and ochratoxin (OTA) in corn samples detected by the fluorescence immunoassay method showed significant differences among the samples. The specific results were as follows: Among all the tested samples, the concentration range of aflatoxin ranged from 5.68 ppb to 2770 ppb. The concentration range of ochratoxin ranged from 6.24 ppb to 247 ppb. The relatively high concentrations in some samples indicated the presence of mildew problems. These detection results provide basic data for the subsequent analysis of SERS and electronic nose data.

The SERS spectral measurement showed that the Raman spectral characteristics in the corn samples were obvious, and there were some intense Raman peaks. The Raman characteristic peaks of aflatoxin and ochratoxin exhibited different peak values and intensities in the SERS spectra of the samples. Samples with different toxin concentrations showed differences in the peak positions and peak intensities of the SERS spectra. Such changes were linearly correlated with the increase in toxin concentration, indicating that the SERS technique is feasible in detecting the toxin content in corn.

The results of the electronic nose detection showed that the odor characteristics of the corn samples had relatively strong differences, and these differences were somewhat related to the toxin content in the samples. The response data of the odor sensors for each sample indicated that the odor components released by the samples with higher toxin concentrations were different from those of the samples with lower toxin concentrations. The sensor response values of the electronic nose had a certain correlation with the types and concentrations of toxins. By analyzing the electronic nose data, it was found that the corn samples with higher toxin contents had more complex odor characteristics and more significant changes in the sensor response values. This indicates that the toxin content will affect the release of odor molecules in corn, thus changing the detection data of the electronic nose.

Model Results

The experimental results showed that the AFB1 and OTA prediction models performed well on the training set, and the correlation coefficient exceeded 0.7. By evaluating the prediction results of the test set, the correlation coefficient of the AFB1 prediction model test set reached 0.70, and the correlation coefficient of the OTA prediction model test set was 0.74, indicating that this model accurately predicted the toxin content in corn and had high accuracy and robustness. The results are shown in Fig. 5.

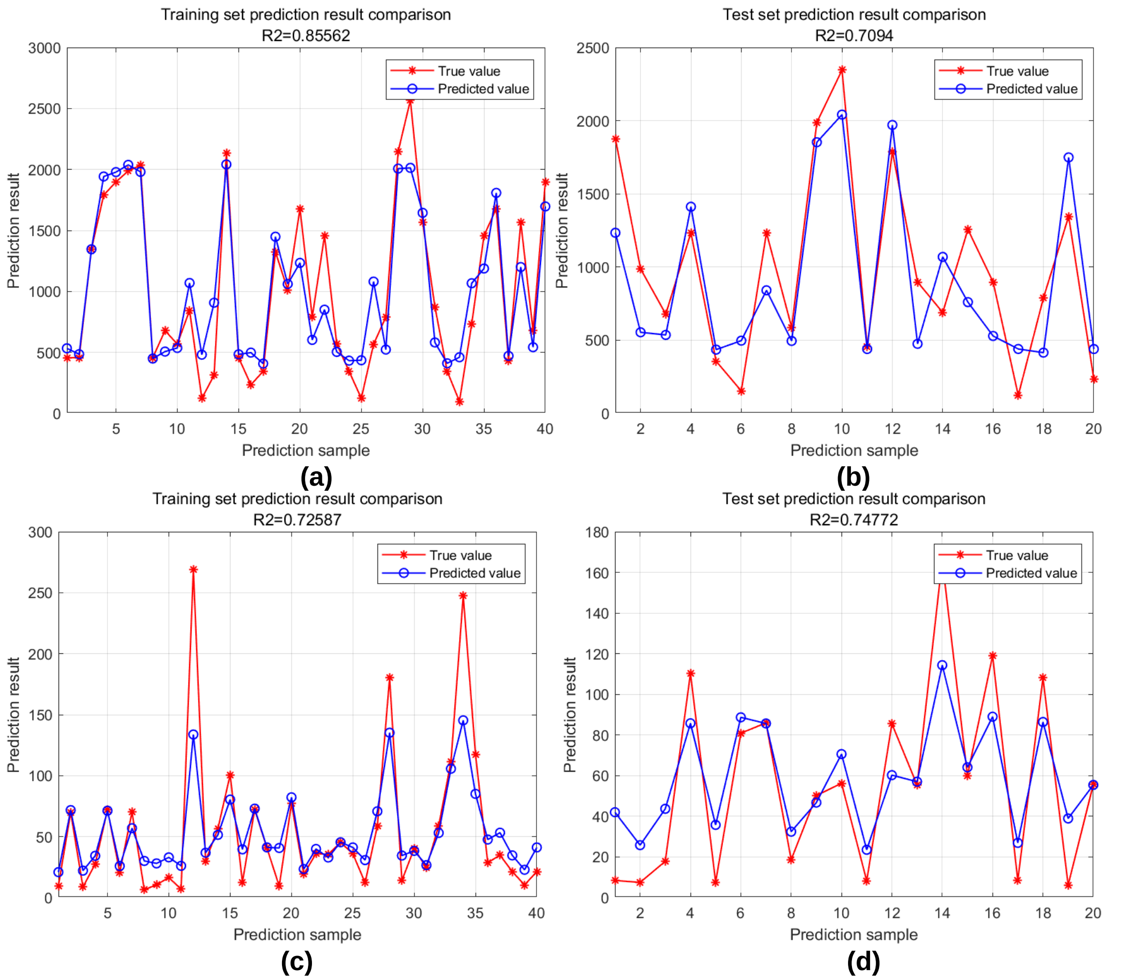


Fig. 5. (a) Results of AFB1 model training set, (b) Results of AFB1 model test set, (c) Results of OTA model training set, (d) Results of OTA model test set

Discussion

The results of this study indicate that the combination of surface-enhanced Raman spectroscopy and electronic nose technology are effective for the detection and prediction of toxins in corn. Some notable points are as follows: SERS technology realizes the detection of low-concentration toxins by enhancing Raman signals. Electronic nose technology provides supplementary information for toxin detection by identifying odor molecules. The combination of these two technologies improves the comprehensive detection ability and compensates for the deficiencies of a single technology. This method quickly and accurately predicts the toxin content in corn and has a high practical application value. Especially in grain quality control and food safety detection, this method provides a real-time and non-destructive detection scheme.

Although the results were favorable, there are still certain challenges. The prediction accuracy of the model in low-concentration toxin samples was insufficient. Future research is needed to enhance the prediction performance by optimizing data preprocessing methods, improving model algorithms, and increasing the sample size.

To summarize, the corn quality detection method based on SERS and electronic nose technology proposed in this study has an efficient application prospect and provides new ideas and technical means for food safety detection. Future research should focus on further optimization of the technology and verification in practical application scenarios, to ensure its reliability and effectiveness under different conditions.

CONCLUSIONS

1. The fusion model established based on the Random Forest (RF) algorithm demonstrated relatively high accuracy and stability in the aspect of corn quality detection. The coefficients of determination for the two prediction models on the test set reached 0.70 and 0.74 respectively.
2. The prediction models performed poorly when dealing with samples with extremely low toxin concentrations. As can be seen in the model for predicting OTA, when the concentration of OTA was lower than 30 ppb, relatively large errors occurred in the model.
3. Since the number of samples was relatively small, the results obtained may lack universality. Subsequently, it is necessary to conduct experiments based on a large number of samples so as to obtain more stable and reliable models.

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