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Article

Electronic nose-based technique for rapid detection and recognition of moldy apples

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Abstract: In this paper, PEN3 electronic nose was used to detect and recognize fresh and moldy apples (inoculated with *Penicilliumexpansum* and *Aspergillusniger*) taken Golden Delicious apples as model subject. Firstly, the apples were divided into two groups: apples only inoculated with different molds (Group A) and mixed apples of inoculated apples with fresh apples (Group B). Then the characteristic gas sensors of the PEN3 electronic nose that were most closely correlated with the flavor information of the moldy apples were optimized and determined, which can simplify the analysis process and improve the accuracy of results. Four pattern recognition methods, includinglinear discriminant analysis (LDA), backpropagation neural network (BPNN), support vector machines (SVM) and radial basis function neural network (RBFNN), were then applied to analyze the data obtained from the characteristic sensors, respectively, aiming at establishing the prediction model of flavor information and fresh/moldyapples. The results showed that only the gas sensors of W1S, W2S, W5S, W1W and W2W in the PEN3 electronic nose exhibited strong signal response to the flavor information, indicating were most closely correlated with the characteristic flavor of apples and thus the data obtained from these characteristic sensors was used for modeling. The results of the four pattern recognition methods showed that BPNN presented the best prediction performance for the training and testing setsfor both the Group A and Group B, with prediction accuracies of 96.29% and 90.00% (Group A), 77.70% and 72.00% (Group B), respectively. Therefore, it first demonstrated that PEN3 electronic nose can not only effectively detect and recognize the fresh and moldy apples, but also can distinguish apples inoculated with different molds.

Keywords: Electronic nose; apple; mildew; pattern recognition; artificial neural network; nondestructive examination

1. Introduction

Apples are widely consumed because of their rich vitamin, water, and dietary fiber contents. The apple industry, with an annual total output of more than 40 million tons, has become the first major fruit industry in China. Unfortunately, because of improper storage after harvesting, apples are vulnerable to diseases induced by various internal and external factors and infections by pathogenic microorganisms, resulting in serious post-harvest losses[1, 2]. A few moldy apples, which can be difficult to find from the surface in time, may lead to greater losses in the absence of a prompt solution. Therefore, development of a rapid and nondestructive detection method for moldy apples

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will help to guarantee the safety and quality of apples during storage and improve the competitiveness of Chinese apples in the global market. And it will help fruit farmers reduce their economic losses and help satisfy consumers by assuring the quality of the apples.

Methods such as gas chromatography [3], gas chromatography–mass spectrometry [4], stable isotope identification, and fluorescence spectroscopy have long been used to analyze the variety and concentrations of single substances in materials under test. However, these methods require expensive instrumentation and fail to evaluate the tested materials comprehensively [5], despite their high accuracy. Most importantly, they are time-consuming and are ineffective in rapid detection applications. The electronic nose technology that imitates the functions of the human olfactory systemhas seen rapid development in recent years.It can realize recognition of the characteristic information of complex flavors [6, 7] and provide superior performance in terms of response time, detection speed, evaluation range, and repeatability [8]. An electronic nose consists of two parts: a gas sensor array and a pattern recognition system [9, 10]. The gas sensor array collects the flavor characteristic information of the sample under test and sends the resulting data to the pattern recognition system. The pattern recognition system then processes the data and outputs the detection results for specific qualities of the samples. Commonly used pattern recognition methods used for electronic noses include principal component analysis (PCA) [11], linear discriminant analysis (LDA) [12, 13], support vector machines (SVM) [14] and artificial neural networks (ANNs) [15, 16].

There have been numerous reports of the application of electronic noses in different agricultural product detection.For example, Russo et al. investigated the classification of red onion varieties using the ISE Nose 2000 electronic nose(Airsense Company, Germany) [17]. The analysis accuracy of the DFA (Deterministic Finite Automaton) model used in their research was as high as 97.5%. Konduru et al. tested onions with sour skin disease using an electronic nose composed of nine metal oxide sensors, and the prediction model established attained an accuracy of 85% [18]. Biondi et al. used a PEN3 electronic nose(Airsense Company, Germany) in a survey of common ring rot and brown rot in potatoes [19]. The prediction model in their research achieved the recognition accuracy of 81.3%. Cheng et al. studied tomato seedlings that had been infected with early blight using a PEN2 electronic nose (Airsense Company, Germany) and obtained an optimum model accuracy of 87.5% [20].In addition, electronic noses have also be applied to mildew detection in grain crops. Yin et al. explored the effects of different features combination characterizations for identifying the moldy maize by using a homemadeelectronic nose [21]. And a positive judgment rate of 96% was obtained with Fisher discrimination analysis.Lippolis et al. used the ISE Nose 2000 electronic nose to wheat mold detection by using the fungal volatile metabolite deoxynivalenol (DON) of wheat as the detection index [22]. The DFA model used in this research yielded a recognition rate of 86.7% for durum wheat.

Until now, researchers are also working on fruit disease detection using electronic noses [23]. For example, Zhu applied the PEN3 electronic nose to classify and identify strawberries that had been artificially inoculated with three pathogens and obtained satisfactory results [24]. In addition, researches with regard to the application of electronic noses to the volatile flavor characteristics information of apples are currently well-established. However, the researches are mostly focused on differences among varieties [25], freshness identification [26], and storage time predictionof the apples [27, 28], along with the detection, analysis, and application of quality and nutrition information [29]. While little attention has been paid to the detection ofmildew in apples, especially discrimination of different mildew.

Therefore, inspired by previous researches, PEN3 electronic nose was used to detect and recognize fresh and moldy apples (artificialinoculation with *Penicilliumexpansum* and *Aspergillusniger* on Golden Delicious apples) in this study. To simplify the analysis process and improve the prediction accuracy, the gas sensor arrays of the PEN3 electronic nose were firstly optimized and determined. And four pattern recognition methods, including LDA, backpropagation neural network (BPNN), SVM and radial basis function neural network (RBFNN), were respectively compared to analyze the characteristic flavor data for establishing the prediction model of flavor information for fresh/moldy apples.

2. Materials and methods

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2.1. Materials

The apples samples used in the experiments were Golden Delicious apples picked from an orchard in the Changping District in Beijing. And the apples were mature and fresh, all had a similar color, and were without surface damage and diseases. The inoculated molds were *Penicillium expansum* and *Aspergillus niger*; potato agar was used as the culture medium (Sinopharm Chemical Reagent Beijign Co., Ltd, China, Analytical grade). PEN3 electronic nose, which has 10 different built-in metallic oxide gas sensors that can realize the detection and identification of various common gases (shown in Table 1), was used for collecting the characteristic flavor of apples. All other solutions were prepared with Milli-Q water (18.2 MΩ/cm resistivity) from a Millipore Milli-Q system (Thermo Scientific EASYpure II, America). All glassware ware was pre-washed three times with Milli-Q water and then dried in an oven.

Table 1. Performance of the sensor arrays of the PEN3 electronic nose.

Nos. in Array	Sensor Name	Reaction compound	Typical Targets
R1	W1C	Aromatic compounds	$C_6H_5CH_3$
R2	W5S	Oxynitride	NO_2
R3	W3C	Aromatic constituents, mainly ammonia	C_6H_6
R4	W6S	Hydrogen	H_2
R5	W5C	Alkanes, aromatic compounds	C_3H_8
R6	W1S	Broad Methane	CH_4
R7	W1W	Sulfides and organic sulfides	H_2S
R8	W2S	Broad Alcohols	C_2H_5OH
R9	W2W	Aromatics, organic sulfides	H_2S
R10	W3S	Alkanes, especially methane	CH_4

2.2. Method of mold inoculation

2.2.1. Culture and purification of the molds

Firstly, the molds were made into suspensions using the pour plate method; these suspensions were then diluted using sterile water into proportions of 1:10, 1:100, and 1:1000. We took 1 ml from each diluted solution and mixed each solution with sterilized but uncondensed potato agar (acting as the culture medium). After being evenly shaken, the mixed solutions were poured into culture dishes and kept until natural coagulation occurred. The mold plates made as described above were then cultured at 25°C using the plate streak method. Finally, the pure colonies of*Penicilliumexpansum* and *Aspergillus niger* were obtained about five days later. The filamentous single colonies obtained were then sealed in sterile environments and preserved in a refrigerator at 2°C for direct use in the future experiments.

2.2.2. Mold inoculation of apples

Firstly, the apple samples were aired naturally on a sterile workbench after being cleaned using alcohol with purity of 75%. Each apple was then drilled with four holes (3mm diameter with a depth of 5mm) located at different positions on the surface using an inoculation needle. The holes were filled with the pure colonies of *Penicillium expansum*and *Aspergillus niger* from the culture dishes by using an inoculation ring and were then covered using sterile parafilm (Bemis Company, Inc., America). The inoculated apples were placed into sterile beakers and further sealed using parafilm.

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The samples were then placed into a thermostat incubator (4℃) and cultured for five days until mildew appeared. Before the experiments began, the inoculated apples were carefully observed to ensure that they had been impregnated by the molds.

2.3. Apples sample set division

Before PEN3 electronic nose measurement, the apple samples were divided into two groups: individual apple only inoculated with/withoutonekind of mold (single sample group, Group A) and mixed apples of inoculated apples with fresh apples (canned sample group,Group B). The canned sample group consisted of fresh apples and moldy apples (inoculated with single molds of *Penicillium expansum*and *Aspergillus niger*, respectively) with a 9:1 ratio, and placed in a sealed can environment. Further, the apple samples of Group A and Group B were randomly divided into training set and testing set, training set A (TA) and testing set A (VA) for Group A and training set B (TB) and testing set B (VB) for Group B, respectively. The training set was used for building the prediction model, and the testing set was used for validating the prediction model.The experimental apple informtionof differernt sample groups or sets of fresh or individual molds infected apples was listed in Table 2.

Table 2. Experimental apple informtion of differernt sample groups

Sample Groups		Training set		Testing set	
		Training samples	Numbers of apples	Training samples	Numbers of apples
Group A	fresh	54	54	10	10
	Penicillium expansum	54	54	10	10
	Aspergillus niger	54	54	10	10
Group B	fresh	49	490	9	90
	Penicillium expansum	45	450	8	72[a] 8[b]
	Aspergillus niger	45	450	8	72[a] 8[b]

[a] fresh apples; [b] moldy apples

2.4. Characteristic data collection of apples using electronic nose

The PEN3 electronic nose is applied to collecting the characteristic flavor of the apples. All the experiments were done in the laboratory fume hood. Briefly, the inoculated apples were placed at room temperature (20 ℃)for 30 min after they were taken out from the incubator. And headspace sampling was then initiated using the PEN3 electronic nose by inserting the sampling and pressure-stabilizing needles into the headspace of the beaker and drawing the flavor from the beaker for 150 s after cleaning for 70 s. Filtered air was used as the carrier gas. Using the PEN3 detection figure, it was found that the response value of the sensor tended to be stable after 50 s (as shown in Figure 1). Therefore, the data acquired from 60 s to 150 s was used as the effective data for the data analysis procedure.

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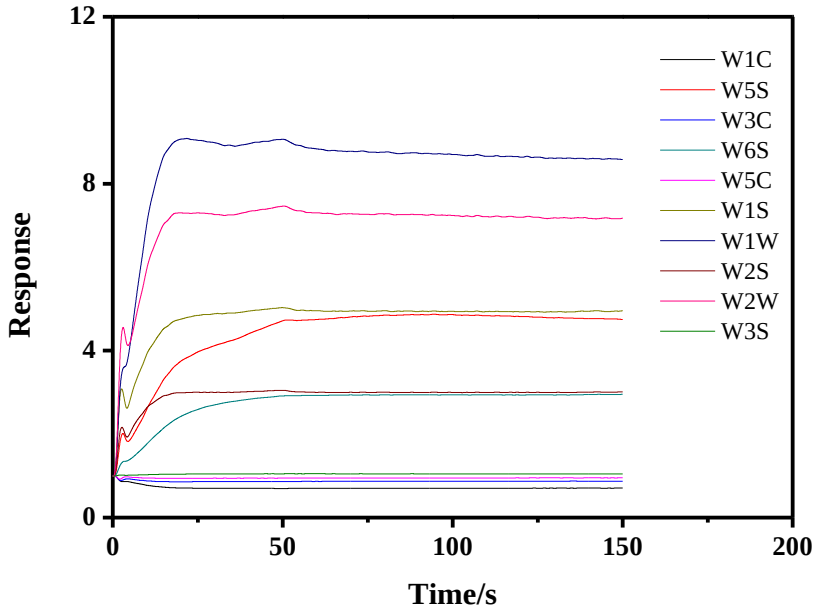

Figure 1. Time-dependent data response of the PEN3 electronic nose

2.5. Data preprocessing

The data preprocessing system is as an important link in the electronic nose system. The sampled odourinforantion signals were transmitted to the data preprocessing system for analysis and processing, giving digital signals (collectedsample data). Then, the average value of the data acquired from 60 s to 150 swas calculated and used as the feature signal value for statistic analysis. And in this study, four pattern recognitionmethods, including LDA, SVM, BPNN and RBFNN, were applied to analyze the data obtained from the characteristic sensors. The data analysisfor SVM was performed usingLibSVM toolbox (Libsvm-3.1,Taiwan), and for LDA, BPNN and RBFNNwas performed usingMatlab 2017 (Math works Inc., USA).

3. Results and disscussion

3.1. Mold culture and inoculation on apple

As described in section 2.2.1, the moldsof *Penicillium expansum*and *Aspergillus niger* were first cultured in the culture dishes with potato agar as the culture mediumby using the plate streak method. Keeping at 25°C and five days later, the filamentous single pure colonies of *Penicillium expansum*, *Aspergillus niger*were obtained (shown in Figure 2b, 2c, respectively). The pure colonies obtained were then sealed in sterile environments and preserved in a refrigerator at 2°C for direct use in the future experiments.

a) b) c)

Figure 2. Cultured molds: a)culture mediumwithout mold b) *Penicilliumexpansum*; c) *Aspergillus niger*. (Conditons: culture at 25°C for 5 days; dish: 90mm）.

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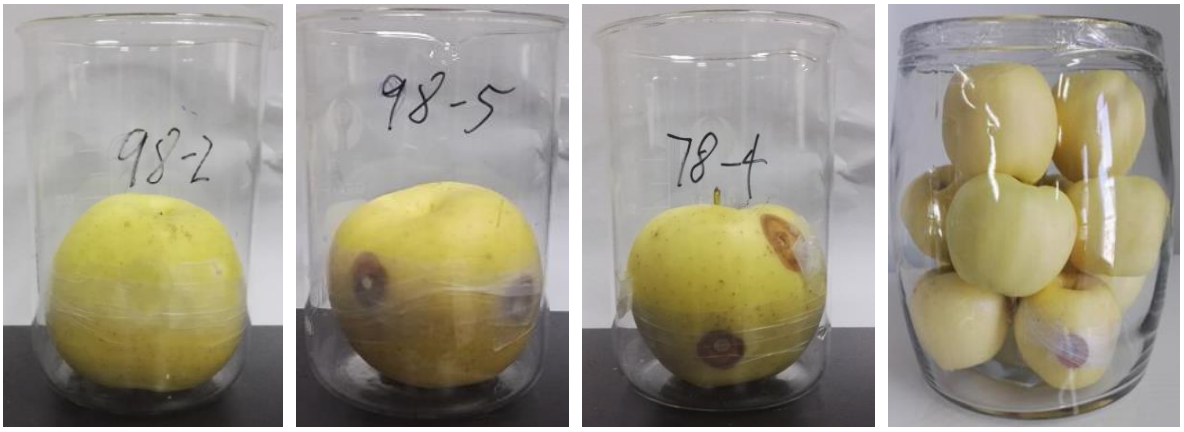
Next, the pretreated apples were inoculated with the cultured pure molds by filling the holes drilled at the apple surface using the inoculation ring (the left holes after samplingcan be seen in Figure 2). The inoculated appleswereplaced into sterile beakers, sealed using further seal films, and kept at 4℃. As shown in Figure 2, the mildew appeared on the apple surface (Figure 3b, 3c, 3d) after five days, which also confirming the apples were successfully impregnated by the differentmolds. And then, the inoculated apples was used for PEN3 electronic nose anlysis.



a) b) c) d)

Figure 3. Singleapple samples inoculated with different molds: a) No mold (fresh apple); b) *Penicillium expansum*; c) *Aspergillus niger*.Cannedapple samples inoculated with single mold (fresh apples: moldy apple, 9:1) d). (Conditons: kept at 4°C for 5 days).

3.2. Determination of Characteristic flavorgas sensors

As shown in Table 2, PEN3 electronic nose has 10 different built-in metallic oxide gas sensors, and it will simultaneously collect 10 sets of data information in one measurement. However, some of these data information are not characteristic flavor information for fresh/moldy apples. To simplify the subsequent analysis process and improve the prediction accuracy, the gas sensor arrays of the PEN3 electronic nose were first optimized to determine the characteristic flavorsensors for fresh and moldyapples. Then the response of PEN3 electronic nose to the fresh and moldyapples (apples inoculated separately with *Aspergillus niger* and *Penicillium expansum*) were explored and the characteristic values were extracted from the collected data of the ten sensors. As shown in Figure 4, it can be noticed that the sensors give different signal for the four sets of samples measured, and only sensors of nos. R2, R6, R7, R8, and R9 (namely W5S, W1S, W1W, W2S and W2W)exhibited strong responses to the four sets of apple samples, indicating these sensors are the characteristic flavor gas sensors for apples. Therefore, the data collected from thesensors of W5S, W1S, W1W, W2S and W2W were used for data analysis in our study.

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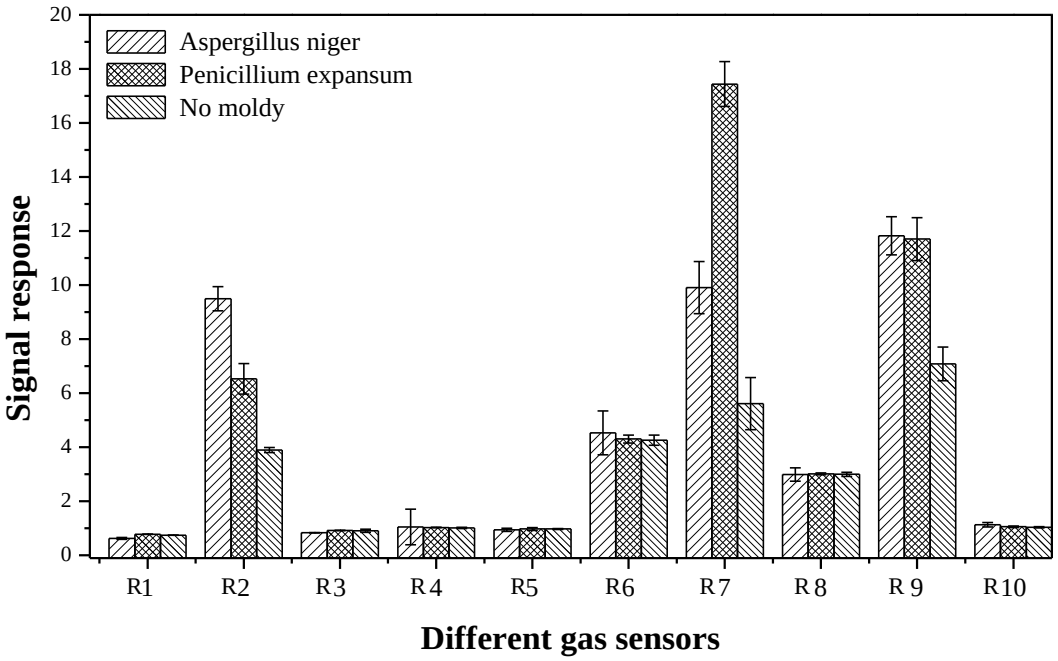

Figure 4. The gas sensors responses of PEN3 electronic noseto apples inoculatedwith different mold.

3.4. Data analysis

Four pattern recognition methods of LDA, BPNN, SVM and RBFNN were respectively applied to analyze the data obtained from the characteristic sensors optimized for the prediction model of flavor information and fresh/moldy apples. For better comparison, an overview of the performance of the four algorithms for moldy apples recognition was listed in Table 3.

Table 3. Recognition accuracies of the four algorithms for the two groups.

Sample Groups	Algorithm	Recognition rate of training set	Recognition rate of testing set
Group A	LDA	79.6%	66.7%
	SVM	94.4%	80.0%
	RBFNN	88.9%	83.3%
	BPNN	96.3%	90.0%
Group B	LDA	68.4%	64.0%
	SVM	70.5%	64.0%
	RBFNN	71.9%	68.0%
	BPNN	77.7%	72.0%

LDA analysis. The purpose of LDA is to find a projection that maps the original sample space to the low-dimensional space, so that the projection of high-dimensional data on the low-dimensional space can make the samples within the class more clustered, and the samples of different categories are separated to the greatest extent. By using LDA algorithm, the test results of prediction accuracies were analyzed and shown in Table 3. As listedin Table 3, the prediction accuracies were all lower than 80.0%, and lower than 70% for sets of TB, VA, VB, indicating that the LDA algorithm showed poor performance, and was not suitable for fresh/moldy apple discrimination.

SVManalysis. SVM is a generalized linear classifier that carries out binary classification on data according to supervised learning, and its decision boundary is the maximum-margin hyperplane that is solved for learning samples.The values obtained from the PEN3 electronic nose are taken as the

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input, and the category as the output.As shown in Table 3, the results indicated that SVM algorithm showed good performance for discrimination of Group Aapple samples inoculated with different molds (testing accuracy 80.0%), but bad recognition performance for Group B apple samples (testing accuracy 64.0%).

RBFNNanalysis. Fistly, the parameters were set for RBFNN analysis. The learning rate was set to 0.08, the momentum factor to 0.1, the maximum iteration epoch to 10000, and target accuracy to 0.02. Then the training data were input into the RBFNN, and the network structure was initialized to train the network. By judging threshold results of the stable error, adjusting the center and the weight of the hidden layer, and increasing the number of training epochs. Finally, the training set was predicted when the error was reduced below the pre-set threshold. The result of the RBFNN iteration process for the moldy apple samples was shown in Figure 5.

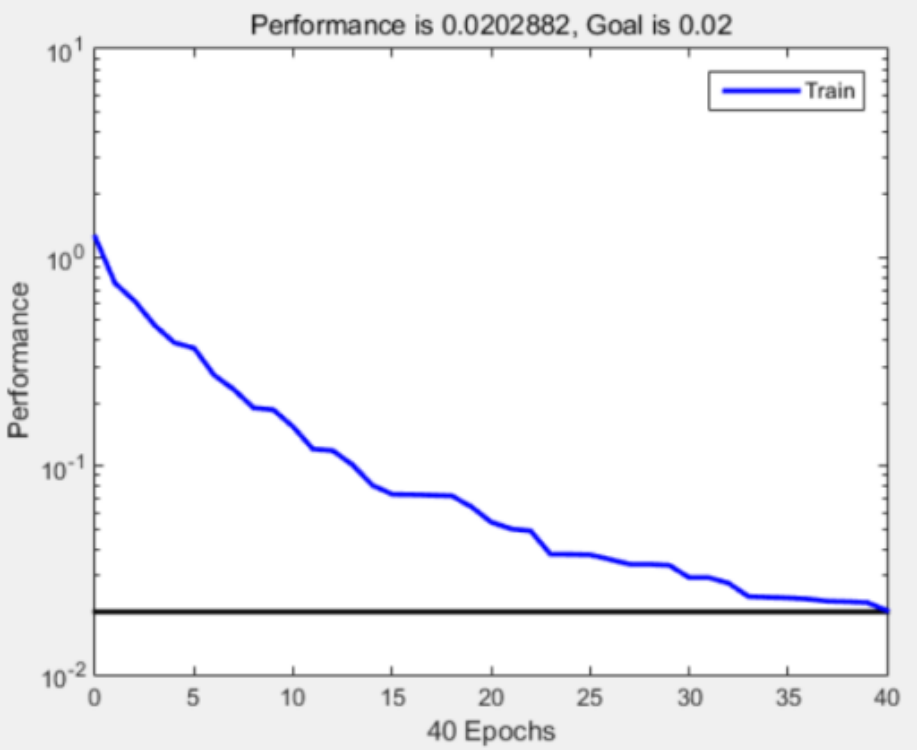

Figure 5. Diagram of the RBFNN iteration process result.

As shown in Figure 5, the training error tends to become stable with a mean square error of 0.020 after 40 training epochs, which meets the maximum error requirements. Furthermore, constantly increasing the training epochs had little effect on the final recognition results obtained after 40 epochs (data not shown), and was time-consuming. Therefore, the designated training epoch number was set at 40 epochs.Then the RBFNN model was constructed for the TA, VA, TB and VB sets, respectively. As shown in Table 3, the testing accuracy was as high as 83.3% for Group A, but was only 68.0% for Group B (a little higher than that of LDA, SVM), indicating the RBFNN algorithm also showed bad recognition performance for discriminating moldy apples of *Penicilliumexpansum* and *Aspergillus niger*, if mixed with fresh apples.

BPNNanalysis. BPNN, which learning processis realized by constantly error backpropagation. In this process, the input information propagates forward, while the errors propagate backward. By a process of constant iteration, training is finally suspended when the error accuracy is reduced to below a preset value or the number of training epochs is maximized. As listed in Table 3, it showed that higher prediction accuracies of 90.0% and testing accuracies of 72.0% were respectively obtaind for Group A and Group B apple samples by using BPNN algorithm. The results also confirmed that the PEN3 electronic nose showed good recognition performance for discriminating fresh and moldy apples, moldy apples inoculated with different molds (*Aspergillus niger*, *Penicilliumexpansum*).

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From the above disscusion, it's obvious that the BPNNalgorithm showed the best performance for recogniston fresh/moldy (*Aspergillus niger*, *Penicilliumexpansum*) apples compared to the other three algorithm, so BPNN algorithm should be firstly considered as the optimized algorithm in the future study. However, the recognition accuracies of the four algorithm for Group B apple samples (canned sample group) were all lower than those of the Group A apple samples (single sample group), this could be explained the collected flavor information of the mildew infected apples was more or less influenced by the mixed fresh apples. But the later was more closer to the real storage environment of apples.

4. Conclusions

In this study, PEN3 electronic nose system was used to detect and distinguish fresh and moldy apples inoculated with *Penicilliumexpansum and Aspergillusniger*. The characteristic sensors of the electronic nose system that most closely correlated with the flavor information of the mildew infected appleswere optimized (designated the W5S, W1S, W1W, W2S, and W2W), which greatly simplifed the analysis procedure and improved the results accuracy.LDA, BPNN, SVM and RBFNNwere respectively used to model the date obtained from the characteristic sensors, and BPNN presented the best recognition accuracy for the two Groups (single sample group and canned sample group), and a higher recognition accuracies was abtained for the single sample group. Therefore, the findings in this study proved that PEN3 electronic nose could not only effectively detect and recognize fresh and moldy apples, but also can distinguish moldy apples inoculated with *Penicilliumexpansum* and *Aspergillusniger*. Furthermore, the electronic nose technology can meet the demands of rapid, low-cost, nondestructive detection merits, which also provide references for developing detection equipment of mildew apple in the future.

Author Contributions: Gang Liang principally conceived the idea for the study, and was in charge of the project management, revising this manuscript, and approval of the manuscript. Wenshen Jia, Hui Tian, Cihui Wan performed the experiments. Hui Tian, Cihui Wan Jing Sun analyzed the data. Gang Liang, Wenshen Jia was responsible for the preparation of this manuscript. All authors participated in some form in the concept, experimentation, writing, and editing of this manuscript.All authors read and approved the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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