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Research Article

Rapid Determination of the Freshness of Lotus Seeds Using Surface Desorption Atmospheric Pressure Chemical Ionization-Mass Spectrometry with Multivariate Analyses

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In order to explore a new method to detect the freshness of lotus seeds, the lotus seeds stored for 0, 1, 2, and 3 years, respectively, were used as experimental materials and analyzed by DAPCI-MS (desorption atmospheric pressure chemical ionization-mass spectrometry). The obtained data were processed by principal component analysis (PCA) and backpropagation artificial neural networks (BP-ANNs). The result showed that DAPCI-MS could obtain abundant chemical material information from the slice surface of lotus seeds. The BP-ANNs model could be applied not only to distinguish fresh and aged lotus seeds with the testing set accuracies of 95.0% and 91.7%, respectively, but also to classify lotus seeds with different storage times with the testing set accuracies of 90.0%, 85.0%, 85.0%, and 90.0%, respectively. The paper developed a fast, convenient, and accurate method for the freshness detection of lotus seed and would provide reliable reference value for rapid authentication of food freshness by the rapid mass spectrometry technique.

1. Introduction

Lotus (*Nelumbo nucifera* Gaertn.) is a perennial aquatic herb of Nymphaeaceae and widely distributed in tropical and subtropical areas [1]. Due to its richness of protein, amino acids, alkaloids, flavonoids, lecithin, and other nutrients, lotus seeds are extensively regarded as a healthy food [2, 3]. Furthermore, lotus is a traditional medicinal plant in Asian countries including China, Thailand, India, Japan, and Korea [4, 5]. In traditional Chinese medical science, lotus seeds have the functions of invigorating the spleen, stopping diarrhea, tonifying the kidney, astringent essence, nourishing the heart, and calming mind [6, 7]. In Korea, the lotus seed is one of the most well-known traditional herbal medicines for the treatment of cardiovascular diseases [8]. Lotus seeds are also used as an antidepressant and an inflammation inhibitor in some Asian countries [9, 10]. Recent studies have shown that lotus seed extracts have the functions of protecting the liver, scavenging free radicals, and antiaging due to their rich flavonoids, polysaccharides, and alkaloids [10–12].

Aging is a very common phenomenon for lotus seeds during storage, and it was shown that the degradation degree of starch increased with the prolongation of storage time of lotus seeds [13]. The study on quality of lotus seeds with different storage times showed that although the amount of starch, fat, and protein remained unchanged roughly, some specific composition varied considerably, such as the great quantity of free fatty acids, produced in the lipid hydrolysis reaction, resulting in the decrease of crude fat content and the increase of fatty acid value [14]. Once the lotus seeds are stored for more than six months, increased hardness and reduced viscosity occur usually, due to respiration, oxidation, and enzyme action [14]. Even with the increase of cooking time or cooking temperature, aged lotus seeds cannot show good taste and quality as fresh lotus seeds [14].

However, it is difficult to distinguish the freshness of lotus seeds with different storage times through their appearances. Therefore, unscrupulous traders sold their aged lotus seeds as fresh ones, which harm the interests of consumers.

Aging also occurs commonly during the storage of other grains [15, 16]. At present, the sensory evaluation method is most commonly used in grain freshness detection (GB/T15682-2008). This method is simple to operate but requires skilled experts trained professionally. In addition, due to the sensory differences of the inspectors, the test results may be accompanied by larger errors [17]. There are also many detection methods, such as the differential scanning calorimeter (DSC) method for the determination of gelatinization degree of lotus seed starch [13], X-ray diffraction method for the detection of wheat starch crystallinity [18], scanning electron microscopy method for structure identification of the rice starch crystal [19], and rapid viscosity analysis (RVA) for the test of rice fluid characteristics [20], depending on measuring instruments. Relative accurate results can be obtained using these methods, but most of them require complicated sample pretreatments, such as the extraction and purification of starch, protein, and other substances. Surface desorption atmospheric pressure chemical ionization-mass spectrometry (DAPCI-MS) technology is a powerful soft ionization technique developed in recent years. It is fast, noninvasive, and real time and able to analyze complex matrix samples. Furthermore, DAPCI-MS does not require toxic reagents and has no secondary pollution. Therefore, it is widely applied in many fields including food quality detection [21–25].

In this study, DAPCI-MS technology was employed to obtain the MS fingerprint of lotus seeds with different storage times, and the obtained data were processed by principal component analysis (PCA) and backpropagation artificial neural networks (BP-ANNs) to construct an identification model of lotus seeds with different freshness. In the experiment, lotus seed slices were examined directly without complicated pretreatment, resulting in no or minor information loss. Therefore, the study could provide an effective reference for the freshness identification of lotus seeds in the market and could be applied in the aging research for other grains.

2. Materials and Methods

2.1. Lotus Seed Samples. The lotus seeds of *N. nucifera* "*guangchangbailian*" were provided by the Guangchang Lotus Science Research Institute (Jiangxi, China). Fresh lotus seeds were removed from seed coat, vacuum-dried to about 13% moisture content, vacuum packaged, and stored in 25~27°C dark as experimental materials. These lotus seeds were sampled after being stored for 0, 1, 2, and 3 years, respectively. Lotus seeds were cut into 2~3 mm thin slices for DAPCI-MS analysis.

2.2. Instruments and Working Conditions. Experiments were performed using a commercial LTQ-XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled with a home-made DAPCI source for ion generation, which has been described previously [21, 25]. As illustrated in Figure 1, a stainless steel needle (SS needle, with an insulator at the end) was inserted coaxially into a fused-silica capillary, and this geometry was carefully arranged so that the SS needle was only about 5 mm prominent. The capillary and the sharp needle were secured coaxially with a union tee (Swagelok, OH, USA) and a silica ferrule. The unoccupied end of the tee piece was connected with a gas line, supplied by high-pressure nitrogen (~0.5 MPa) and carried methanol/H_2O mixture vapor as the reactive reagent. These precursor gases were ejected from the pinhole through the tee tube. When a high voltage (3.5 kV, with a discharge current of about 0.1 mA) was applied to the SS needle, a corona discharge occurred to generate primary cluster ions of the reactive reagents in ambient air. This source was carefully aligned with the LTQ mass spectrometer, and the electrode tip was placed ~5 mm away from the inlet orifice. The distance between the needle tip and the sample surface was 1~3 mm. The generated primary ions were accelerated by the highly localized electric field and the nebulizing flow and subsequently bombarded the sample surface to produce the ions of analytes at ambient surroundings.

The DAPCI source and the LTQ mass spectrometer were both run in the positive ion mode. The temperature of the heated capillary was set at 150°C. Other parameters were default recommended by the instrument manufacturer. All mass spectra were collected with an average time of 1.6 min, while background subtraction was performed on the spectra. Collision-induced dissociation (CID) experiments, up to MS^3, were performed on the characteristic ions to identify the structures of the compounds, with a collision energy (CE) of 20–30% and a mass-to-charge window width of 1.5 units.

2.3. Principal Component Analysis (PCA). Using Matlab (version 7.0, Mathworks, Inc., Natick, MA, USA) software "princomp" function, the mass spectrometry data quality ratio (m/z 50~500) was obtained, and the corresponding signal intensity formed 240 test sample vectors (1×451) from each 60 samples of four groups (stored for 0, 1, 2, and 3 years, respectively). All the test sample vectors composed a sample matrix (240×451) and were introduced into the Matlab package for PCA analysis. New variables were obtained that represented the information in the original data, and to simplify the data, we utilized a few principal components unrelated to each other, by calculating eigenvalues, eigenvectors, and cumulative contribution rates, and the information of original variables was retained as completely as possible.

2.4. BP-ANNs Model. The BP artificial neural network is a multilayer feedforward neural network, in which signals are transmitted forwardly, and errors are delivered back [26]. Fifty principal components (contribution rate reached to 99.99%) extracted by PCA were treated as the input layer of the neural network, which contained 50 input layer nodes, 48 hidden layer nodes, and 2~4 output layer nodes,

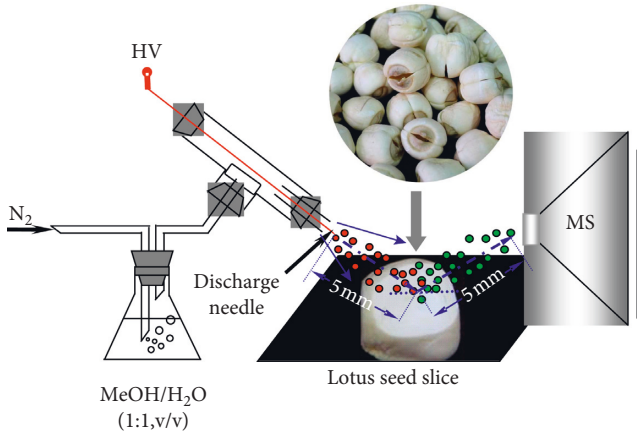

Figure 1: Schematic illustration of DAPCI-MS of lotus seeds.

respectively. In the input layer, the learning rate was 0.1, and the error goal was 0.01, while the maximum training number of iterations was set at 1000 times. In this study, to construct a BP-ANNs model, 160 samples (40 for each group) were selected randomly as the training set, and other 80 samples (20 for each group) were chosen as the test set. In the classification analysis of freshness of lotus seeds, lotus seeds with different storage times were served as output layers, and the output was represented by 1 in the *N* method with the output nodes being 2. In the classification recognition of lotus seeds under 4 different treatments, the output was represented as 1000, 0100, 0010, and 0001, and the number of output nodes was 4.

3. Results and Discussion

3.1. DAPCI-MS Analysis of Lotus Seeds. As shown in Figure 2, the spectrum of lotus seeds stored at different times has high similarity. With the extension of storage time, only a few ions showed slight differences, such as the signal strength of *m/z* 61 and *m/z* 296 decreased slightly, while the signal intensity of *m/z* 149 and *m/z* 251 increased considerably. The DAPCI-MS fingerprints of aged lotus seeds showed there were more small peaks at *m/z* 100–150, suggesting small molecular compounds were more abundant than fresh lotus seeds.

In the storage of grains, due to the oxidation and hydrolysis process, a lot of small molecular substances, such as aldehyde, ketone, and volatile carbonyl compounds, were transformed from lipid compounds including linoleic acid and oleic acid and released usually some unpleasant odor [27, 28]. DAPCI-MS is sensitive to small molecular substances, especially volatile substances. In the DAPCI-MS spectrum, peaks of substances of aged lotus seeds appeared were smaller molecules than those of fresh ones, which was consistent with the existing research results.

The mass spectra of *m/z* 296 were analyzed by tandem mass spectrometry, and the results are shown in Figure 3. Fragment ions *m/z* 265 and *m/z* 250 were formed when the protonated parent ions *m/z* 296 lost the CH_3O or CH_3CH_2OH group after CID, respectively. According to their characteristics, it was inferred that these fragments were from nuciferine which was a common compound in lotus seeds, and the result was consistent with the previous analysis by HPLC [26]. Nuciferine is a valuable bioactive substance with anti-AIDS and antipoliovirus effects [29, 30]. MS results showed that the content of *m/z* 296 decreased with the prolongation of storage time, which may be due to the enzymatic degradation of microorganisms during storage.

3.2. PCA Analysis. Top 10 principal components were extracted with the most informative and the highest contribution rate. The cumulative contribution of the top 3 principal components rate was 87.11%, and that of the top 10 was 98.98%, suggesting that these mass spectrometry data were highly correlated, and the vast majority of variable information can be covered with only a small amount of principal components. After many experimental calculations, the classification was not ideal when the top 10 principal components were used as the input of neural network, probably owing to some variables with a smaller contribution rate that affected the results of discrimination. In this method, PCA was the onset of the neural network model, and the top 50 principal components were the input layer of BP-ANNs. Compared with the 451 original variables, irrelevant variables were greatly reduced and the amount of calculation was simplified.

To distinguish lotus seeds with different storage times, the DAPCI-MS fingerprints of lotus seeds were introduced into Matlab (version 7.0, Mathworks, Inc., Natick, MA, USA) for PCA data processing and analysis, and a three-dimensional PCA score figure could be produced. As shown in Figure 4, the cumulative contribution of PC1, PC2, and PC3 was 95.12%, 1.86%, and 1.06%, respectively. The cumulative contribution of the top 3 principal components rate was 98.04%. It was indicated that PC1 could be used to describe the direction of the largest variable in the data set, and the direction of PC1 was able to distinguish lotus seed samples at different storage times. Therefore, the components of alcohol extracts from lotus seeds in different storage periods were significantly different, and they could be distinguished in different regions of the three-dimensional PCA space.

3.3. Discrimination Analysis of the BP-ANNs Model. Fifty principal components (contribution rate reached 99.99%) were extracted by PCA and served as the input layer of a neural network, to establish a prediction model of a neural network with three layers. Through continuous training, 50 input layer nodes and 48 hidden layer nodes were obtained in the network. Because of the existence of a certain error range between test results and the target output, these test classification results were displayed regionally and more intuitively (Figure 5). In these figures, the horizontal and vertical coordinates had no practical meaning, but they could identify the target output area of lotus seeds with different storage times. 160 random (40 of each treatment) samples were used as the training set, and 80 samples (20 of each treatment) were used as a test set. After 10 training and

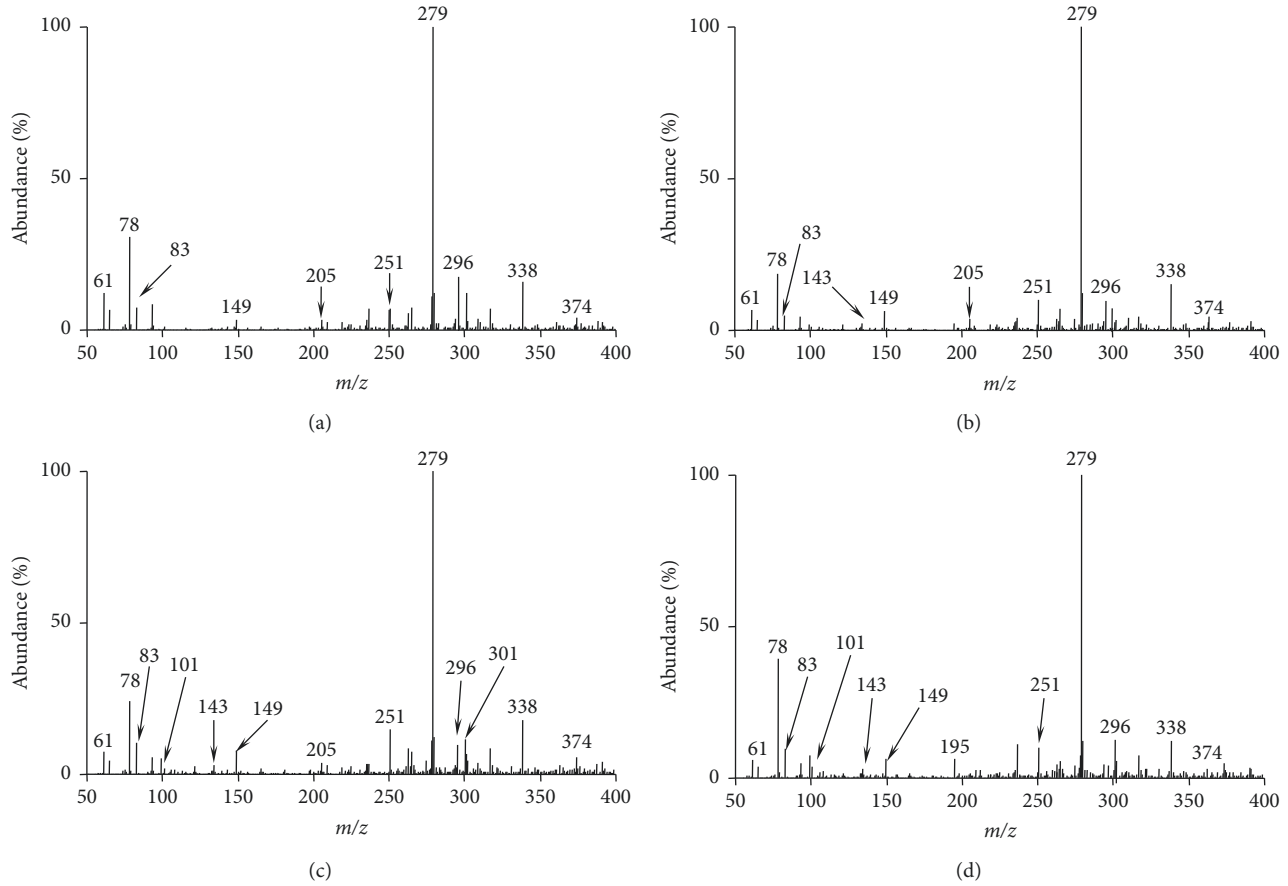

Figure 2: Mass spectra of lotus seeds with different storage times by surface desorption atmospheric pressure chemical ionization-mass spectrometry (DAPCI-MS) under the positive ion mode. Lotus seeds were stored for (a) 0 years, (b) 1 year, (c) 2 years, and (d) 3 years.

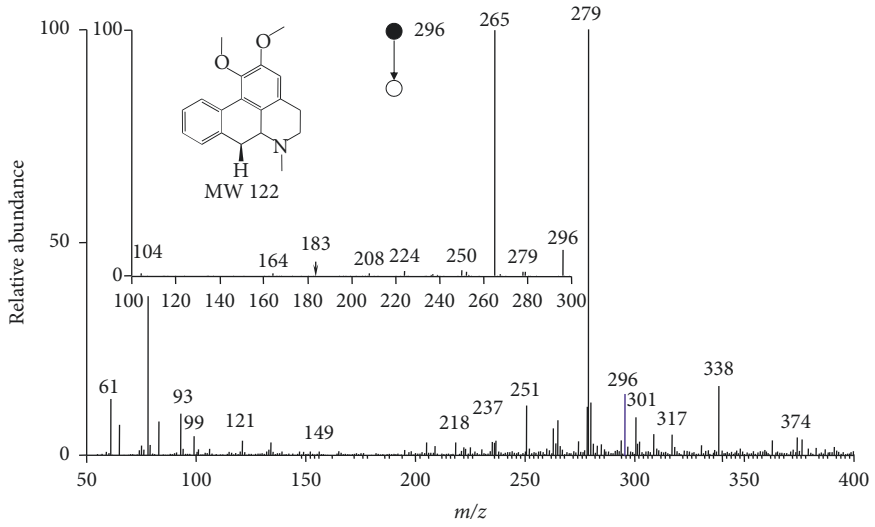

Figure 3: Mass spectra and MS/MS of nuciferine of lotus seeds by DAPCI-MS.

testing, the iterations were 37 to discriminate fresh and aged lotus seeds with a mean square error (MS^E) of 0.0001, while the iterations of lotus seeds with different storage times were 30 with a mean square error (MS^E) of 0.01757.

3.4. Classification of Fresh and Aged Lotus Seeds. In total, the data of 240 lotus seeds (60 lotus seeds for each treatment) were analyzed by PCA. PCA could reduce mainly the dimension of data, provide an experimental sample matrix

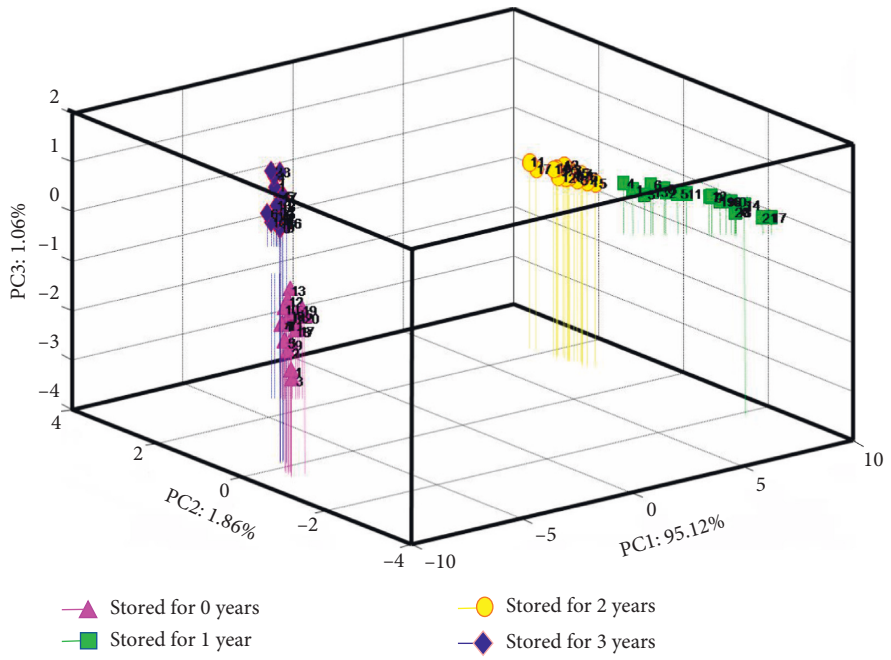

Figure 4: 3D PCA plot of lotus extracts by DAPCI-MS.

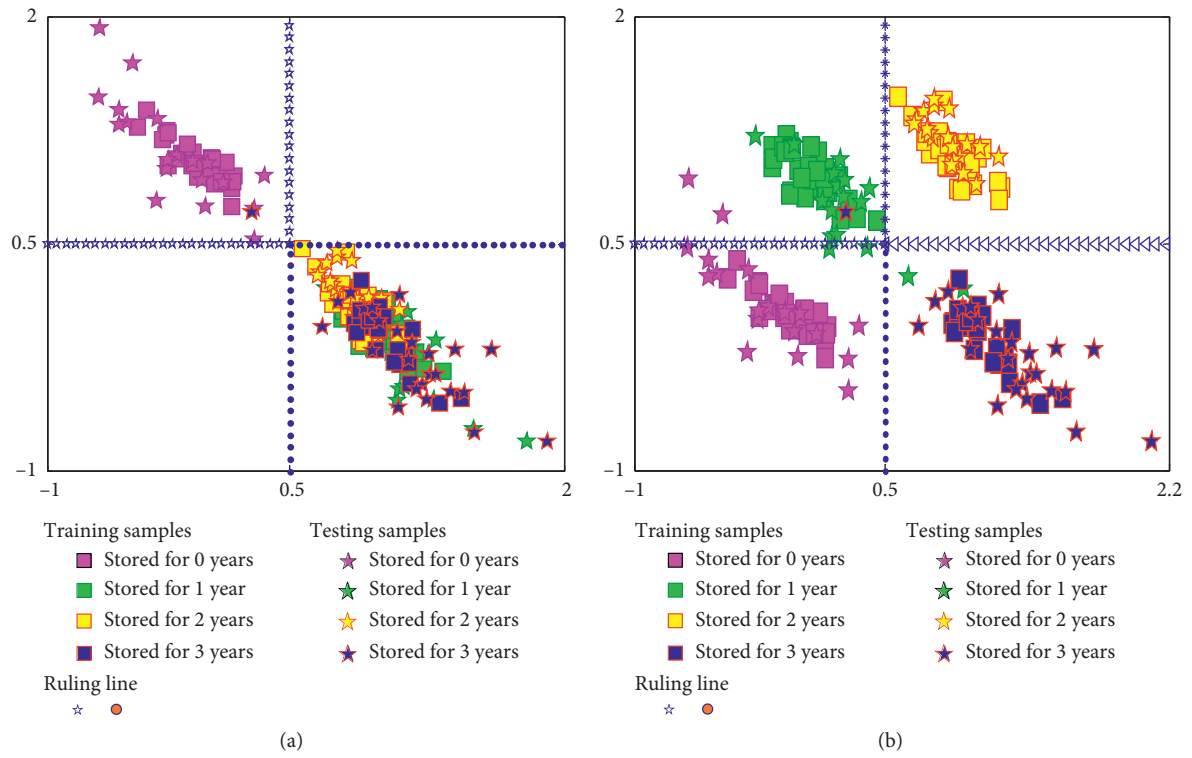

Figure 5: 2D classification results of freshness of lotus seeds (a) and result of 4 kinds of lotus seeds based on BP-ANNs (b).

from the charge ratio and signal strength of mass spectrometry and obtain the principal components that represented the most information of original variables. The cumulative contribution rate of PCA of the top 50 principal components is 94.3%, which fully represents the original information of the samples. Therefore, the top 50 variables were used as the input layer of BP-ANNs.

In order to quickly identify the freshness of lotus seeds, the samples were divided into two categories for learning. The target output of the fresh lotus seeds (stored for 0 years) was set as 10, and that of the aged seeds (stored for 1 year, 2 years, and 3 years, respectively) was 01. The classification and the prediction results from BP-ANNs were mapped into the two-dimensional plane, and the judgment interval of

Table 1: Classification result of different lotus seeds based on BP-ANNs.

Lotus seeds	Fresh	Aged	Stored for 0 years	Stored for 1 year	Stored for 2 years	Stored for 3 years
Training sets accuracy	100	92.5	100	100	100	96.0
Test sets accuracy	95.0	91.7	82.4	85.0	100	84.0

fresh or aged was determined by the same type of decision line. As shown in Figure 5(a), fresh and aged lotus seeds could be clearly distinguished. As shown in Table 1, the accuracy rate of training samples of fresh and aged seeds was 100% and 92.5%, respectively, while that of test samples was 95% and 91.7%, respectively. It was indicated that this method could be used in effective and rapid identification of freshness of lotus seeds.

3.5. Classification of Lotus Seeds with Different Storage Times. To further examine the differences among lotus seeds with various storage times, the samples were divided into four categories for the network learning. The target outputs were set as 1000, 0100, 0010, and 0001, respectively, to correspond with lotus seeds that were being stored for 0, 1, 2, and 3 years. Network classification and predicted results of lotus seeds under four treatments were mapped into a two-dimensional plane, and the judgment interval of each kind of seeds was decided by two adjacent judge lines. As shown in Figure 5(b), lotus seeds under different treatments were gathered in different regions. Furthermore, the accuracy rates for the four groups of lotus seeds were all more than 96%, and the accuracy rates of test samples were all above 80%. It was indicated that this method could be used effectively to identify lotus seeds of different freshness.

Metabolic activities still are ongoing during the storage of lotus seeds, including the respiration, oxidation, and enzyme action, leading to a series of physical and chemical changes [13]. It was reported that the amount of starch, fat, and protein remained unchanged roughly during the storage period of lotus seeds, and some specific composition varied considerably; for example, many of free fatty acids were produced in the lipid hydrolysis reaction and resulted in the decrease of crude fat content and the increase of fatty acid value [14]. In this study, the lotus seeds with different freshness could be classified by the BP-ANNs model accurately, which suggested that some complicated chemical changes could be detected efficiently by DAPCI-MS. Therefore, lotus seeds with a different freshness could be distinguished effectively by DAPCI-MS combined with BP-ANN.

3.6. Analysis Speed and Stability. In this study, the spectral scan time was set as 100 ms, and each lotus seed examined was sampled 6 times continuously. The total detection time of a seed was about 2 min, and the relative standard deviation (RSD) was 7.5%. Therefore, using the DAPCI-MS technology, the freshness of lotus seeds could be distinguished quickly and accurately without any sample pretreatment.

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Yunyang Chi and Liping Luo contributed equally to this work.

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Supplementary Materials

Raw data of lotus seeds stored for different years using surface desorption atmospheric pressure chemical ionization mass spectrometry (DAPCI-MS) under the positive ion mode. There are 6 biological replicates in lotus seeds stored for 3, 1, and 0 years, 6 technical replicates per biological replicate, and 1 data point includes *m/z* and intensity 2 columns. There are 5 biological replicates in lotus seeds stored for 2 years, 6 technical replicates per biological replicate, and 1 data point includes *m/z* and intensity 2 columns. (*Supplementary Materials*)

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